UNIVERSIDAD AUTÓNOMA DE MADRID

DEPARTMENT OF MOLECULAR BIOLOGY

PhD Thesis

The role of Mnk1 kinase in pancreas biology and exocrine diseases

JAROSŁAW CENDROWSKI

MADRID, 2013

AUTONOMOUS UNIVERSITY OF MADRID

FACULTY OF SCIENCES

DEPARTMENT OF MOLECULAR BIOLOGY

The role of Mnk1 kinase in pancreas biology and exocrine diseases

Doctoral thesis submitted to the Autonomous University of Madrid

for the degree of Doctor of Philosophy by

MSc in Biotechnology,

Jarosław Cendrowski

Thesis Director

Prof. Dr. Francisco X. Real Arribas

EPITHELIAL CARCINOGENESIS GROUP

MOLECULAR PATHOLOGY PROGRAMME

SPANISH NATIONAL CANCER RESEARCH CENTRE

Dr. Francisco X. Real Arribas, Head of the Epithelial Carcinogenesis Group at Spanish National Cancer Research Center (CNIO)

CERTIFIES

That Mr Jaroslaw Cendrowski, Master in Biotechnology by the Warsaw University of Life Sciences, has completed his Doctoral Thesis "The role of Mnk1 kinase in pancreas biology and exocrine diseases" and meets the necessary requirements to obtain the PhD in Molecular Biosciences. To this purpose, he will defend his Doctoral Thesis at the Universidad Autónoma de Madrid. The work has been carried out under my supervision and hereby I authorize its defence.

I hereby issue this certificate in Madrid on February 31st 2013

Francisco X. Real
PhD Thesis Director

Marcos Malumbres
PhD Thesis Tutor

This thesis, submitted for the degree of Doctor of Philosophy at the Autonomous University of Madrid, has been carried out and completed in the Epithelial Carcinogenesis Group at the Spanish National Cancer Research Centre (CNIO), under the supervision of Prof. Dr. Francisco X. Real Arribas

This work was supported by La Caixa/CNIO International PhD Fellowship, and grants from Ministerio de Economía y Competitividad, Madrid, Spain: SAF2007-60860 – Cancer de pancreas exocrino: papel de los componentes acinar y ductal y desarrollo de modelos animales; SAF2011-29530 – Control transcripcional de la diferenciacion de las celulas acinares y cancer de pancreas; and CONSOLIDER ONCOBIO.

1
If you set out on a journey let it be long
wandering that seems to have no aim groping your way blindly
so you learn the roughness of the earth not only with your eyes
but by touch
so you confront the world with your whole skin

(...)

7
Więc jeśli będzie podróż niech będzie to podróż długa
powtórka świata elementarna podróż
rozmowa z żywiołami pytanie bez odpowiedzi
pakt wymuszony po walce

wielkie pojednanie

—Zbigniew Herbert, "Journey" („Podróż”)

Dedicated to my beloved wife

Acknowledgements

First of all, I would like to thank my thesis director Paco Real for his enormous support and patience during this time that I have spent in his laboratory: You were always there to help me with advice and motivation, but most of all I want to thank you for showing me how to find excitement in science.

Moreover I would like to acknowledge the members of my my PhD thesis committee, Dr. Marisol Soengas, Dr. Amparo Cano and Dr. Ángel Nebreda for very important feedback during our meetings.

I want to thank our collaborators:
Julia Mayerle, Marcus Lerch and Matthias Sendler, for hosting me in their lab and for big help with experiments,
Marta Cañamero and Antonio Salas for histological analyses.

I am also very grateful to all members (current and former) of Epithelial Carcinogenesis Group that created a great atmosphere in the lab: Laia (for being the best bench neighbor ever), Marinela (for being warm-hearted and helpful from the first day in the lab until the last), Marta (for sharing sweets and for help and collaboration at the end of my project), Miriam (for filling our "fishtank" with joy and optimism), Natalia (por ayuda, energía y las broncas que si que las merecería), Yoli (por toda tu ayuda y paciencia), Jose María (for being there when I needed help), Victor (for creating great atmosphere and helping me to push my project forward), Cristina (for Montoya-evenings, massages and cold hands when I needed them), Luis (also for massages and for philosophic discussions), Xavi (por ser tu), Carme (per ser tu també), Francesc ("… for the music" and for being my biggest fan), Espe (por las horas de "Huesitos" and for being my second biggest fan), Lina (for our experiment jokes and weekends in the lab), Paola (for wise advices and for the smell of good coffee in the afternoons), Ariel (for tech-support and for reminding me I´m not young anymore), Andreia (for helping me adapting to the lab reality), Dani (for being so joyful and helpful), Julie (for your cheering laughter and coffee breaks).

I want to acknowledge other people at CNIO that made my PhD life better and easier:

"La Caixas" from the first (and the best) La Caixa Fellowship call (Aga, Eva, Kerstin, Marta, Miljana, Sara, Silvia, Jarek and Maciek) for all the trips, parties, lunches, dinners and whatever else we did together to have some relax from work.

All the members of our polish enclave at CNIO (Aga M., Jarek, Maciek, Aga Ch., Marta, Magda W., Magda Z.): Dzięki za sprawienie że czułem się w Madrycie jak w domu.

Diego, Manu and Chimo for a lot of help with fascinating world of confocal microscopy and Marta Cañamero for showing me that the pancreas can be fun.

To all other people from CNIO (that are too many to mention) who helped me somehow at work or simply shared with me the difficulties of everyday in science.

Chciałbym ogromnie podziękować moim rodzicom i mojemu bratu za ich wsparcie, mimo tego że nie było mnie z nimi tak długo: Dziekuję, że we mnie wierzyliście i trzymaliście za mnie kciuki.

Na koniec, brak mi słów żeby opisać jak bardzo dziękuję mojej żonie, Kasi, za okazaną cierpliwość, wsparcie w trudnych chwilach i wyrozumiałość: Bez Ciebie byłoby mi o wiele ciężej.

Table of contents

TABLE OF CONTENTS

Summary

Summary

In this dissertation I describe the role of Mnk1 kinase in exocrine pancreas physiology, pancreatitis and pancreatic cancer. I show that this kinase is expressed at exceptionally high levels in the developing and adult mouse pancreas, where it is a specific marker of acinar cell lineage. Mnk1 is embedded in acinar cell transcription program as a direct PTF1 target. Owing to this, its expression is down-regulated similarly to that of other acinar markers upon caerulein-induced acute pancreatitis – a condition that also leads to phosphorylation of Mnk1 and of its substrate eIF4E. To address the role of Mnk1 in pancreatic acinar cell response to acute pancreatitis we used *Mnk1*$^{-/-}$ mice. Although pancreata of these mice display no histological abnormalities, they show higher expression of acinar gene transcripts and elevated digestive enzyme protein content. On the other hand, they have reduced basal levels of p-eIF4E and decreased expression of c-Myc and Ccnd1 proteins, whose biosynthesis is known to be positively regulated by p-eIF4E. Importantly, *Mnk1*$^{-/-}$ mice do not show eIF4E phosphorylation upon caerulein-induced acute pancreatitis and have impaired induction of c-Myc protein expression. Consistently, upon induction of pancreatitis with caerulein, *Mnk1*$^{-/-}$ mice display elevated serum amylase levels, increased MPO^{+} inflammatory cell infiltration, stronger suppression of the acinar transcription program, and reduced acinar cell proliferation. These findings indicate that Mnk1 contributes to pancreatic regeneration during acute pancreatitis. The elevated severity of acute pancreatitis in *Mnk1*$^{-/-}$ mice can be explained by secretory defects that lead to impaired down-regulation of enzymatic protein content and accumulation of active carboxypeptidase A1 in acinar cells. In response to prolonged stress, such as multiple episodes of acute pancreatitis, *Mnk1*$^{-/-}$ mice show reduced levels of Ptf1a – a master regulator of acinar transcription program. Consistently, Mnk1 knock-down in 266-6 cells results in down-regulation of acinar gene expression, what is intensified upon H_2O_2 treatment. Moreover activity of this kinase is modulated by Kras^{G12V} signaling in acinar cells. During PDAC development, Mnk1 expression is down-regulated below detection upon acinar-to-ductal metaplasia but is detected at low levels in tumor cells. In the absence of Mnk1, *Ptf1a*$^{+/Cre}$*;Kras*$^{+/G12V}$ mice develop more PDAC precursor lesions but less metaplastic tubular complexes. Collectively, these data suggest that Mnk1 is important for acinar cell homeostasis in response to pancreatitis or oncogenic signaling, playing a suppressory role in early steps of PDAC development.

Resumen

En este trabajo se describe la función de la quinasa Mnk1 en la fisiología exocrina del páncreas, durante pancreatitis y en cáncer de páncreas. Los niveles de esta quinasa son excepcionalmente elevados tanto en desarrollo como en páncreas adulto, siendo un marcador específico de células acinares. Mnk1 pertenece al programa transcripcional de las células acinares, y está regulado directamente por PTF1. De esta manera, su expresión disminuye de manera similar a otros marcadores acinares en respuesta a pancreatitis aguda producida por tratamiento con ceruleína – situación que tiene como resultado la fosforilación de Mnk1 y de su diana eIF4E. Con el objetivo de estudiar el papel de Mnk1 en la respuesta de células acinares a pancreatitis aguda utilizamos ratones *Mnk1*$^{-/-}$. Aunque el páncreas de estos ratones no presenta alteraciones histológicas, se observan mayores niveles de transcripción de genes acinares y mayor contenido de enzimas digestivas a nivel proteico. Por el contrario, se detectan niveles basales inferiores de fosforilación en eIF4E y menor expresión de c-Myc y Ccnd1, cuya biosíntesis está regulada positivamente por p-eIF4E. Es importante destacar que tras pancreatitis aguda con ceruleína, los ratones *Mnk1*$^{-/-}$ no presentan fosforilación de eIF4E y la inducción de c-Myc está impedida. Consistente con este resultado, tras inducción de pancreatitis con ceruleína, los ratones *Mnk1*$^{-/-}$ presentan mayores niveles de amilasa en suero, aumento de infiltrados inflamatorios MPO+, mayor supresión del programa transcripcional acinar, y menor proliferación celular acinar. Estos resultados indican que Mnk1 contribuye a la regeneración del páncreas durante pancreatitis aguda. La mayor severidad de la pancreatitis aguda en ratones *Mnk1*$^{-/-}$ podría explicarse por los defectos en secreción que conllevan alteraciones en la reducción de los niveles enzimáticos y la acumulación de la forma activa de carboxipeptidasa A1 en células acinares. En respuesta a estrés prolongado, como son múltiples episodios de pancreatitis aguda, los ratones *Mnk1*$^{-/-}$ presentan menores niveles de Ptf1a – elemento fundamental en la regulación del programa transcripcional acinar. De manera similar, el silenciamiento de Mnk1 en las células 266-6 da como resultado una disminución en la expresión de genes acinares, que se ve agravado por tratamiento con H_2O_2. Asimismo, la ruta de señalización de Kras^{G12V} regula la actividad de esta quinasa en células acinares. Durante el desarrollo de PDAC, la expresión de Mnk1 disminuye de manera que los niveles son indetectables en la metaplasia acino-ductal, aunque sí se observa expresión en células tumorales. En ausencia de Mnk1, ratones *Ptf1a*$^{+/Cre}$*;Kras*$^{+/G12V}$ desarrollan más

lesiones precursoras de PDAC aunque se observan menos complejos tubulares metaplásticos. En conjunto, estos datos sugieren que Mnk1 es importante para la homeostasis de las células acinares en respuesta a pancreatitis o señalización oncogénica, teniendo un papel supresor en las etapas iniciales de desarrollo de PDAC.

Abbreviations

ABBREVIATIONS

Amy2	Amylase 2
ADM	Acinar-to-ductal metaplasia
AP	Acute pancreatitis
ARE	Adenylate-uridylate-rich elements
BrdU	Bromodeoxyuridine
BSA	Bovine Serum Albumine
CAC	Centroacinar cell
CCK	Cholecystokinin
Ccnd1	Cyclin D1
Cel	Carboxylester lipase
ChIP	Chromatin innumoprecipiation
clCpa1	Carboxypeptidase A1
c-Myc	Cellular myelocytomatosis oncogene
CP	Chronic pancreatitis
Cpa1	Procarboxypeptidase A1
$cPLA_2$	Cytoplasmic phospholipase A2
Ctrb1	Chymotrypsinogen 1
Ecad	E-cadherin
EGFR	Epidermal growth factor receptor
eIF4E	Eucariotic translation initiation factor E
eIF4G	Eucariotic translation initiation factor G
Ela1	Elastase 1
ER	Endoplasmic reticulum
Erk1/2	Extracellular signal-regulated kinase 1/2
Hnf1α	Hepatocyte nuclear factor 1 homeobox α
IFN	Interferon
JNK	Jun N-terminal kinase
Ki67	Antigen identified by monoclonal antibody Ki-67
KRAS	Kirsten rat sarcoma viral oncogene homolog
Krt19	Keratin 19
Krt7	Keratin 7
MAPK	Mitogen activated protein kinases
MDLs	Metaplastic ductal lesions
Mist1	Muscle, intestine and stomach expression 1
Mknk1	MAP kinase-interacting kinase 1 gene
MMLs	Mucinous metaplastic lesions
Mnk1	MAP kinase-interacting kinase 1
Mnk2	MAP kinase-interacting kinase 2
Mnks	MAP kinase-interacting kinases
MPCs	Multipotent progenitor cells
MPO	Myeloperoxidase
mTOR	Mammalian target or rapamycin
Muc1	Mucin 1

NES	Nuclear export signal
NLS	Nuclear localization signal
Nr5a2	Nuclear receptor subfamily 5, group A, member 2
PanIN	Pancreatic intraepithelial neoplasms
PDAC	Pancreatic ductal adenocarcinoma
Pi3K	Phosphoinositide 3-kinase
PMN	Polymorphonuclear leukocytes
PDL	Pancreatic duct ligation
PTF1	Pancreas specific transcription factor complex 1
Rbpj	Recombination signal binding protein for immunoglobulin kappa J
Rbpjl	Recombination signal binding protein for immunoglobulin kappa J region-like
RT-qPCR	Reverse transcriptase - quantitative PCR
Spry2	Sprouty 2
TCs	Tubular complexes
TGFβ	Transforming growth factor β
TMAs	Tissue microarrays
TNFα	Tumor necrosis factor α
Try	Trypsinogen
UTR	untranslated region of mRNA

Introduction

1. PANCREAS BIOLOGY

1.1. Pancreas anatomy and physiology

1.1.1. General considerations

The pancreas is a retroperitoneal secretory organ. Its name is derived from Greek words: “pan” – meaning “all” and “creas” – meaning “flesh”, reflecting its unusual composition – it does not contain any cartilage, bones nor hence (Buchler et al., 2002). It is localized in the abdomen between the spleen, stomach and small intestine (Fig. I1A) and has an elongated structure in which 4 parts are distinguished (from proximal to distal): head, neck, body and tail. This organization is well defined in humans and less apparent in smaller animals like rodents (Slack et al., 1995). In the pancreas two major compartments can be distinguished. The endocrine compartment is responsible for production and secretion of metabolism regulating hormones, whereas the exocrine compartment produces digestive enzymes and secretes them into duodenum.

1.1.2. The endocrine pancreas

The endocrine component of the pancreas is organized into compact structures called islets of Langerhans (Fig. I1B) which are constituted by 5 types of hormone producing cells: α-cells (producing glucagon), β-cells (insulin), δ-cells (somatostatin), ε-cells (ghrelin) and PP-cells (pancreatic polypeptide) (Fig. I1C). Rodent islets of Langerhans are organized such that the core of each islet is formed by β cells (the most abundant), surrounded by the other cell types. This structure is similar but not so evident, in humans (Gittes, 2009). Islets of Langerhans are embedded in the parenchyma of exocrine tissue and constitute around 5% of total pancreas mass (Benitez, Goodyear and Kim, 2012).

1.1.3. The exocrine pancreas

The exocrine pancreas consists of 3 distinguishable epithelial cell types: acinar, constituting 85% of the whole organ, centroacinar (CAC) and ductal cells (Pandol, 2010) (Fig. I1B and D). An exocrine functional unit is composed by acinar cells forming a structure resembling a cluster of berries – acinus (term derived from Latin), and a ductule (Fig. I1D). Acinar cells produce a wide variety of digestive enzymes and secrete them into the lumen of the acinus. This drains into a ductule which extends into

interlobular ducts and further into the main pancreatic ductal system. These structures are lined with ciliated polarized cells of cuboidal shape that also secrete mucins and ions into the lumen which mix with the acinar enzymes and form pancreatic juice. The best known markers of ductal cells in the pancreas are cytokeratins Krt7 and Krt19. CACs reside at the distal end of acini (Fig. I1D) and have some ductal characteristics, like the expression of carbonic anhydrase that produces bicarbonate (Steward, Ishiguro and Case, 2005), but they also seem to have progenitor features and may give rise to other cell types in the adult pancreas (Rovira et al., 2010).

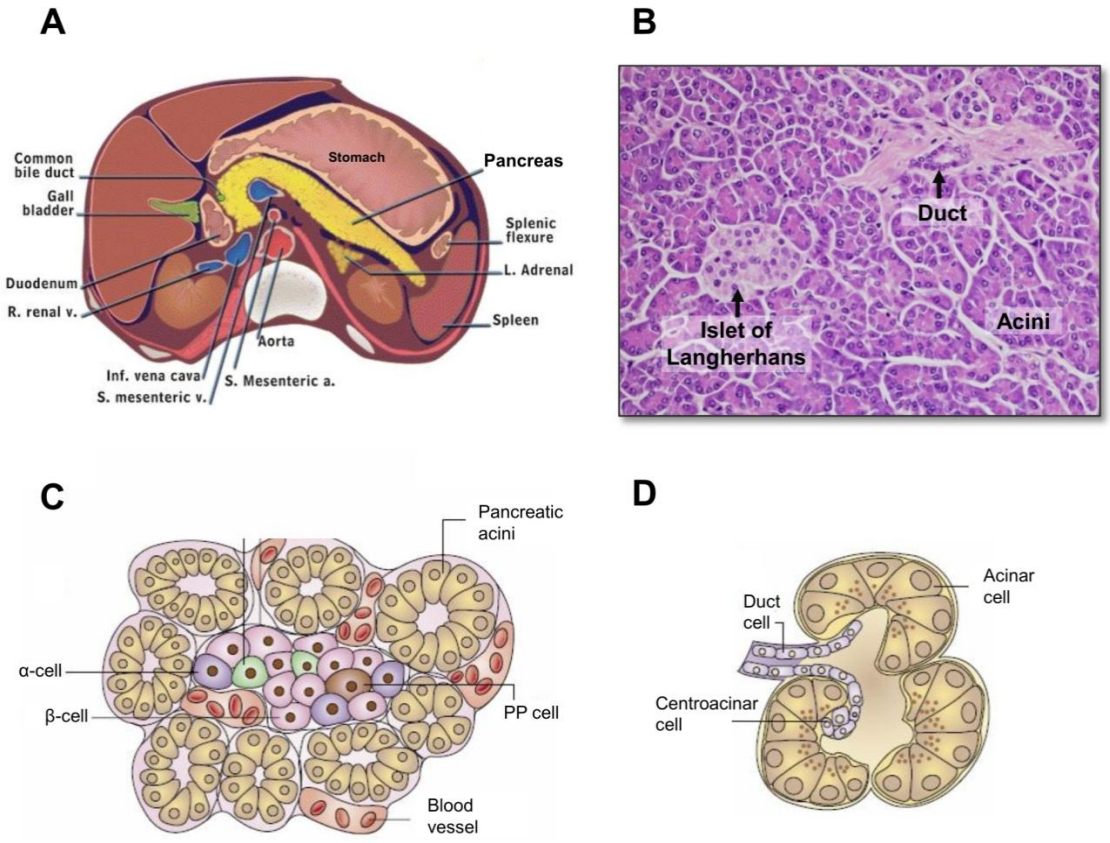


Figure I1. Anatomy and hisology of the pancreas. (A) Cross-sectional anatomy of the pancreas. *Adapted from Pandol, 2010*. (B) Hematoxylin-eosin staining of pancreatic section showing endocrine (Islet of Langerhans) and exocrine (Duct and Acini) compartments. (C) Schematic representation of islet of Langerhans embedded in exocrine parenchyma. (D) Schematic representation of an acinus connected to a ductule. *Adapted from Bardeesy and Depinho, 2002*

1.2. Pancreas development in mouse

1.2.1. Mouse pancreas embryology

Pancreas development has been very thoroughly studied in mice (Fig. I2A). Development of the mouse organ can be divided in 3 phases: primary transition – from

embryonic day 9.5 (E9.5) to E12.5; secondary transition – from E12.5 to birth - and postnatal period. At E9.5, pancreas development starts from thickening of endoderm at duodenal region and evagination of a dorsal pancreatic bud. Soon after that (E9.75) the ventral bud forms (Benitez, Goodyear and Kim, 2012). Both parts grow towards each other and, while the progenitors proliferate, continuous tubular structures are formed (Kesavan et al., 2009; Villasenor et al., 2010), partially mediated by signaling through Cdc42 Rho-GTPase (Kesavan et al., 2009) and by activity of Pdx1 transcription factor (Wescott et al., 2009). Simultaneously, as the pancreas grows in a tree-resembling shape, the compartmentalization of pancreatic progenitors into "trunk" and "tip" domains occurs (Fig. I2A). Pancreatic multipotent progenitor cells (MPCs) reside at the periphery of the growing organ (in the "tips") and proliferate outwards, giving rise to unipotent (acinar) precursors and bipotent (endocrine and ductal) that stay in the trunk domain. This continues until secondary transition when (at around E13) "tip" progenitors lose their multipotency and become strictly acinar precursors (Zhou et al., 2007) (Fig. I2A and B). The progenitor specification is regulated by extrinsic signals from surrounding mesenchyme and penetrating blood vessels that crosstalk with intrinsic regulation of differentiation driven by pancreas specific transcription program (Puri et al., 2010; Magenheim et al., 2011).

1.2.2. Pancreatic multipotent progenitor cells (MPCs)

From pancreatic bud formation until the end of the primary transition, pancreatic multipotent progenitor cell identity is maintained by several crucial transcription factors, such as Pdx1, Ptf1a, Sox9, c-Myc, Hnf1β, Nkx6.1, Hes1 and others (Fig. I2B).

Pdx1 is first found in prepancreatic endoderm domain at E8.5, thus before the formation of pancreatic buds starts, and is believed to initiate pancreas development, as inactivation of Pdx1 results in complete inhibition of pancreas formation (Offield et al., 1996). After the secondary transition, Pdx1 is present at high levels only in endocrine precursors whereas it is less abundant in acinar and ductal precursors (Ohlsson et al., 1993; Guz et al., 1995). One of the functions of Pdx1 in pancreas-committed progenitors is to initiate the expression of Ptf1a.

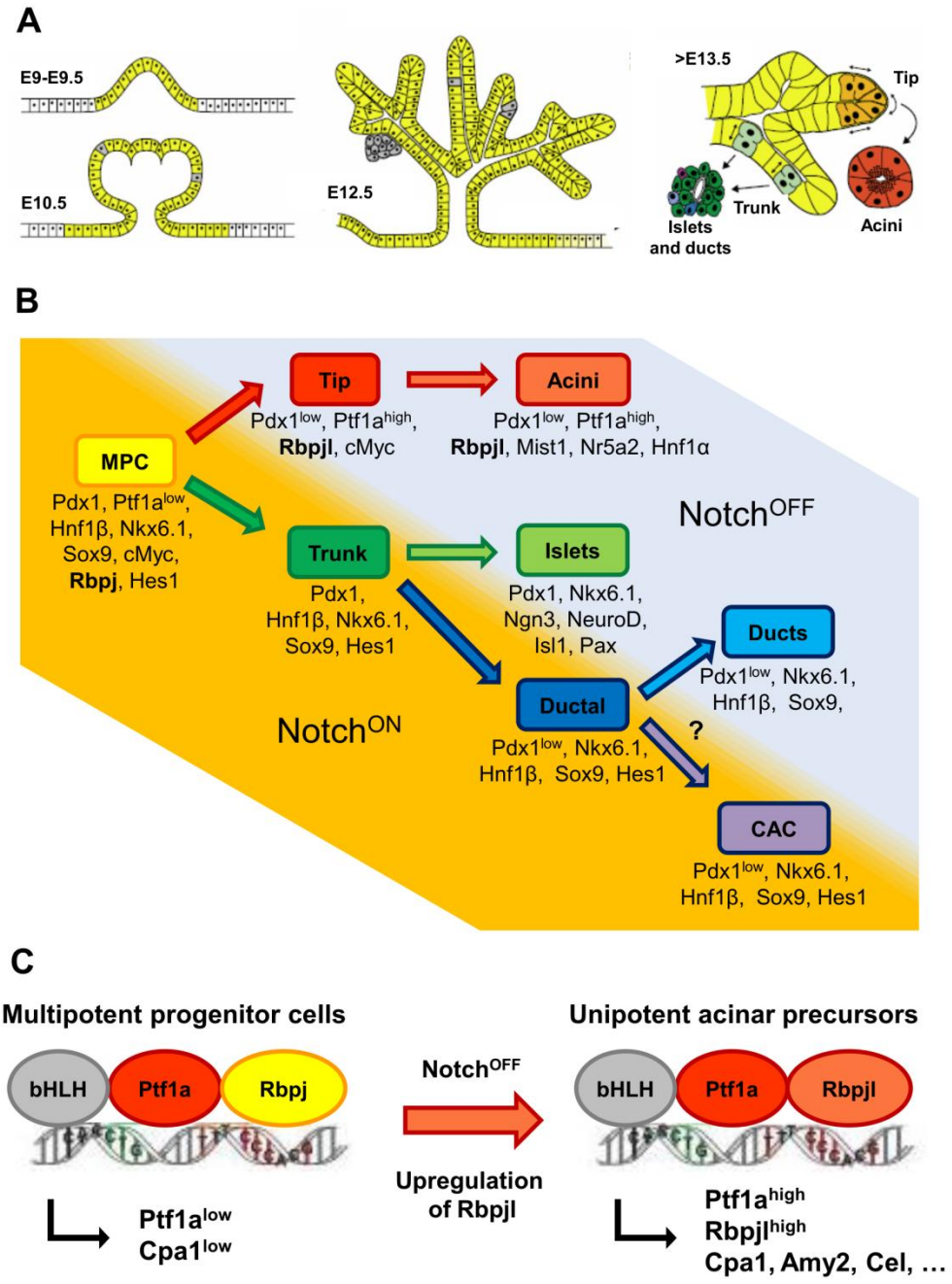


Figure I2. Pancreas development and cell type specification. (A) Sequential stages of pancreas development in mice. *Adapted from Kim and MacDonald, 2002* (B) Shematic representation of mouse pancreatic cell type specification with emphasis on involvement of transcription factors and Notch signaling. (C) Rbpjl replaces Rbpj in the PTF1 complex during acinar cell maturation. *Adapted from Masui et al., 2007.*

Ptf1a is a pancreas- and neural-specific class B bHLH transcription factor. In the pancreas it is expressed in MPCs from the beginning of the organ formation, however at later stages of development it is only present in acinar precursors and in mature acinar

cells (Krapp et al., 1996; Krapp et al., 1998). In its absence, the development of dorsal pancreatic bud is strongly impaired and only a rudimentary duct is formed whereas the formation of the ventral part is completely inhibited, because initially committed Pdx1 expressing progenitors revert to the intestinal fate (Kawaguchi et al., 2002). Ptf1a is a member of the PTF1 heterotrimeric complex that in MPCs also contains one of the common class A bHLH proteins and Rbpj – a mammalian homologue of *Drosophila melanogaster* Suppressor of Hairless, a mediator of canonical Notch siganlling (Beres et al., 2006). During the primary transition, Rbpj-containing PTF1 complex (called PTF1-J) is required for growth and morphogenesis of pancreatic epithelium and Rbpj inactivation leads to pancreas agenesis, similar to Ptf1a inactivation (Masui et al., 2007). In addition, PTF1-J directly activates expression of some genes that, at later stages of pancreas development, become acinar-specific like Ptf1a itself (Masui et al., 2007) and some exocrine digestive enzymes. A good example is Carboxypeptidase A1 (Cpa1) that is regarded as a marker of MPCs (Zhou et al., 2007).

Sox9 and **c-Myc** are not pancreas specific but are important for the maintenance of the progenitor pool during pancreas develepment. Inactivation of Sox9 results in a severe pancreatic hypoplasia (Seymour et al., 2007); however, c-Myc deletion has similar effect only on acinar and ductal lineages, not affecting to the same extent the endocrine cell growth (Nakhai et al., 2008).

Much evidence has been gathered regarding the role of Notch signaling in multipotent progenitors. Its main downstream effector, the transcritptional repressor **Hes1**, is co-expressed with Pdx1 in MPCs in the initial pancreatic buds and at the periphery of the pancreatic epithelium until E13 (Seymour et al., 2007) and it maintains the undifferentiated state of these cells by suppressing the expression of lineage-specific transcription factors (Jensen et al., 2000; Esni et al., 2004) (Fig. I2B). This ensures the expansion of multipotent progenitors before they specify for particular cell lineages.

1.2.3. Pancreatic cell type specification

In progenitors that commit to **acinar** fate (thus also in “tip” precursors during the secondary transition) PTF1-J binds and activates Rbpjl, an Rbpj paralogue (Masui et al., 2010 (1)). As this protein accumulates, it replaces Rbpj in the complex now called PTF1-L (Fig. I2C). PTF1-L binds and activates the promoters of acinar specific genes,

including those that were regulated by the "J" complex, and supports acinar cell differentiation (Masui et al., 2010). In the acinar precursors, Ptf1a expression also increases due to an autoregulatory loop. These cells maintain lower levels of Pdx1 that is necessary for their differentiation (Hale et al., 2005) and c-Myc that is required for their proliferation and survival (Nakhai et al., 2008; Bonal et al., 2009). Inactivation of c-Myc during the secondary transition leads to impairment of exocrine pancreas development due to acinar cell atrophy and transdifferentiation into adipocytes. On the other hand acinar precursors lose the expression of Sox9 and Hnf1β transcription factors that are responsible for specification of other lineages.

The bipotent endocrine/ductal precursors occupy the "trunk" domain of the pancreatic epithelium and express high levels of **Nkx6.1,** a transcription factor that antagonizes the proacinar function of Ptf1a by restraining its autoregulation driven overexpression (Schaffer et al., 2010). Owing to that, these cells maintain Sox9 and Hnf1 β expression and may commit either to endocrine or ductal lineages (Solar et al., 2010; Kopinke and Murtaugh, 2010). Nkx6.1 expression is sustained in both cell types and is detected in adult ducts and endocrine cells. Unipotent endocrine precursors arise scattered in the tubular epithelium and their differentiation is driven by transient expression of Ngn3 transcription factor that activates a group of other transcriptional regulators (such as NeuroD, Isl1, Pax and others) governing further commitment to different types of endocrine cells and the formation of islets of Langerhans (Gu et al., 2002; Benitez, Goodyear and Kim, 2012). By contrast, no master regulator of ductal cell differentiation has so far been discovered and it is possible that the ductal phenotype results from a default setting of $Sox9^{+}$, $Hnf1\beta^{+}$, $Nkx6.1^{+}$ cells that do not commit to acinar or endocrine fate by Ptf1a or Ngn3, respectively (MacDonald, Swift and Real, 2010). There is no conclusive evidence on how CACs arise, however as they have some ductal cell characteristics and express Hes1 it is possible that they originate from ductal precursors that maintain active Notch signaling. Another possibility is that they arise from acinar precursors that reactivated Notch (Rovira et al., 2010).

1.3. Pancreatic acinar cell biology

1.3.1. Acinar cell function in food digestion

Pancreatic acinar cells produce and secrete most of the digestive enzymes that are responsible for food digestion in the small intestine. To meet a high demand for these enzymes to match mammalian metabolism, acinar cells have the highest rate of protein synthesis of all mammalian cell types (Case, 1978). It is achieved owing to a very high rate of transcription of genes coding for digestive enzymes, a very abundant rough endoplasmic reticulum (ER) and a well designed system for storage and secretion (Fig. I3A).

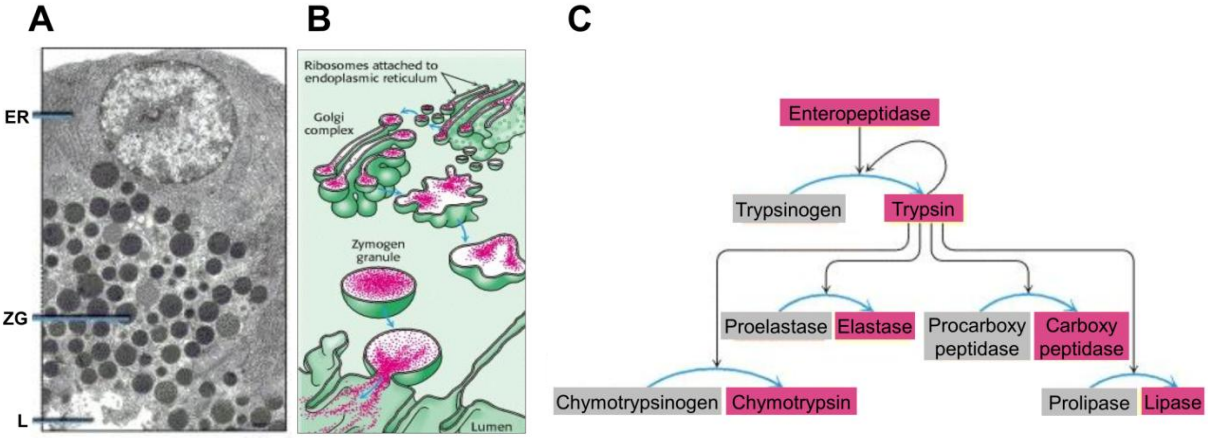


Figure I3. Acinar cell morphology and zymogen production and activation. (A) Ultrastructure of pancreatic acinar cell showing prominent endoplasmic reticulum – ER, abundant zymogen granules – ZG and apical surface of the acinar cell that forms lumen – L. *Adapted from Pandol, 2010.* (B) Depiction of digestive enzyme transport between different subcellular compartments inside the acinar cell. (C) Schematic representation of zymogen activation cascade that occurs after digestive enzyme release into the duodenum. *Adapted from Berg, Tymoczko and Stryer, 2002.*

Digestive enzyme transcripts constitute around 80% of all acinar cell mRNAs (MacDonald, Swift and Real, 2010). Due to hydrophobic signal sequences on N-terminus the enzymes, when translated, are transported into the lumen of the ER where they are post-translationaly modified. Further modifications occur in the Golgi complex; subsequently, acinar enzymes also undergo concentration and packaging into highly specialized storage structures called zymogen granules (Farquhar and Palade, 1998) (Fig. I3A and B). Apart from being involved in protein synthesis, the ER is also a repository of Ca^{2+} ions whose flux into the cytoplasm mediates zymogen granule secretion (Petersen and Tepikin, 2008). Upon neurohumoral stimulation of receptors on

the surface of acinar cells, cytosolic Ca^{2+} concentration increases, leading to secretion of zymogen granules into the lumen and the pancreatic ductal system. This occurs through exocytosis whereby the actin-myosin system moves the granules to the apical surface where they fuse with plasma membrane.

1.3.2. Zymogen activation

Acinar cells contain proteolytic, amylolytic, lipolytic and nucleolytic digestive enzymes (Whitcomb and Lowe 2007). Some of them, as α-amylase or lipase, are present in cells in their active forms. However, the majority of the enzymes are proteases that are kept inactive as proenzymes, what is facilitated by the acidic pH of the zymogen granules. They are activated in the duodenum through a cascade of enzymatic reactions (Fig. I3C). First, enteropeptidase – a peptidase present in the duodenal lumen activates trypsinogen by hydrolysis yielding trypsin that catalyses the activation of other proenzymes (including remaining trypsinogen) such as procarboxypeptidase (to carboxypeptidase), chymotrypsynogen (to trypsinogen), proelastase (to elastase) and others (Whitcomb and Lowe 2007).

1.3.3. Plasticity of the acinar cell

Acinar cells display a significant plasticity (Puri et al., 2010) and depending on conditions they can acquire features of ductal cells, endocrine cells, adipocytes or hepatocytes (Fig. I4A). Human and rodent isolated acini cannot be kept in culture due to stress-induced cell death and transdifferentiation into cells with ductal features (Vila et al., 1994; Means et al., 2005; Houbracken et al., 2011). This switch from acinar to ductal differentiation program is also frequently observed in vivo (in human samples and animal models) in metaplastic ductal lesions (MDLs) that are found in pancreatitis and in pancreatic cancer specimens. These lesions can be flat duct-like tubular complexes (TCs) or mucinous metaplastic lesions (MMLs) (Fig. I4B). In humans, the origin of MDLs has not been firmly established. Using lineage tracing in mice, upon caerulein-induced acute and chronic pancreatitis, direct proof was provided that some of these lesions result from acinar-to-ductal transdifferentiation (Strobel et al., 2007). Moreover, in mouse models of pancreatic cancer over-activation of Kras signaling specifically in acinar cells leads to formation of TCs, MMLs as well as pretumoral lesions with ductal characteristics (Wagner et al., 1998; Guerra et al., 2007; Grippo and Sandgren 2012).

Metaplastic cells lose the expression of Ptf1a, Rbpjl and, as a result, of all the digestive enzymes.

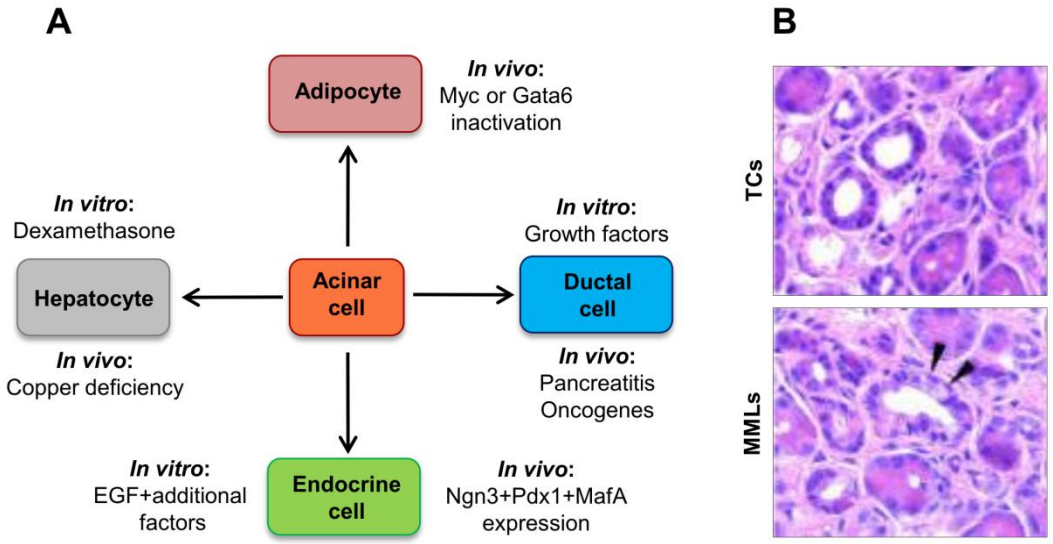


Figure I4. Acinar cell plasticity. (A) Schematic representation of pancreatic acinar cell plasticity manifested by their ability to transdifferentiate *in vitro* or *in vivo* into ductal cells, endocrine cells, hepatocytes or adipocytes. (B) Hematoxylin-eosin staining of mouse pancreatic sections showing 2 types of metaplastic lesions that can arise as a result of acinar-to-ductal transdifferentiation: flat duct-like tubular complexes – TCs and mucinous metaplastic lesions – MMLs (arrowheads indicate mucin). *Adapted from Strobel et al., 2007*.

In some conditions, acinar cells can also transdifferentiate into other cell types: hepatocytes (in mice fed with copper deficient diet (Rao et al., 1988) and in vitro upon treatment with dexamethasone) (Lardon et al., 2004; Al-Adsani., 2010); endocrine as shown also in vitro (Baeyens et al., 2009) and in vivo (Zhou et al., 2005), or adipocytes upon c-Myc or Gata6 inactivation during pancreas development (Bonal et al., 2009; Martinelli et al., 2012) (Fig. I4A).

There are situations in which, despite the stimuli that favor acinar-to-ductal transdifferentiation, the cells do not lose completely their original identity and partially retain the acinar transcription program. This is observed in human pancreatitis patients and in mouse models of acute and chronic pancreatitis where acinar cells have reduced expression of acinar-specific transcription factors and digestive enzymes and can recover the full acinar phenotype after the damage is over (Pinho et al., 2011; Molero et al., 2012). Partial downregulation of acinar gene expression without ductal transdifferentaition also may occur upon oncogenic signalling in acinar cell carcinoma

(ACC) (a rare human pancreatic tumor) and in acinar cells and tumors from Ela1-Myc mice (Sandgren et al., 1991).

To sum up, acinar cells are plastic and depending on the context they can irreversibly transdifferentiate into several different cell types and reversibly downregulate their phenotype. Since digestive enzymes produced by acinar cells can be very harmful for pancreatic tissue and for other organs of the organism, in response to stress their production has to be rapidly downregulated and in the case of persisted insult completely shut down. This is probably the main reason for their plasticity. This regulation can occur at different levels: gene transcription, mRNA stability, mRNA translation, protein turnover and secretion.

1.3.4. The acinar transcription program

During cell type specification in the developing pancreas, acinar cells establish a specific transcription program that drives the exceptionally high expression of digestive enzymes and proteins involved in zymogen storage and secretion. It is turned on gradually during organ development and in the adult animal it is regulated upon alterations in dietary nutrients and suppressed in response to cellular stress. Acinar-specific genes contain in their promoters so called pancreas consensus elements (PCE) that are recognized by acinar specific transcription factors. As mentioned above (1.2.3), acinar cell differentiation is primarily induced by the "late" PTF1 complex, the main driver of acinar phenotype (Petrucco et al., 1990; Keller et al., 1990). Its components **Ptf1a** and **Rbpjl** bind to an E-box (CACCTG) and TC-box (TTTCCC), respectively (Beres et al., 2006) in the consensus elements of genes coding for digestive enzymes, secretion machinery proteins and other transcription factors to potently induce their expression (Masui et al., 2010). They also bind to their own promoters, ensuring the robustness of the system (Masui et al., 2007; Masui et al., 2008). All acinar specific genes are transcriptional targets of Ptf1a, however Rbpjl can be replaced by Rbpj at some target sites (Masui et al., 2010). While Ptf1a is important for initiating the acinar phenotype, its cooperation with Rbpjl enhances mitochondrial metabolism and cytoplasmic energy stores, completes the apparatus for intracellular transport, packaging and regulated secretion and maximizes secretory protein synthesis (Masui et

al., 2010). A number of additional transcription factors important for full acinar cell maturation have been discovered, among others Mist1, Hnf1α and Nr5a2.

Mist1 is a bHLH transcription factor that controls the expression of genes involved in secretion in different exocrine cell types including zymogenic cells of the stomach, Ig-secreting plasma cells, alveolar breast lobular cells and acinar cells of salivary glands and the pancreas. In pancreatic acinar cells, these genes regulate zymogen granule formation and transport, Ca^{2+} distribution and cell polarity. In *Mist1*$^{-/-}$ mice, pancreatic acini are disorganized with loss of apical-basal polarity and the cells have reduced amount of secretory proteins, defective calcium signaling and show secretory defects (Luo et al., 2005).

Hnf1α is involved in regulation of expression of tissue specific genes in different epithelial cells for example in liver (Potonglio et al., 1996) where it regulates lipid and glucose metabolism. In acinar cells it positively regulates the acinar transcription programme and its absence in mice leads to reduced levels of Ptf1a protein and of its target transcripts. Moreover Hnf1a is important for pancreas organogenesis since *Hnf1a*$^{-/-}$ mice contain dispersed acinar cells with lower secretory capacity. However, Hnf1a does not regulate acinar genes directly and its effects are at least partially mediated by Nr5a2 that is its direct target (Molero et al., 2012).

Nr5a2 was first shown to bind directly and positively regulate carboxyl-ester lipase (*Cel*) gene (Fayard et al., 2003); this was recently confirmed in a broader study that identified *Cel* among other direct targets of Nr5a2 in the mouse pancreas. These targets are genes coding for acinar specific transcription factors (as *Rbpjl*), acinar digestive enzymes and proteins involved in zymogen secretion and in mitochondrial metabolism (Holmstrom et al. 2011). Nr5a2 binds to its specific DNA sequences in the promoter of these genes and possibly interacts with PTF1-L complex. Upon conditional inactivation of *Nr5a2* in adult mice, pancreatic acinar cells produce and secrete less digestive enzymes (Holmstrom et al. 2011).

The above described transcription factors have been shown to be important for maintenance of acinar cell phenotype during development and in the adult upon stress. Together with the central regulators Ptf1a and Rbpjl, as well as with others not

described here, they lead to a highly organized transcription program. This organization allows for tight control of acinar gene transcription that is also important during its suppression upon stress.

1.3.5. Post-transcriptional regulation of protein biosynthesis in acinar cells

As opposed to transcriptional regulation, less is known about additional mechanisms of control on further levels of acinar specific gene expression. Very few studies have addressed transcript stability regulation in pancreatic acinar cells. The most direct analysis was performed in Actinomycin D-treated rats in which, under a protein-free diet, the half-life of mRNAs of anionic trypsinogen izozymes I and II (but not of cationic trypsinogen) was increased (Carreira et al., 1996). More is known about translational regulation in these cells. CCK stimulation in physiological range (Bragado et al., 2000; Crozier et al., 2006) and high protein diet (Hashimoto and Hara, 2003; Hashi et al., 2005) lead to an overall increase in mRNA translation, whereas starvation (Sans et al., 2004) or secretagogue-induced acute pancreatitis (Sans et al., 2003) lead to a decrease in translation.

Regulation of enzymatic protein turnover in acinar cells has been addressed only in the case of Cel, that was shown to be ubiquitinated and degraded by proteasome in AR42J rat acinar cells upon stress induced by secretion inhibition (Le Petit-Thévenin et al., 2001). On the other hand, zymogen degradation through autophagy has been studied more thoroughly (Vaccaro, 2012). In the work of Grasso and colleages a novel selective form of autophagy in acinar cells was identified and named zymophagy, as it was described as a process to specifically detect and degrade zymogen granules containing prematurely activated enzymes before they can harm the organ (Grasso et al., 2011).

Digestive enzyme abundance in acinar cells is also affected by changes in zymogen secretion, which is stimulated by physiological levels of secretagogues like choleocystokinin (CCK) and acetylcholine (ACh) upon food intake (Williams, 2010a) and can be inhibited by acute supramaximal stimulation by CCK or its analogues (Saluja and Lerch, 2007). Considering that zymogen secretion is modulated by intracellular oscilation of Ca^{2+} and rapid changes of actin cytoskeleton, this might be the fastest mean of intracellular enzymatic protein regulation. Intake of food acts mainly via neural pathways regulating release of hormones; however, there is increasing evidence for a

direct regulatory role of some nutrients (Williams, 2010a). Molecular pathways involved in regulation of acinar enzyme secretion have been broadly investigated in vitro.

1.3.6. Studies of pancreatic acinar cell biology in cells cultured in vitro

As described above, acinar cells isolated from pancreas cannot be kept in culture due to cell death and loss of acinar phenotype. Thus it has not been possible to establish cell lines that would closely resemble normal pancreatic acinar cells. However, short term experiments can be performed on freshly isolated rodent acinar cells and this approach has been widely used to address the role of genes and pathways in acinar cell function. Additionally, there are a few cancer cell lines that retain some characteristics of pancreatic acinar cells, such as rat AR42J (Jessop et al., 1980) and mouse 266-6 (Ornitz et al., 1985) lines obtained from acinar tumors. They have been mainly used to study regulation of acinar cell differentiation, however the results of these studies cannot be easily extrapolated to normal acinar cells.

Studies in isolated rodent acinar cells unveiled much about their physiology regarding zymogen secretion and stress-induced intracellular enzyme activation. A very common approach is to isolate functionally intact rodent acini and treat them with secretagogues. As observed already 4 decades ago, increasing concentrations of CCK and its analogues such as caerulein and pentagastrin result in a biphasic response in amylase release into the medium (Williams et al,. 1978). At first, increasing ligand concentration induces an increase in amylase release until the optimal concentration (for most secretagogues around 100 pM). Treatment with higher doses (so called supramaximal concentration) produces a submaximal release not involving changes in ATP levels or Ca2+ oscillations (Williams et al,. 1978) but rather reflecting actin cytoskeleton reorganisation that result in secretion blockage (Schäfer et al., 1998). In parallel, it leads to intracellular trypsin activation with linear dynamics, showing higher activation at supramaximal doses (Saluja et al., 1999). As shown by Schafer and colleagues supramaximal concentrations of CCK and its analogues, as well as osmotic stress (induced with sorbitol), activate p38 MAP kinase pathway that partially regulates stress-induced acinar cell actin cytoskeleton reorganization (Schäfer et al., 1998).

2. DISEASES OF EXOCRINE PANCREAS

The main diseases of exocrine pancreas are pancreatitis and pancreatic cancer. There is vast evidence that acinar cells play a central role in the pathogenesis of both disorders: in pancreatitis through the damage caused by intracellular activation of acinar digestive enzymes and in pancreatic cancer via acinar cell plasticity underlying ductal tumor formation.

2.1. Acute and chronic pancreatitis

Pancreatitis is a necroinflammatory disease that can manifest as an acute or chronic condition with a wide range of severity (Fig. I5). Histologically, **acute pancreatitis (AP)** is characterized by acinar cell necrosis and parenchymal infiltration by inflammatory cells. Although the disease is usually mild, in 10-15% cases it can be severe leading to multiorgan dysfunction and risk of death. **Chronic pancreatitis (CP)** is associated with broad acinar atrophy and fibrosis that can affect both exocrine and endocrine function. Similarly to acute pancreatitis, it is associated with alcohol abuse and can be caused by genetic factors. Although in the past they used to be described as two distinct conditions, it is now clear that repeated episodes of AP lead to chronic disease. This can occur due to genetic predisposition and/or persistence of pancreatitis-inducing environmental factors (Vonlaufen et al., 2008; Whitcomb, 2012).

Most typical for CP is a high extent of fibrosis (Fig. I5A), the formation of which is believed to be mediated by pancreatic stellate cells (PSC) that are scattered in the pancreatic parenchyma (Apte et al., 2003; Phillips et al., 2003). In normal state, they are quiescent while in response to chronic injury and inflammation they become activated, acquire myofibroblast features, proliferate and secrete the extracellular matrix components. Moreover these cells produce matrix metalloproteinase-2 (MMP2) that degrades basement membrane and facilitates deposition of fibrillar collagen (Phillips et al., 2003).

2.1.1. Factors involved in pancreatitis

Different environmental, genetic and other factors have been shown to be involved in pancreatitis development and most of them have been proven to directly or indirectly lead to zymogen activation and inflammation, regardless of the etiology (Vonlaufen et

al., 2008). The intracellular enzyme activation is mainly catalyzed by cathepsin B from lysosomes that fuse with zymogen granules (Lerch et al., 1993). The most important non-genetic factors are gallstone migration, alcohol, and tobacco. The former leads to pancreatic duct obstruction that blocks digestive enzyme flux through the ductal system what is believed to impede acinar cell exocytosis and as a consequence to cause accumulation of zymogen granules and their fusion with lysosomes (Vonlaufen et al., 2008). Alcohol also negatively affects acinar cell exocytosis, but through effects on cytoskeleton (Ponnappa et al., 1987; Siegmund et al., 2004) and destabilization of the membrane of zymogen granules, facilitating their interaction with lysosomal content (Wilson et al., 1990). Both gallstone migration and alcohol may cause AP that can progress to chronic disease.

Although most cases of chronic pancreatitis are "sporadic", a rare syndrome of familial chronic pancreatitis has been described, that is inherited as an autosomal dominant trait, characterized by recurrent bouts of acute pancreatitis initiated during childhood. It is caused by mutations in genes coding for proteins involved in zymogen activation, namely trypsinogen (cationic – *PRSS1* and anionic – *PRSS2*) leading to enzyme autoactivation (Whitcomb et al., 1996; Sahin-Toth et al., 2000; Teich et al., 2004) and in its intracellular inhibitor (serine peptidase inhibitor – *SPINK1*) that lead to lack of inhibitory activity towards trypsin, hence inability to block the activation cascade upon insult (Kume et al., 2006, Király et al., 2007). Moreover, pancreatitis has been associated with inactivating mutations in chymotrypsinogen C (*CTRBC*) (Rodendahl et al., 2008) – an enzyme that is known to degrade trypsin and is considered to be protective against pancreatic injury (Nemoda et al., 2006; Szmola et al., 2007). In addition chronic pancreatitis may evolve in patients suffering from cystic fibrosis (CF) which is a complex hereditary disease caused by mutation in *CFTR* gene. CFTR is a receptor that in the pancreas is found in ductal cells and regulates secretion of bicarbonate into the lumen (Sharer et al., 1998; Cohn et al., 2005).

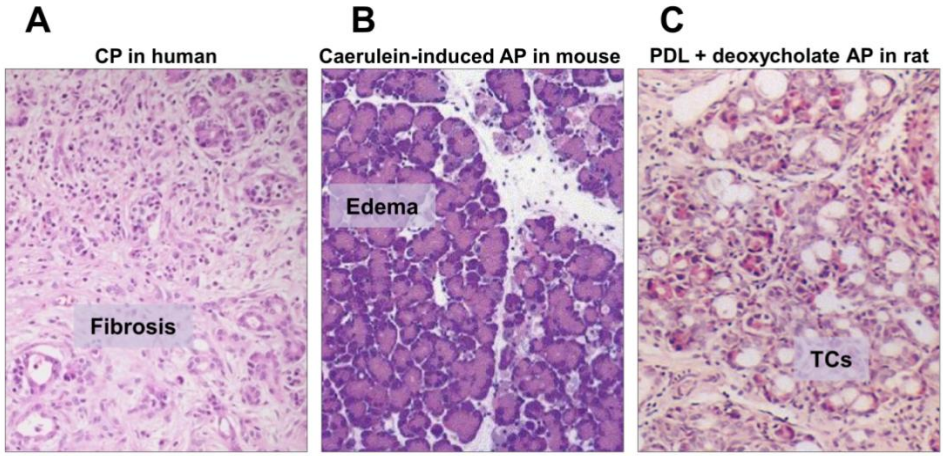


Figure I5. Histological changes in the exocrine pancreas upon pancreatitis. (A-C) Hematoxylin-eosin staining of pancreatic sections showing: (A) CP in human – with high extent of fibrosis; (B) caerulein-induced AP in mouse – with edema; (C) PDL and deoxycholate induced AP in rat – with abundant TCs.

2.1.2. Animal models of pancreatitis

A significant improvement in our understanding of the molecular basis of acute pancreatitis has been provided by animal studies. Over the past 3 decades, acute or chronic pancreatitis have been induced in rodents by various factors including secretagogues, L-arginine, deoxycholate, alcohol, mechanical obstruction, and genetic modifications.

Caerulein. As described above (1.3.6), choleocystokinin and its analogues induce premature trypsin activation and cellular damage in acini isolated in vitro. They are also potent inducers of acute pancreatitis in vivo in rodents (Adler, Kern and Scheele, 1986) (Fig. I5B). The classical protocol of caerulein administration in mouse (7 hourly doses of 50μg/kg) leads to edema, leakage of pancreatic enzymes, inflammation and acinar cell necrosis as well as pulmonary inflammation. In this model, morphological changes in acinar cells resemble those observed in acute pancreatitis in humans (Willemer and Adler, 1989; Willemer et al., 1989), however the outcome is a mild, self-resolving, acute pancreatitis with low mortality (Su, Cuthbertson and Christophi, 2006). The simple and reproducible nature of caerulein-induced pancreatitis has made it the most "popular" experimental model used (see below). Additional variants of caerulein administration have been used (i.e. two inductions over two days) with distinct outcomes.

L-Arginine. L-arginine administration leads to a severe necrotizing pancreatitis with a 2.5% of mortality in rats (Mizunuma et al., 1984; Hegyi et al., 2004). In contrast to caerulein, the mechanisms of L-arginine action on pancreas are poorly known. It has been proposed that L-arginine may induce free radicals (Czako et al., 1998) or a disarrangement of the rough endoplasmatic reticulum (Kishino and Kawamura, 1984) and produce ER stress (Kubisch et al., 2006) but no conclusive data have been gathered so far.

Deoxycholate. When administered to rats through the pancreatic duct, deoxycholate induces a severe pancreatitis with inflammation and fibrosis and around 25% mortality rate (Kotani et al. 1999, Tu et al. 2012), but since the mechanism of damage induction is unknown this drug has not been commonly used to study acute pancreatitis.

Obstructive pancreatitis. Closed duodenal loop (CDL) and pancreatic duct ligation (PDL) lead to an acute pancreatitis associated with systemic effects similar to those observed in the clinic resulting from obstruction of ductal secretory flow. The combination of mechanical and chemical methods can lead to a very severe acute pancreatitis (Kotani et al. 1999) (Fig. I5C).

Alcohol. The effect of alcohol on pancreatitis identified in humans has been poorly modelled in rodents as very high doses are necesary for induction of damage (Pandol et al., 1999; Werner et al., 2002). On the other hand alcohol has been shown to greatly enhance the effect of secretagogues (Quon et al., 1992; Foitzik et al., 1994; Luten et al., 1994).

Prolonged or repetitive episodes of acute pancreatitis as described above can result in development of chronic lesions (Aghdassi et al., 2001). Repetitive caerulein-induced acute pancreatitis for several weeks or low dose daily caerulein administration leads to chronic inflammation and replacement of acinar parenchyma with tubular complexes (TCs) (Weaver et al., 1994); daily administration of L-arginine produces fibrotic tissue which replaces acinar cells that undergo necrosis (Lu et al., 2002). However, these protocols do not fully reproduce the fibrogenic chronic pancreatitis observed in humans.

Genetic modifiers of pancreatitis. Trypsin over-activation is a very early event in pancreatitis in animal models (Rao et al., 1988). Two studies addressed directly the

ability of trypsin to induce pancreatitis (Archer et al., 2006; Gaiser et al., 2011). Archer and colleagues generated a mouse expressing self-activating trypsin (*PRSS1*^{R122H}) in pancreatic acinar cells under the control of the elastase promoter. The mice displayed early-onset acinar cell injury and pancreas inflammation (with activation of Jnk and Erk MAP kinase pathways) that progressed with age to develop chronic lesions with acinar cell dedifferentiation and extensive fibrosis. Since in this model trypsinogen is activated during embryonic development, it cannot serve to conclude about the role of trypsin upon acute stress. To address the role of acute trypsinogen activation, Gaiser and colleagues used an inducible system allowing to turn on the endogenous expression of *PRSS1*^{R122H} in the acinar cells of adult mouse. Activating trypsin resulted in severe acute pancreatitis with caspase-3 mediated apoptosis that did not lead to chronic inflammation or fibrosis. The authors reasoned that trypsin-induced apoptosis facilitated resolution of acute inflammation rather than causing chronic damage (Gaiser et al., 2011).

2.1.3. Mechanisms of acute caerulein-induced pancreatitis in rodents

Secretagogue hyperstimulation (mainly by caerulein) is the most commonly used approach to investigate pancreatitis. In rats and bigger rodents it is generally performed as an intravenous infusion whereas in mice, which are too small for infusion, it is usually performed as 7 hourly intraperitoneal injections. There are some differences in dynamics and severerity of the response between rats and mice which might be due, in part, to different ways of drug administration. Pancreatic acinar cells are believed to be the initiation site of the pancreatitis in this model. After the initial injection, early events in acinar cells occur and they are potentiated by subsequent injections (or constant infusion) which induces further acinar cell response (Fig. I6). As a result, the repetitive injections lead to acinar cell damage that further leads to late events related to inflammation and systemic response (Saluja and Lerch, 2007). Early events include a blockade in zymogen secretion, co-localization of zymogen granules and lysosomes, activation of trypsinogen and other enzymes, and acinar injury. Late events are induced by chemokines and cytokines released by stressed acinar cells what attracts leukocytes. They in turn secrete cytokines that feedback to acinar cells and provoke more systemic symptoms.

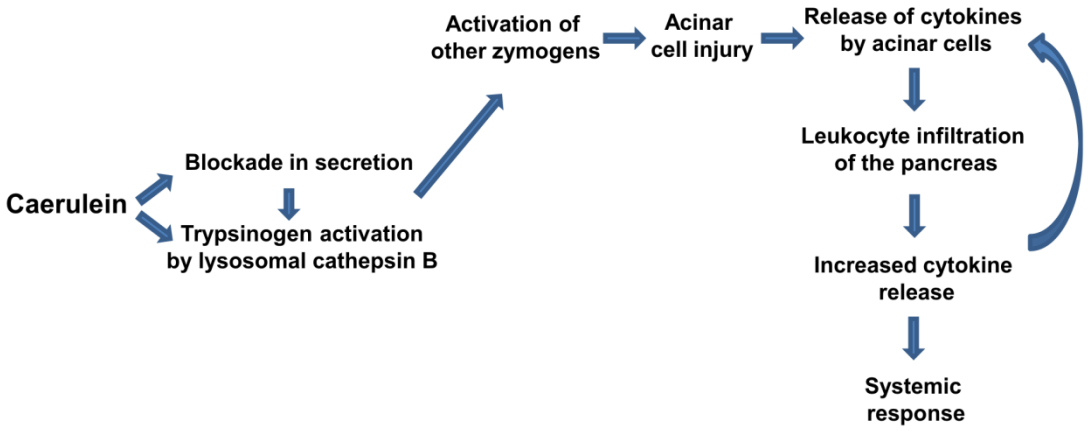


Figure I6. Pancreatitis induction by caerulein. Schematic representation of events underlying acinar cell damage and subsequent inflammatory and systemic response during caerulein-induced acute pancreatitis in rodents.

Secretagogues can induce both, trypsinogen activation and secretion blockade; those that are unable to induce the latter (such as bombesin and CCK-JMV-180) fail to produce pancreatitis (Powers et al., 1993; Grady et al., 1996), pointing to its mechanistic relevance. Intracellular trypsinogen is activated by cathepsin B upon fusion of zymogen granules and lysosomes as early as 10 min after caerulein infusion in rats (Grady et al., 1996) and 30-60 min in mice (Halangk et al., 2000). Trypsinogen activation can also be assessed by measuring free trypsinogen activation peptide (TAP), a cleavage product the increase of which is also detected very early (15 min of treatment in rats) (Grady et al., 1996). It is generally assumed that trypsin and cathepsin B together activate other proenzymes; however, the extent of activation and its dynamics in vivo has not been studied so far. Acinar cell injury leads to leakage of acinar enzymes into the plasma. An increase in amylase serum activity (hyperamylasemia) is detected subsequent to trypsinogen activiation and cannot be detected in rats until 30 min later (Grady et al., 1996). Multiple caerulein injections in mice lead to hyperamylasemia and the highest amylase leakage is reached 1 h after the last injection (Halangk et al., 2000). Similarly, other hallmarks of acinar cell damage follow trypsin activation and increase with time.

First modest immune cell infiltration is observed as early as 1 h after the first caerulein injection (Mayerle et al., 2005). Early during acute pancreatitis, acinar cells release

cytokines, like tumor necrosis factor (TNF)-α, IL-6, IL-1β, MOB-1, MIP-2, and chemokine KC (Gukovskaya et al., 1997; Han et al., 1999; Grady et al., 1997) as a result of NF-κB activation (Gukovsky et al., 1998; Steine et al., 1999). These cytokines attract inflammatory cells, mainly neutrophils, amplifying injury and contributing to the severity of pancreatitis (Zaninovic et al., 2000).

In the acute caerulein mouse model, acinar cell damage is associated with a rapid downregulation of acinar cell transcription programme (Molero et al., 2007; Molero et al., 2012) that is, in part, related to transient activation of the Notch pathway (Jensen et al., 2006; Siveke et al., 2008). Both in mice and rats, a downregulation of Ptf1a and Rbpjl expression is rapidly followed by downregulation of mRNA levels of digestive enzymes whose expression is lowest 1 day after pancreatitis and is gradually restored with histological recovery (Molero et al., 2012).

Many of the above aspects are regulated through different signalling pathways activated very early by caerulein i.e. protein kinase C (PKC) that modulates the NF-κB signaling (Satoh et al., 2004; Ramnath et al., 2010); phosphatidylinositol 3-kinase – (PI3K) responsible for colocalisation of cathepsin B with zymogens (Singh et al,. 2011) and inhibition of translation via mTOR – 4E-BP – eIF4E pathway (Sans et al., 2003); JNK, ERK and p38 MAP kinases whose role in pancreatitis is complex and not fully understood (Dabrowski et al., 1996; Grady et al., 1996; Wagner et al., 1999; Mazzon et al., 2012). These pathways are important mediators in tumorigenesis and are most likely also activated in caerulein induced chronic pancreatitis that is an important cofactor in pancreatic ductal adenocarcinoma development (Guerra et al., 2007).

2.2. Tumors of the exocrine pancreas

2.2.1. Classification

A number of different neoplasms can arise in the exocrine pancreas and they are classified according to their morphological characteristics. The most common form, accounting for 85% of all pancreatic tumors is Pancreatic Ductal Adenocarcinoma (PDAC) (Hezel et al., 2006) which will be discussed in detail in following chapters. Other types are less frequent, exhibit an overall better prognosis and are not the main focus of discussion. Intraductal papillary mucious neoplasms (IPMNs) account for 1-3% of

pancreatic tumors but their incidence is increasing. They are formed by columnar, mucin-producing cells that grow inside the ductal system. IPMNs evolve from adenoma to dysplasia, carcinoma in situ and finaly to invasive carcinoma and they are often found associated with PDAC (Bassi et al., 2008). Other rare pancreatic tumors show characteristics of other pancreatic cell types. Acinar cell carcinomas (ACCs) and pancreatoblastomas are examples of tumors with acinar differentiation features. ACCs are solid, usually unifocal differentiated tumors that secrete acinar digestive enzymes (Lowery et al., 2011). A large fraction of these tumours arise as a result of genetic alterations in Wnt/β-catenin signaling pathway (Klimstra 2007). Pancreatoblastoma is rare and it is the most common pancreatic neoplasia in childhood. It is similar to ACC in terms of Wnt/β-catenin pathway activation; however, it has a distinct morphology (Mulkeen, Yoo and Cha, 2006). Solid pseudopapillary neoplasms (SPNs) do not have any type of pancreatic cellular differentiation and their cellular origin is unknown. They may be solid or cystic and in some cases they metastasize to liver or peritoneum what is associated with a better outcome and higher long-term survival (Martin et al., 2002).

2.2.2. PDAC: pathology and treatment

PDAC is a devastating disease: five year survival rate of patients is <5% (Siegel et al., 2013; Ferlay et al. 2013). During 2012, 39084 cases were diagnosed in the UE and 38885 deaths were recorded (Ferlay et al.2013); this very high overall mortality has not changed in the last 50 years (Siegel et al., 2013). This situation persists despite the recent advances in our understanding of the biology of this cancer.

Most PDACs (60%–70%) arise in the head or neck of the pancreas, leading to obstructive cholestasis and, less frequently, duodenal obstruction or gastrointestinal bleeding. It can also be accompanied by acute pancreatitis and dysglycemia (Hidalgo, 2010). Patients generally present with deep upper abdominal pain and non-specific systemic manifestations such as asthenia, anorexia, and weight loss. These patients have a very poor prognosis, this being mainly due to prevalent local invasions and distal metastasis. Perineural, vascular or lymphatic invasions as well as lymph-node, liver, peritoneum or lung metastases are commonly observed (Stathis and Moore, 2010). In patients with resectable tumors, adjuvant chemotherapy increases the 5-year survival up to 20% and median survival is approximately 23 months, indicating that early

detection is the best way of improving the outcome of patients with PDAC (Stathis and Moore, 2010). However, as of now, most patients are diagnosed at a late stage and are not candidate for radical surgery. Moreover, tumor dissemination occurs early as the 2-year survival of patients with a 2-cm tumor is only 20% (Hernández-Muñoz et al., 2008).

Predominantly, PDAC is found as an infiltrating ductal adenocarcinoma producing a firm, sclerotic mass with poorly defined boundaries that sometimes contains cystic features (Fig. I7A). Microscopically, it contains infiltrating ductal glands with an intense desmoplastic reaction. The stroma is so abundant that, often, only a minority of the cells in the tumor mass are neoplastic (Maitra and Hruban, 2008) (Fig. I7B). They can be identified by immunohistochemistry through the expression of keratins 7, 8, 13, 18, and 19 (Hruban, Klimstra and Pitman, 2006), MUC1, MUC3, MUC4, and MUC5AC mucins, and CEA (Iacobuzio-Donahue et al., 2003).

Advanced PDAC is currently uncurable. The main drugs used for the treatment of advanced disease are gemcitabine, erlotinib, Abraxane, and the combination therapy FOLFIRINOX (Saif et al., 2011). It has been proposed that the dense tumor stroma may severely hamper the accessibility of drugs to neoplastic cells, suggesting that targeting the stroma may be a useful therapeutic strategy (Maitra and Hruban, 2008).

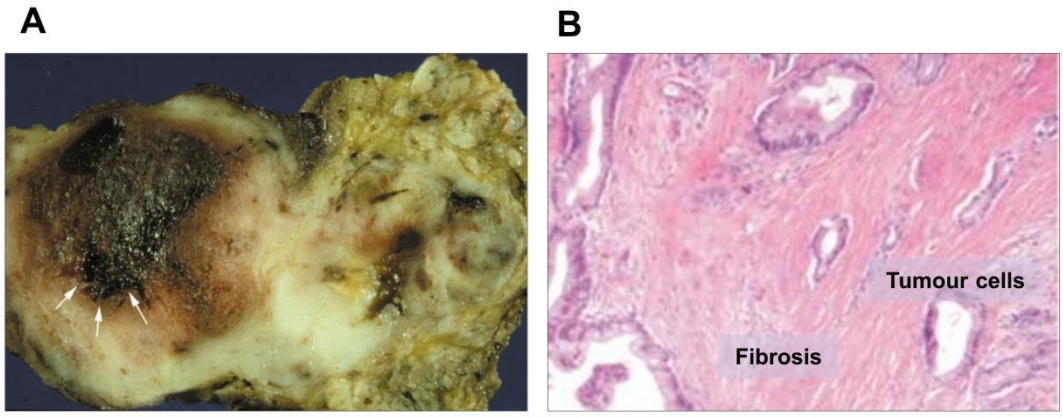


Figure I7. Pancreatic ductal adenocarcinoma (PDAC) macroscopic and microscopic aspect. (A) Resection specimen of undifferentiated pancreatic ductal adenocarcinoma showing cystic features with with hemorrhage indicated by white arrows. (B) Hematoxylin-eosin staining of human PDAC with very abundant fibrotic tissue. *Adapted from Kosmahl et al., 2005*.

2.2.3. PDAC etiology

The main risk factors associated with PDAC are advanced age, smoking (doubles the risk of PDAC), high meat and fat diet, obesity, diabetes mellitus and chronic pancreatitis (Raimondi, Maisonneuve and Lowenfels 2009). Individuals with a family history of PDAC (around 10%) have an increased risk of developing PDAC and the risk increases with the number of affected relatives (Amundadottir et al., 2004; Klein et al., 2004). Germline mutations in *BRCA1*, *BRCA2*, and *PALB2* (associated with breast, ovary and prostate cancers), *P16/CDKN2A* (melanoma), *PRSS1* (autosomal dominant hereditary chronic pancreatitis), *STK11/LKB1* (gastrointestinal tract and breast cancers), *ATM*, *hMLH1* and *hMSH2* have been associated with PDAC in the context of familial cancer syndromes (Maitra and Hruban, 2008; Roberts et al. 2012). Genome-wide association studies have recently identified new PDAC susceptibility loci in the vicinity of *ABO* (9q34), *KLF5* and *KLF12* (13q22.1), *NR5A2* (1q32.1), *TERT* (5p15.33) and *SBF2* (11p15.4) (Amundadottir et al., 2009; Petersen et al., 2010; Wu et al., 2012).

Association of PDAC with CP is particularly important from the clinical standpoint (Lowenfels et al., 1993; Malka et al., 2002) as this condition increases PDAC development by 14-fold (Lowenfels et al., 1993). This association is even stronger when only familial chronic pancreatitis patients are considered (Rebours et al., 2008).

2.2.4. PDAC progression model

PDAC is believed to arise from low grade precursor lesions of ductal characteristics called Pancreatic Intraepithelial Neoplasms (PanINs) (Fig. I8A). According to this model, normal duct cells evolve to PanIN-1A (hyperplastic epithelium with no dysplasia) that progresses to PanIN-1B (papillary hyperplasia) then to PanIN-2 (mild dysplasia) and finally to PanIN-3 (severe dysplasia with nuclear atypia), the latter being the direct precursor of invasive carcinoma. PanIN-1 lesions are often found in old people without PDAC whereas PanIN-3 lesions are almost exclusively detected in PDAC patients, suggesting that PanIN-2 is a turning point in the progression to carcinoma (Sipos et al., 2009). Increasing grade of the lesions is paralleled by accumulation of genetic alterations like telomere shortening (Hong et al., 2010) and gene mutations (Hezel, Kimmelman, Stanger, Bardeesy and Depinho, 2006). Activating mutation in *KRAS* codons 12/13 and inactivation of *P16/CDKN2A* are found in most PDAC (around 90% and

almost 100%, respectively). Less frequent are the inactivation of *TP53* and *SMAD4/DPC4* (60-80% of tumors). Analysis of prevalence of these mutations suggested a chronological order of their occurrence whereby *KRAS* activation is an initial event that is followed by *P16/CDKN2A* and subsequent *TP53* and *SMAD4/DPC4* inactivations (Maitra and Hruban, 2008) (Fig. I8A).

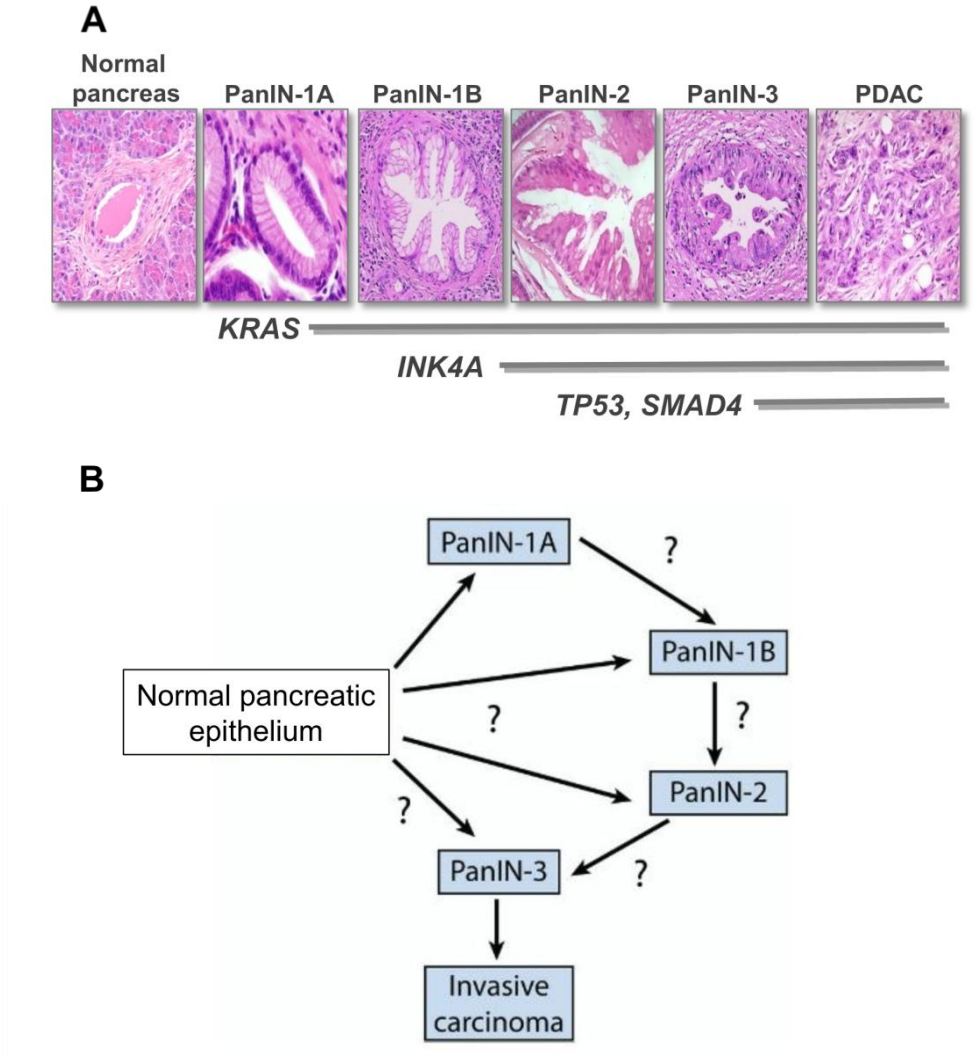


Figure I8. PDAC progression. (A) Pancreatic Ductal Adenocarcinoma (PDAC) linear progression model according to which ductal cells that acquire *KRAS* activating mutations proliferate and form PanIN-1A lesions which due to mutations in tumor suppressors gradually progress into lesions of higher grade (PanIN-1B and PanIN-2 and PanIN-3) to finally form ductal adenocarcinoma. (B) An alternative model of PDAC progression suggesting that different types of PanIN lesions might arise directly from pancreatic epithelium and low grade lesions might arise through mechanisms independent from those leading to carcinoma formation. *Adapted from Real, 2003 and Real et al., 2008*.

Since the linear model was based on interpretation of cross-sectional data from human PDAC samples, it is likely an oversimplification (Rooman and Real, 2012). There is no formal direct evidence that PanIN-1 progresses to higher grade lesions and is not simply an independent event. It is also possible that different events may lead to carcinoma formation and that different precursor lesion characteristics are determined by the cell of origin (Fig. I8B). Similarly, no formal proof has been found to support the hypothesis that PDAC originates from ductal cells (Rooman and Real, 2012). This was an assumption based on the ductal phenotype of PanINs and carcinomas, made when there were no existing tools to address this issue experimentally. During the last decade, however, a lot of data from animal models of pancreatic cancer have been obtained that argue against this hypothesis.

2.2.5. Mouse models of pancreatic cancer

Experiments with genetically engineered mice (GEM) have challenged the notion that ductal cells are the cell of origin of PDAC. Conditional expression of mutant *KRas^{G12V}* in mouse pancreatic MPCs at the time of pancreas formation using Cre recombinase under the regulatory elements of *Pdx1* or *Ptf1a* leads to the development of all the spectrum of PanINs as well as PDAC in the adult (Aguirre et al., 2003; Hingorani et al., 2003). This confirmed that *Kras* is a potent oncogene in the pancreas but these models did not allow answering the question of the cell of origin of PDAC. So far, it has not been possible to induce PDAC by targeting mutant *Kras* to ductal cells. Transgenic mice expressing active Kras under keratin 19 promoter (*Krt19*-Kras^{G12V}) developed hyperplastic ductal lesions with periductal lymphocytic infiltration but no tumors (Brembeck et al., 2003) and conditional, tamoxifen inducible expression of *Kras^{G12D}* using *Krt19*-Cre^{ERT} resulted in development of PanIN-1A but no other lesions (Ray et al., 2011). Recently Kopp and colleagues proved that mutant *Kras* does not lead to PDAC development when expressed in both ductal and CACs using inducible Cre recombinase under the control of the *Sox9* promoter (Kopp et al., 2012). Conversely, activation of mutant *Kras* in acinar cells results in acinar-to-ductal mateplasia (ADM) and development of ductal tumors (Guerra et al., 2007), effects that are also observed in *Ela1-Tgfα* and *Ela1-c-Myc* transgenic mice (Sandgren et al., 1991; Wagner et al., 1998). To investigate in more detail the capacity of mutant *Kras* to induce ductal tumors from acinar cells, Guerra and colleagues applied an inducible system to temporally control *Kras^{G12V}* expression from its endogenous locus

only in acinar cells using Cre recombinase regulated by doxycyclin from the *Ela1* promoter (*Ela1-tTa/tetO-Cre;Kras*^{LSLG12Vgeo}) (Guerra et al., 2007). This approach allowed concluding that mutant Kras activation in embryonic, but not in adult, acinar cells leads to ADM, PanINs and PDAC. Interestingly, when Kras is activated in adult acinar cells and a concominant CP is induced, PDAC ensues. This was a very important finding as it pointed out that recurrent tissue damage creates a permissive context for the oncogenic effects of *Kras* in adult acini, in agreement with data from patients with CP. More recently the same group uncovered that the role of inflammation in this model is to suppress senescence induced in adult acini by oncogenic stress (Guerra et al., 2011).

Other studies addressed the role of different tumor suppressor genes and inflammatory mediators in *Kras*-driven pancreatic tumorigenesis using compound mutant mice combining oncogenic *Kras* with inactivation of tumor suppressors in the epithelium: upon inactivation of *p16*^{Ink4a}*/p19*^{Arf} or *Trp53*, expression of dominant-negative forms of *Trp53*, or inactivation of *Tgfbr2*, tumor progression was faster and animals developed invasive and metastatic PDACs with some specific characteristics associated with each genotype (Aguirre et al., 2003; Bardeesy et al., 2006; Hingorani et al., 2005; Ijicji et al,. 2006).

The role of inflammation in PDAC progression has recently been a focus of many studies. Two groups reported simultaneously that Stat3 signalling is crucial for tumor formation (Fukuda et al., 2011; Lesina et al., 2011). Its activation supports inflammation and acinar cell proliferation as well as MMP-7 expression during neoplastic development (Fukuda et al., 2011). Moreover, it is an important mediator of IL-6 trans-signaling that promotes PanIN progression and PDAC formation (Lesina et al., 2011). Mice lacking Stat3, its phosphorylatable tyrosine (*Stat3*^{Y705}), or either *IL-6* or *MMP7* genes had delayed tumor formation and reduced tumor burden in a *Ptf1a*$^{+/Cre}$*;Kras*$^{+/G12D}$ background (Fukuda et al., 2011; Lesina et al., 2011). These data emphasize the importance of Stat3 phosphorylation in pancreatic cells in response to inflammatory mediators such as IL-6 that promote PDAC development. Meanwhile, other studies focused on cytokines produced by neoplastic cells such as Cxcr2 that is important for proper tumor-stroma interaction in tumors lacking Tgfrb2 and granulocyte-macrophage colony-stimulating factor (GM-CSF), the production of which by PanIN cells promotes progression to PDAC

(Ijicji et al,. 2011; Pylayeva-Gupta et al., 2012). Moreover, GM-CSF was shown to be responsible for recruitment of $Gr1^{+}$;$CD11b^{+}$ myeloid-derived suppressor cells (MDSCs) by $Kras^{G12D}$-transformed pancreatic tumor cells in the early stages of tumor development (Bayne et al., 2012). This suppressed CD8+ T cell-mediated antitumor immune responses, thereby allowing for tumor growth.

3. MNK KINASES

3.1. Mnk protein kinases (Mnks)

3.1.1. Discovery of Mnks

Mnk (MAP kinase-interacting kinase or MAP kinase signal-integrating kinase) protein kinases were discovered independently by two research groups (Fukunaga and Hunter, 1997; Waskiewicz et al., 1997) that applied different screening strategies searching for ERK-regulated proteins – bacterial expression libraries and yeast two-hybrid. These studies identified 2 novel kinases Mnk1 (Fukunaga and Hunter, 1997; Waskiewicz et al., 1997) and Mnk2 (Waskiewicz et al., 1997) – products of *Mknk1* and *Mknk2* genes respectively – that were phosphorylated by ERK1/2 and p38 MAP α/β but not by JNK enzymes. The yeast two-hybrid approach allowed to show that Mnk1 strongly interacted with both ERK and p38 MAP kinase whereas Mnk2 bound stably only to ERK2 (Waskiewicz et al., 1997). Moreover these studies described that stimulating ERK (with phorbol esters or serum) or p38 MAP kinase (with proinflammatory cytokines such as TNFα or IL-1β or hyperosmolarity) led to activation of Mnk1 which was blocked by the MEK inhibitor PD098059 or the p38 MAP kinase inhibitor SB202190 (Fukunaga and Hunter, 1997).

3.1.2. Mnks: homology to other kinases and structural information

At the amino acid level, Mnks are most closely related to other kinases that are substrates of MAP kinases, especially to calmodulin-dependent kinase subfamily like MK2 or pK3 and p90RSK (Fukunaga and Hunter, 1997). A common feature of these kinases is an activation loop domain, through which MAP kinase regulation occurs, that contains phosphorylatable Thr residues followed by Pro – the so called "T-loop" (Fig. I9A). Mnks contain 2 such Thr in this domain and their mutation results in enzyme inactivation (Goto et al., 2009). There are two specific features in the amino acid

sequence of Mnks that distinguish them from other protein kinases. First, instead of a DFG motif located N-terminally from the T-loop, they contain a DFD sequence. Second, the activation loop in Mnks is flanked by 2 short insert sequences that are not found in other kinases from their subfamily (Buxade et al., 2008). When crystal structures of the catalytic domains of Mnk1 and Mnk2 were solved, it became apparent that in non-phosphorylated form the activation loop of Mnks has an unusual open conformation and that G to D substitution in DFD motif may interfere with ATP binding (Jauch et al., 2005; Jauch et al., 2006) (Fig. I9B). These features imply a relatively low basal enzymatic activity of these kinases when not phosphorylated. In the case of Mnk1 the activation loop functions in an autoinhibitory fashion due to specific residues in the second insert sequence described above (Jauch et al., 2006).

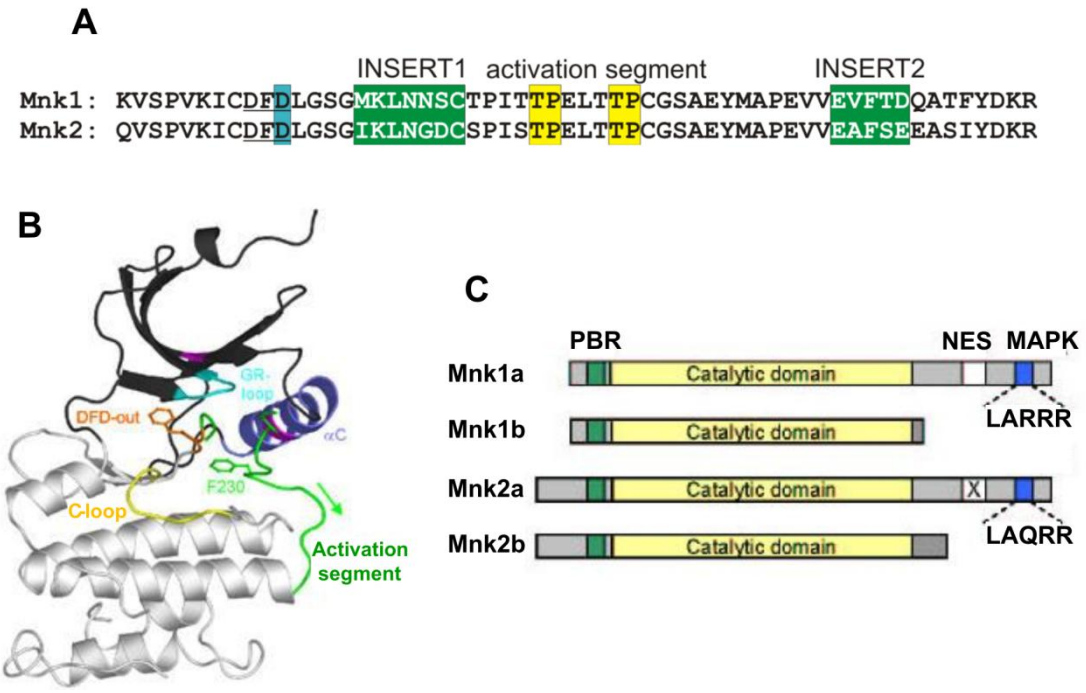


Figure I9. Mnk kinases domain organisation. (A) Sequence of the activation domain of Mnk1 and Mnk2 containing the DFD motif, the two Mnk-specific inserts and the two Thr residues that are phosphorylated by MAP kinases. (B) Ribbon plot of Mnk1 showing its autoinhibitory conformation with disorganized activation segment and long C-terminus occluding catalytic site. (C) Differences in domain organization between Mnks: "b" isoforms are shorter and lack the NES and MAPK domains; Mnk2a has non-functional NES and aminoacid substitution contributing to stable binding to upstream kinases. *Adapted fom Buxade et al., 2008 and Jauch et al., 2006*

3.1.3. Mnk isoforms and distribution of functional domains

Two splicing variants have been described for Mnk1 and Mnk2 yielding "a" and "b" isoforms of each protein (Fig. I9C). Mnk1a and Mnk2a are longer and contain all essential domains (from N- to C-terminus): polybasic region (PBR), catalytic domain, nuclear export signal domain (NES) and MAP kinase binding domain (Buxade et al., 2008). The last domain, however, is only fully functional in Mnk1a. Mnk1b (O'Loghlen et al., 2004) and Mnk2b (Slentz-Kesler et al., 2000) proteins have shorter C-terminus – lacking the MAP kinase binding motif and the NES. So far the existence of "b" isoforms has only been proven in human cells.

The most N-terminal domain of all Mnk isoforms, PBR, is responsible for protein-protein interactions. Two proteins binding to Mnks through this region have been described: EIF4G, a scaffold protein for translation initiation factors (Waskiewicz et al., 1999; Pyronnet et al., 1999), and importin α (karyopherin), mediating nuclear import (Parra-Palau et al., 2003). Interaction with importin α (through the nuclear localisation signal – NLS) allows these kinases to enter the nucleus and Mnk1b and Mnk2b are mainly nuclear (Parra-Palau et al., 2003; O'Loghlen et al., 2004). On the other hand Mnk1a is predominantly cytoplasmic, as it also contains C-terminal NES (Parra-Palau et al., 2003; Scheper et al., 2003). Mnk2a contains a non-functional NES-related sequence, however it is also mainly found in the cytoplasm due to its long C-terminal domain that interferes with binding of importin α to the NLS (Buxade et al., 2008; Goto et al., 2009).

3.1.4. Mnk1/2 activity regulation

Due to structural differences, Mnk isoforms have different activities. Mnk1a displays a low basal activity and is highly responsive to stimuli that activate ERK or p38 (Waskiewicz et al., 1997; Waskiewicz et al., 1999) whereas Mnk2a has high basal activity which is poorly enhanced by stimulation (Scheper et al., 2001). Unlike Mnk1a, Mnk2a binds stably to phosphorylated ERK1/2 due to a single amino acid substitution in the MAP kinase binding domain (Fig. I9C). Moreover, Mnk2a stabilizes phosphorylated ERK (Parra et al., 2005). This implies that ERK inhibitors can block Mnk1a activation but have little effect on Mnk2a which is already associated with residual phosphorylated ERK (Waskiewicz et al., 1997). In this regard, Mnk1b, which as described above lacks MAP kinase binding domain, is more similar to Mnk2a than Mnk1a since it shows high

basal activity (O'Loghlen et al., 2004; O'Loghlen et al., 2007). Moreover Mnk1b does not require stimulation from MAP kinases and is affected neither by ERK nor by p38 inhibitors, possibly due to its very short C-terminus that is not able to interfere with the catalytic domain (Waskiewicz et al., 1999), as normally occurs in longer Mnk isoforms (discussed below). Similarly to Mnk1b, Mnk2b (that also lacks MAP kinase binding domain) does not respond to ERK or p38 activating stimuli; however, it has very low basal activity (Scheper et al., 2003).

Low basal activity of Mnk1a is a consequence of the unique properties of its long C-terminus that occludes access to the catalytic domain (Parra et al., 2005; Goto et al., 2009). However, upon interaction with upstream kinases Mnk1a acquires a different conformation and becomes active. In the case of Mnk2a, which contains a similar C-terminus, this interference does not occur due to stable binding to ERK and to differences in the first insert of the catalytic domain (Parra et al., 2005). In both kinases the C-terminus also occludes T-loop whose phosphorylation occurs only upon binding to MAP kinases.

Thr344 (the third phosphorylatable residue crucial for their activity) plays a very important role in the activation of "a" isoforms in response to upstream signaling (Goto et al., 2009). This residue is located in the C-terminus and modulates its conformation, controlling the access to the catalytic domain and T-loop (Goto et al., 2009). It has been shown to be phosphorylated by ERK1/2 and mutation of this residue to the phosphomimetic Asp in mouse cells yields a constitutively active kinases (O'Loghlen et al., 2004).

3.2. Mnk substrates

3.2.1. eIF4E

The first Mnk1/2 substrate described was eukaryotic translation initiation factor 4E (**eIF4E**) (Waskiewicz et al., 1997), one of the subunits of eIF4F translation initiation complex (Fig. I10). eIF4E is a mRNA 5'-cap structure (m^7GDP) binding protein responsible for the association of eIF4F to mRNAs that are subject to cap-dependent translation. eIF4E is the least abundant translation initiation factor in the cytosol, making it a rate limiting factor in protein synthesis (Hiremath et al., 1985; Duncan et al.,

1987). It binds to the eIF4F complex through eIF4G – a scaffold protein to which all other subunits bind. eIF4G also recruits Mnks that, upon activation, can phosphorylate eIF4E at Ser209 (Pyronnet et al., 1999). Studies with knockout mice have unequivocally shown that Mnk1 and Mnk2 are solely responsible for eIF4E phosphorylation (Ueda et al., 2004); however, they have not elucidated the functional significance of this process (For review see Scheper and Proud, 2002).

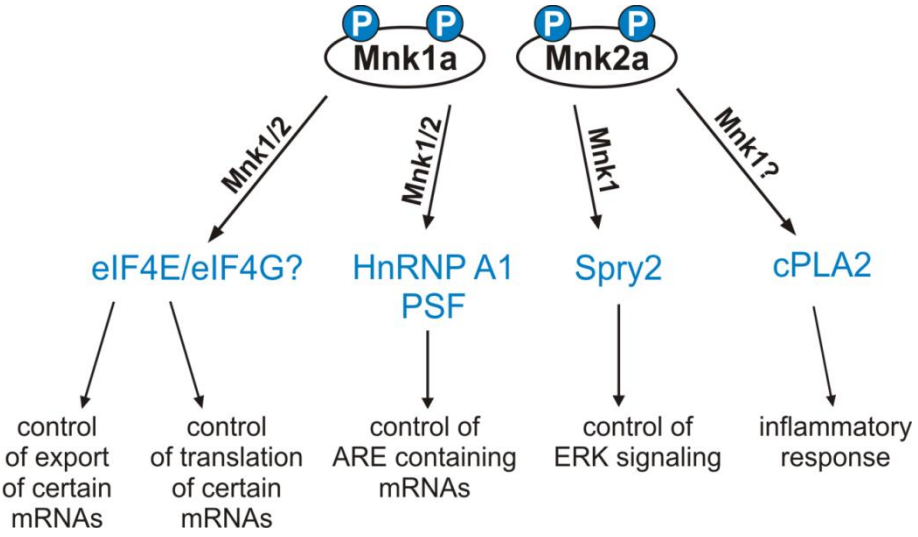


Figure I10. Mnk substrates. Schematic depiction of confirmed and putative Mnk substrates indicating their roles in cellular processes. Question marks indicate that there is no formal evidence that Mnk1 is the kinase that phosphorylates $cPLA_2$ or eIF4G. *Adapted fom Buxade et al., 2008*

Different biochemical and structural approaches were applied to address the role of eIF4E phosphorylation in translation initiation. Some reports provided data that phosphorylation increases the affinity of eIF4E for capped mRNA (Minich et al., 1994; Marcotrigiano et al., 1997; Shibata et al., 1998) while others suggested the opposite (Scheper et al., 2002; Tomoo et al., 2002; Niedwiecka et al., 2002). On the other hand Mnk pharmacological inhibition in cellular systems does not affect overall translation (Knauf et al., 2001; Saghir et al., 2001). As suggested by Scheper and Proud eIF4E phosphorylation may induce disasembly of eIF4F complex from mRNAs the translation of which has already been initiated, allowing subsequent binding to the cap of the same or different mRNA (Scheper and Proud, 2002). The efficiency of this mechanism is dependent on the tertiary structure of the 5' end of given mRNAs, implying that translation of different transcripts would be differentially affected by this

phosphorylation. The currently accepted hypothesis is that p-eIF4E increases the translation rate of mRNAs with long and complex 5' UTR.

Apart from effects on translation initiation, p-eIF4E also contributes to nuclear export of some mRNAs through the recognition of specific regions in 3' end of these transcripts.

3.2.2. Other Mnk substrates

A number of additional proteins have been proposed to be Mnk substrates and most of them are also involved in mRNA biology (Fig. I10):

- **HnRNP A1** is a component of heterogeneous nuclear RNA-ribonucleoprotein complexes that shuttles between the nucleus and cytoplasm and is involved in mRNA splice site recognition, polyadenylation, cleavage, stability and transport. It binds to AU rich elements (ARE) present in 3' end of cytokine or "immediate-early" gene transcripts. HnRNP A1 can be phosphorylated by Mnks resulting in a positive regulation of TNFα biosynthesis in T cells (Buxade et al., 2005) or in accumulation of this ribonucleoprotein in stress granules (SGs) – cytoplasmic domains that contain translationally arrested mRNAs (Guil et al., 2006). Thus, Mnk-HnRNP A1 pathway differentially regulates the translation of mRNAs coding for TNFα (and possibly other genes) vs. those mRNAs targeted to SGs.

- **PSF** (polypyrimidine tract-binding protein-associated splicing factor) – was found in a proteomic approach aimed at identifying putative Mnk targets pulled-down together with mRNA cap (Buxade et al., 2007). Similarly to HnRNP A1, PSF is an ARE-binding protein and it regulates mRNA splicing and stability. It was identified by above mentioned approach as it was bound to 3' ends of mRNAs pulled down together with 5' cap. It can be phosphorylated by Mnks but the consequences of this modification are unknown (Buxade et al., 2007).

- **Sprouty 2** is the only non-mRNA-binding protein among confirmed Mnk targets and its Mnk-catalysed phosphorylation is not involved in the regulation of protein biosynthesis. Spry2 is a negative modulator of ERK signaling and is known to be subject to Tyr and Ser phosphorylation in response to growth factor stimulation (reviewed in Kim and Bar-Sagi, 2004). Mnk1 phosphorylates Spry2 at Ser112 and Ser121, decreasing its phosphorylation at Tyr55 which targets it for c-Cbl mediated proteasome degradation,

thereby stabilizing the protein. Thus, Mnk1 increases the half-life of Spry2 providing a negative feedback mechanism that inhibits ERK signaling (Dasilva et al., 2006).

- **cPLA$_2$** (cytoplasmic phospholipase A2) is a putative Mnk target, that is involved in production of eicosanoids which regulate immunity and inflammation. It can be phosphorylated at Ser505 and Ser727 via p38 MAP kinase α/β and this could be mediated, in part, by Mnk1 (Hefner et al., 2000). This study suggested that Mnk1 can phosphorylate cPLA2 (only at Ser727) in vitro; however, it is not known whether this kinase is responsible for in vivo cPLA2 phosphorylation.

- **eIF4G**, as described above, structurally mediates the binding of Mnk1/2-eIF4E and it was also initially proposed to be a target of these kinases (Pyronnet et al., 1999). However, no direct evidence thereof has been provided. Mnk1 and Mnk2 phosphorylation by MAP kinases allows their binding to eIF4G, preceding Mnk1-eIF4E interaction (Mayya Shveygert et al., 2010; Dobrikov et al., 2011). So far, only indirect regulation of its activity has been described for Mnk2 that has been reported to negatively affect eIF4G phosphorylation by mTOR (Shou-Ih et al., 2012).

3.3. Cellular functions of Mnks

3.3.1. Animal models to study Mnk1/2 cellular function

Mnk proteins are mammalian homologues of Drosophila protein LK6 which was initially identified as a short lived serine/threonin kinase that associates with microtubules and centrosomes (Kidd and Raff, 1997). Excessive LK6 activity obtained through overexpression of an activated form of this kinase leads to microtubule abnormalities and is deleterious to fruitflies. Only recently a study has been carried out that addressed whether in mammalian cells Mnk proteins also associate with microtubules. Active human Mnk1 can localize to centrosomes, spindle microtubules and the midbody and be required for abscission after cell division: reduced Mnk1 activity resulted in formation of multinucleated cells (Rannou et al., 2012). LK6 has also been found in a genetic screen as a regulator of RAS-MEK-ERK pathway (Huang and Rubin, 2000). Given that Spry2 is a Mnk substrate, it is hypothesized that dSpry2 might contribute to this function. As Mnks, LK6 phosphorylates eIF4E in fruitflies at Ser251 (corresponding to Ser209 in mouse and human) (Parra-Palau et al., 2005) and its replacement by Ala has a negative effect on

growth and viability (Lachance et al., 2002). Further studies in Drosophila have shown that increased phosphorylation of eIF4E by active LK6 mutant increases cell size and number (Arquier et al., 2005). However, LK6 seems to be dispensable for normal growth and development and its loss has an effect only upon diet with reduced amino acid content or induction of oxidative stress, when it leads to decrease in cell number and size (Reiling et al., 2005).

In contrast to findings in Drosophila, introduction of the S209A eIF4E mutation in mouse cells does not lead to an obvious growth phenotype (Furic et al., 2010). Ueda and colleages have developed *Mnk1*$^{-/-}$, *Mnk2*$^{-/-}$ and *Mnk1*$^{-/-}$;*Mnk2*$^{-/-}$ mice and have shown that these kinases are dispensable for mouse growth and development (Ueda et al., 2004). However, using *Mnk1*$^{-/-}$, *Mnk2*$^{-/-}$ and double-deficient MEFs they have demonstrated that eIF4E phosphorylation is dependent on both kinases, Mnk2 being responsible for basal and Mnk1 for stimulated phosphorylation. Together, the in vivo data pointed out that the main physiological function of Mnk1/2 in eukaryotic cells is probably a response to stress conditions or oncogenic context.

3.3.2. Role of Mnks in cellular stress

The notion that LK6 might be involved in the response of cells to nutrition and oxidative stress was followed in mouse. Using MEFs from Mnk knock-out mice, it has been shown that H_2O_2 increases eIF4E phosphorylation through Mnk1 possibly regulating translation (Shenberger et al., 2007) and that Mnk1 and Mnk2 loss sensitizes cells to serum withdrawal and arsenic trioxide (As_2O_3)-induced apoptosis (Chrestensen et al., 2007; Dolniak et al., 2008).

3.3.3. Role of Mnks in inflammatory cells

As mentioned before, Mnk kinases are involved in TNFα production in T lymphocytes through the phosphorylation of ARE binding proteins (Buxade et al., 2005; Guil et al., 2006). However, their role in different inflammatory cells is broader. In macrophages, they are involved in biosynthesis of proinflamatory cytokines (such as TNFα, IL-1β, IL-6, IL-8 and MCP-1) upon signaling from different TLR receptors (Andersson et al., 2006; Cherla et al., 2006; Rowlett et al., 2007). The importance of Mnk1 in macrophages has also been raised by Xu and colleagues who showed that the MAPK-Mnk1-eIF4E signaling

cascade is activated by TLR4 to induce polarization towards M1macrophages (Xu et al., 2012).

Apart from being involved in upstream regulation of cytokine production, Mnks can also positively mediate cytokine-induced growth and differentiation signals. Mnk1 takes part in translation induction of IFN stimulated genes (Joshi et al. 2009; Joshi et al., 2011). Moreover, Mnk1 may participate in IL-2 an IL-15 induced activation of Ets1 posttranscriptional expression in human natural killer (NK) cells (Grund et al., 2005).

Together, these studies suggest an important role of Mnks (mainly Mnk1) in inflammatory responses both regulating cytokine production as well as the response to cytokine stimulation. Mice lacking these kinases have no obvious hematopoietic phenotype but it is conceivable that absence of Mnk1, Mnk2 or both may have a suppressor effect on immune response during inflammation. Indeed, a recent study has described that - while dispensable for T cell development - both Mnks are important for the response to experimental autoimmune encephalomyelitis (Gorentla et al. 2013).

3.3.4. Role of Mnks in cell survival and cancer

The best characterized Mnk substrate, eIF4E, is a downstream effector of PI3K-AKT-mTOR pathway essential for cell growth (Sonenberg, 2008). It can be sequestered in the cytoplasm by its cellular inhibitors eIF4E binding proteins (4E-BPs), that compete with eIF4G in binding to this factor. 4E-BPs (mainly 4E-BP1) are among the most important targets of mTORC1 complex. Upon PI3K-Akt pathway activation, mTORC1 phosphorylates 4E-BPs, inducing their dissociation from eIF4E, increasing its availability for eIF4F and - as a consequence - elevating global cap-dependent translation rate. A limitation in the robustness of this pathway is the abundance of eIF4E itself. It is elevated in many tumor types what have been proposed to be associated with worse prognosis and aggressive, poorly differentiated tumors (De Benedetti et al., 1999; Bjornsti and Houghton, 2004). Moreover, its overexpression - alone or in combination with c-Myc or E1A - is able to transform normal cells both in vitro and in vivo (Lazaris-Karatzas et al., 1990; Lazaris-Karatzas et al., 1992; Ruggero et al., 2004; Wendel et al., 2004).

Some of the oncogenic effects of eIF4E overexpression may be attributed to a general increase in translation. However, many studies have suggested that this is dependent on its activation by Mnks. Ser209 phosphorylation seems to exert its effect on eIF4E in three ways: 1), by increasing its affinity for long 5' UTR containing transcripts such as Mcl-1 in the c-Myc driven mouse lymphoma model (Wendel et al., 2007), HIF-1 in a wide variety of human cancer cell lines (Jin et al., 2008), VEGF in MDA-MB-435 breast cancer cells (Korneeva et al., 2010), and SMAD2 in human gloglastoma cells (Grzmil et al., 2011); 2)^{n} by an IRES-dependent translation switch of some mRNAs such as ODC in *Kras*^{G12V} transformed RIE-1 cells (Origanti and Schantz, 2007) and c-Myc in rapamycin-treated multiple myeloma cells (Shi et al., 2012); 3 by stimulating the 3' UTR mediated nuclear export of transcripts such as HDM2 (Phillips and Blaydes, 2008) and cyclin D1 (CCND1) (Weater et al., 2010), both in human breast cancer cells.

The eIF4F complex assembly, and hence the Mnk-eIF4E association, has been shown to be regulated during the cell cycle (Pyronnet et al., 2001). Wendel and colleagues pointed out that Mnk1 activity is necessary for eIF4E to promote c-Myc-driven lymphoma formation by increasing cap-dependent translation of Mcl-1 (Wendel et al., 2007). The most convincing data regarding tumor dependency on Mnk1 came from using genetically engineered mouse models. Mice expressing a non-phosphorylatable mutant of eIF4E (*eIF4E*$^{S209A/S209A}$) are resistant to prostate cancer resulting from Pten loss (Furic et al., 2010) and Mnk1/2 double knockout mice show delayed tumor formation in a Pten-deficient lymphoma model (Ueda et al., 2010).

Together, the above described studies suggest an important role of Mnks in cancer cell survival and proliferation and point to eIF4E as a crucial mediator in this process. It is thus not surprising that a substantial interest has been placed recently on the possible use of Mnk inhibitors in anti-cancer therapy (reviewed in Hou et al., 2012).

Objectives

Unraveling the molecular mechanisms that control acinar phenotype in homeostasis and upon oncogenic signaling and injury could provide a better understanding of processes underlying Kras driven PDAC development. The main objective of this thesis was to describe the role of Mnk1 in pancreas biology, as this kinase could be involved therein.

The specific goals for this thesis were:

1. To investigate whether Mnk1 expression is restricted to specific pancreatic cell lineages in developing and adult pancreas and under which conditions this kinase is activated in the pancreas.

2. To dissect the role of Mnk1 in the pancreas by analyzing the effect of its inactivation on mouse pancreas development, physiology and response to acute and chronic damage.

3. To assess the relationship between Mnk1 and oncogenic Kras signaling in the pancreas and investigate the involvement of this kinase in pancreatic ductal adenocarcinoma formation.

Objetivos

La identificación de los mecanismos moleculares que controlan el fenotipo acinar en homeostasis, así como durante la señalización oncogénica y daño tisular, ayudaría a entender el desarrollo de PDAC dirigido por Kras. El objetivo principal de esta tesis doctoral fue investigar el papel de Mnk1 en la biología del páncreas, ya que esta quinasa podría estar involucrada en estos procesos.

Los objetivos específicos planteados en esta tesis fueron:

1. Investigar si la expresión de Mnk1 está restringida a líneas celulares específicas en el páncreas tanto en desarrollo como en páncreas adulto, así como bajo qué circunstancias esta quinasa se activa en dicho tejido.

2. Estudiar la función de Mnk1 en el páncreas mediante el análisis del efecto que su desaparición produce en un modelo murino durante desarrollo, en la fisiología pancreática y en respuesta a pancreatitis aguda y crónica.

3. Describir la relación entre Mnk1 y la ruta de señalización oncogénica de Kras en el páncreas e investigar la participación de esta quinasa en la formación de adenocarcinoma pancreático ductal.

Materials and Methods

1. IN VIVO PROCEDURES

1.1. Mouse strains

Mouse strains used in the experiments were of C57BL/6 or mixed background with following genotypes: wild type, *Mnk1*$^{-/-}$, *Mnk1*$^{-/-}$*;Mnk2*$^{-/-}$, *Ptf1a*$^{+/CreERT2}$*;Kras*$^{+/G12Vgeo}$, *Ptf1a*$^{+/CreERT2}$*;Kras*$^{+/G12Vge}$*;Mnk1*$^{-/-}$ *and Ela1-Myc. Mnk1*$^{-/-}$ and *Mnk2*$^{-/-}$ mice were obtained from Professor Christopher Proud, School for Biological Sciences, University of Southapmton. Other strains were available at CNIO. Mice were bred and maintained under sterile and pathogen-free conditions. Experiments were approved by the Animal Ethical Committee of Instituto de Salud Carlos III and performed following guidelines for Ethical Conduct in the Care and Use of Animals as stated in The International Guiding Principles for Biomedical Research involving Animals, developed by the Council for International Organizations of Medical Sciences.

1.2. Caerulein-induced acute pancreatitis

Acute pancreatitis was induced by seven hourly intraperitoneal injections of caerulein (Sigma) (50µg/kg of mouse) and animals were sacrificed by cervical dislocation either during the treatment: at 0.5h, 4h, 8h after first injection (1, 4 and 8 injections respectively) or at 1 day, 2 days and 7 days after the treatment. The pancreas was quickly collected: one piece homogenized instantly (for RNA analysis), one piece snap-frozen on liquid nitrogen and stored at -80°C (for protein analysis and quantification of pancreatic amylase and trypsin activity) and the rest of the pancreas was placed in 4% PBS-buffered formaldehyde (for histology). In all the cases blood was collected from live animal just before the euthanasia. In animals sacrificed 2 days and 7 days post-treatment a single intraperitoneal injection of BrdU (20 mg/kg of animal) was performed 12h before sacrifice. A minimum of 3 mice were included for each genotype and experimental time point.

1.3. Caerulein-induced chronic pancreatitis

Chronic pancreatitis was induced by weekly induction of acute pancreatitis for 8 weeks. During the procedure, blood samples were collected from live animals at 4 weeks and 8 weeks, each time 5 days after last acute pancreatitis. At 8 weeks (5 days after last acute pancreatitis) animals were sacrificed by cervical dislocation and the pancreas samples

for RNA, protein and histology analysis were collected as described in 1.2. A minimum of 4 mice was included for each genotype.

2. HISTOLOGICAL ANALYSIS

2.1. Histopathology

Mouse pancreatic tissues were fixed in 4% PBS-buffered formaldehyde, embedded in paraffin and serially sectioned (3µm). Chosen sections were deparaffinized and stained with hematoxylin-eosin.

2.2. Immunohistochemistry

Pancreatic tissue sections from *Ela1-tTa/tetO-Cre;Kras*$^{+/LSLG12Vgeo}$*;p16*$^{-/-}$ and *Ela1-tTa/tetO-Cre;Kras*$^{+/LSLG12Vgeo}$*; p53*$^{lox/lox}$ mice were kindly provided by M. Barbacid and C. Guerra (CNIO). Other mouse tissue sections were prepared as follows. Paraffin embedded tissue sections were deparaffinized, rehydrated and boiled in 10mM Sodium Citrate Buffer (pH6.0) in a preheated steamer for 10 min to retrieve the antigens. Next, sections were washed in distilled water and incubated for 30 min with 3% hydrogen peroxide in methanol, after which they were washed again with water and blocked for 30 min with 2% BSA in Phosphate Buffer Saline (PBS) with 0.5% Triton X-100. After blocking, the sections were incubated with primary antibodies in PBS with 1% BSA and 0.1% Triton X-100 for 1h at room temperature. For detection of BrdU positive cells the incubation buffer also contained DNAse I and 4mM $MgCl_2$. Antibody dilutions used: rabbit anti-Mnk1 (Cell Signaling #2195, 1:400), rabbit anti-Amy2 (Sigma-Aldrich A8273, 1:200), rabbit anti-Cpa1 (AbD Serotec 1810-0006, 1:400), rabbit anti-MPO (Dako A 0398, 1:1000), rabbit anti-Ki67 (Novocastra NCL-Ki67p, 1:1000), and mouse anti-BrdU (Developmental Studies Hybridoma Bank Ab-G3G4, 1:1000). Next, sections were washed in PBS with 0.1% Triton X-100 three times for 10min and incubated for 30min with EnVision+ HRP labeled secondary anti-rabbit antibodies (Dako). After this, sections were washed again and the signal was obtained using DAB+ Chromogen system (Dako). Finally, sections were rinsed with water, counterstained for 2min with Carazzi´s Hematoxylin solution DC (Panreac), dehydrated with increasing concentrations of alcohol and with Xylol and mounted using DePeX mounting medium (Gurr).

2.3. Immunofluorescence

Tissue sections were processed as described in previous paragraph, but without using hydrogen peroxide, and incubated with primary antibodies: rabbit anti-Mnk1 (Cell Signaling #2195, 1:200), goat anti-Cpa1 (RnD Systems AF2765, 1:100), rabbit anti-Cpa1 (AbD Serotec 1810-0006, 1:200), rabbit anti-Try (Abcam ab6193, 1:3000), rabbit anti-Muc1 (CT-1, 1:500) (Pemberton et al., 1992), mouse anti-E-Cad (BD Laboratories 610182, 1:1000). After washing, the sections were incubated with fluorescently labeled secondary antibodies (1:200): Cy3 anti-rabbit, Cy3 anti-mouse, Cy2 anti-goat (Jackson ImmunoResearch Laboratories, Inc.), Alexa 488 anti-rabbit (Invitrogen). Next, the sections were washed 2 times with PBS, incubated for 5min with DAPI (0.5ug/mL in PBS), washed again 1 time with PBS and mounted using ProLong® Gold Antifade Reagent (Life Technologies)

3. RNA ANALYSIS

3.1. RNA isolation

From mouse tissue samples:

Pieces of different mouse tissues were homogenized using T10 basic ULTRA-TURRAX homogenizer (IKA) in a guanidine thiocyanate buffer (4M Guanidine thiocyanate, 0.1M Tris-HCl, 1% 2-Mercaptoethanol, pH 7.5, prepared in a DEPC treated water) and total RNA was extracted using Phenol-Chloroform method.

From cell lines:

Total RNA from cultured cells was isolated using GenElute™ Mammalian Total RNA Miniprep Kit (Sigma-Aldrich). The material for isolation was collected either by cell trypsinisation and lysis of pelleted cells or by lysing the cells directly on the culture dish.

3.2. Reverse transcriptase and quantitative PCR (RT-qPCR)

Samples after total RNA isolation were subjected to DNase treatment using DNA-free™ DNase Treatment & Removal Reagents (Ambion). mRNA was transcribed with TaqMan® Reverse Transcription Reagents kit (Invitrogen) using 20ng of total RNA per 1ul of reaction mix. Quantitative PCRs were performed using SYBR® Green PCR Master Mix in the 7900 HT Real-Time PCR System (both Applied Biosystems). Expression of each mRNA was normalized with Hprt levels and calculated as fold change over control sample. Primers used to analyze mRNA expression levels are listed in Table 1.

Transcript	Forward	Reverse
Hprt	GGCCAGACTTTGTTGGATTTG	TGCGCTCATCTTAGGCTTTGT
Mnk1	GCAAATACGAGTTTCCTGAC	AGAGAGTCAAGTCCATGGTG
Mnk2	CTACTGACAGCTTCTCAGGC	CTTGACAGCATATTCCTGGT
eIF4E	AATCCCCCACCTGCAGAAGA	CGAAGGTTTGCTTGCCAAGT
hnRNPA1	GGCCGATGAAGGGAGGAAAC	TAGCTACTGCTGCTGCTGGA
Spry2	CCCAGCAGGTACACGTCTTG	GGCAGACCGTGGAGTCTTTC
Ptf1a	ACAAGCCGCTAATGTGCGAGA	TTGGAGAGGCGCTTTTCGT
Rbpjl	ATGCCAAGGTGGCTGAGAAAT	CTTGGTCTTGCATTGGCTTCA
Hnf1a	TAATAGGGCGGAGTGCAT	GGTCCGTTATAGGTGTCCAT
Nr5a2	CTGCTGGACTACACGGTTTGC	CTGCCTGCTTGCTGATTGC
Mist1	AGGGAGTGATCTGGGCCTTC	CTGGAGTCGTCCCTTAGCCA
Amy2	TGGCGTCAAATCAGGAACATG	AAAGTGGCTGACAAAGCCCAG
Cpa1	TACACCCACAAAACGAATCGC	GCCACGGTAAGTTTCTGAGCA
Cel	AAGTTGCCCGTGAAAAAGCAG	ATGGTAGCAAATAGGTGGCCG
Ela1	CGTGGTTGCAGGCTATGACAT	TTGTTAGCCAGGATGGTTCCC
Ctrb1	GCAAGACCAAATACAATGCCC	TGCGCAGATCATCACATCG
c-Myc	CCTAGTGCTGCATGAGGAGAC	CCTCATCTTCTTGCTCTTCTTCA
Ccnd1	GCATCTACACTGACAACTCT	GATGGTCTGCTTGTTCTCAT
Krt7	CACGAACAAGGTGGAGTTGGA	TGTCTGAGATCTGCGACTGCA
Krt19	CCTCCCGAGATTACAACCACT	GGCGAGCATTGTCAATCTGT

Table 1. List of primers used for RT-qPCR.

4. PROTEIN ANALYSIS

4.1. Pancreatic protein lysate preparation

Pieces of mouse pancreata were collected immediately after animal scarification, snap frozen in dry ice or liquid nitrogen and stored at -80°C. The frozen samples were lysed with ice-cold modified RIPA buffer (50mM Tris-HCl pH 7.4, 150mM NaCl, 1mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) with cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail (Roche), Phosphatase Inhibitor Cocktail 3 (Sigma-Aldrich) and 0.2mM orthovanadate. Next, they were sonicated with probe-type sonicator (30

pulses), centrifuged 15min 15 000g and the supernatants were collected and stored at -80°C. Protein concentration in the supernatants was determined with Bradford method using BioRad Protein Assay Solution (BioRad).

4.2. SDS-PAGE-western blotting

Western blotting samples were prepared by mixing the homogenates with 5x concentrated sample buffer, boiled for 5min at 100°C. 50µg of total protein (or 2µg in case of Amy2, Cpa1 and Cel expression analysis) was separated on polyacrilamide gel (10-12%) and transferred to nitrocellulose membranes by electroblotting. The membranes were blocked with 5% milk in Tween-containing Tris-buffered Saline (TBS-Tween) (4mM Tris base, 100mM NaCl, Tween-20 0.1%, pH 7.5), washed in TBS-Tween, incubated for 2h with the indicated primary antibodies (in TBS-Tween with 5% milk), washed again and incubated for 1h with HRP-labeled secondary antibody. Signal was revealed with the Amersham™ ECL™ Western Blotting Detection Reagents (GE Healthcare) and bands were visualized using Amersham™ Hyperfilm ECL™ (GE Healthcare). Identification of the band of interest was assessed by size as compared with Dual Color Precision Plus Protein™ Standard (BioRad) molecular weight marker. Quantitation of protein expression was determined by densitometry analysis of digitalized images using ImageJ software (NIH, Bethesda, MD,). Levels of analysed proteins were normalized against levels of actin or vinculin proteins and represented as relative fold change over levels in control samples.

Antibodies used were: rabbit anti-Mnk1 (#2195, 1:1000), p-Mnk1 (#2111, 1:500), eIF4E (#9742 1:750), p-eIF4E (#9741, 1:500), Erk1/2 (#9101, 1:1000) and p-Erk1/2 (#9101, 1:500) (Cell Signaling), rabbit anti-Ptf1a (B. Bréant, U. Paris VI, Paris, France, 1:400), mouse anti-Vinculin (V9131, 1:4000) and β-Actin (A2228, 1:4000) and rabbit anti-Amy2 (A8273, 1:100) (Sigma-Aldrich), rabbit anti-Cpa1 (1810-0006, 1:1000) and mouse anti-Ctrb1 (2100-0657, 1:1000) (AbD Serotec), rabbit anti-Cel (ab87431, 1:1000) (Abcam), rabbit anti-c-Myc (06-340, 1:400) and mouse anti-Ras (07-524, 1:2000) (Millipore), mouse anti-Ccnd1 (DCS-6, 1:500) (Thermo Scientific).

4.3. Chromatin Immunoprecipitation followed by quantitative PCR (ChIP-qPCR)

Chromatin Immunoprecipitation in pancreatic extracts was performed as described (Beres et al., 2006). Briefly Pancreas pieces were homogenized using Dounce homogenizer and intact nuclei were extracted by centrifugation in sucrose gradient. Next, chromatin was crosslinked to proteins with 1% formaldehyde for 10min at 30°C (the reaction was stopped by adding glycine to final concentration of 125mM). The crosslinked DNA was washed and sonicated using Biorupotr bath sonicator (Diagenode Inc.). Immunoprecipitation was performed at 4°C, over-night, with A agarose beads using around 100ug of chromatin per 1ug of anti-Ptf1a antibody. Precipitated protein-DNA complexes were decrosslinked, DNA was purified using QIAquick PCR Purification Kit (QIAGEN) and quantitative PCRs were performed as described in 3.2 using primers listed in Table 2.

Genomic region	Forward	Reverse
***Rbpjl* prom**	TGCTGGGTCTGGCTTCTACT	CCGATCCTCACACTGGATTT
Ptf1a prom	TGTGTTATGATTCCCACGGACT	GAGCCTAGAGATGGGCTGTG
***Ela1* prom**	TTGACTTAAAATTTGTTCATTTGT	ACCCTCTTTATACGGCTCTT
***Cpa1* prom**	CCATGGTCAAGGGTGAAAGC	TCTGGGGCCTTTTTAAACAC
***Ctrb1* prom**	GCTGGCCACTACCAATGTTC	CTGAGGCTCTTTTATGTCCC
***R28S* prom**	CTGGGACATAGTGGGTGCTT	GAGCCTAGAGATGGGCTGTG
***Mknk1* -175bp**	CGTTCTGGCTCGCAGAAGTAA	CAGGCGTGGCATATCAGAGC
***Mknk1* +1kb**	TGTTGACCACAGGCCTTGTG	CCCTTTAGAAGGCCCGAAGC
***Mknk1* +1.8kb**	CATGGTACAGGACTGGCACAT	GGGCCCTCCTTAGCCATGAA
***Mknk1* +3.9kb**	ATGCCTGCATGACCAGATTCC	TCCCCCAGTGTGCTATGCTC
***Mknk1* +5.6kb**	GGGTTTCCCACACCTGTCTC	CCTTGCTTGCAACTCTTGCTG
***Mknk1* +8.6kb**	AACAGCACCATGCCTACAGAA	GGGTAACAGTGCCAGCAAGA
***Mknk1* +9.9kb**	TTGCCAGGCAGGTTTTCTGT	GTGGGGAGCAGGGGAAGAT
***Mknk1* +11.3kb**	AGACAGTCCTCTGACGGAGC	AGGTGACAATCTCCTCCCGT

Table 2. List of primers used for ChIP-qPCR.

5. BIOCHEMICAL ASSAYS

5.1. Assessment of pancreatic amylase activity

Pancreas pieces were homogenized in ice-cold Sodium Phosphate Buffer (50mM NaH_2PO_4, 120mM NaCl, pH 7.3) with 0.01% Soybean trypsin inhibitor (Sigma) using T10 basic ULTRA-TURRAX homogenizer (IKA). Next, Triton X-100 was added to final concentration of 0.2% and the homogenates were incubated for 20min at 37°C. Lysates were mixed, centrifuged 10sec at 15000g. Amylase activity was measured using Reflotron® Pancreatic Amylase system (Roche) in lysates diluted 1/200 in saline. Amylase activity was calculated as Units (U) related to DNA content, measured using Nanodrop (Thermo Scientific) in concentrated samples.

5.2. Assessment of serum amylase activity

Mouse blood samples (not more than 50ul) were collected from Jugular vein of non-anesthetized animals, allowed to coagulate for 2h at room temperature and were centrifuged for 10min at 200g in order to separate serum from coagulates. Serum samples were transferred to separate tubes and amylase activity was measured using Reflotron® Pancreatic Amylase system (Roche).

5.3. Measurement of pancreatic trypsin activity

Pancreas pieces were homogenized using T10 basic ULTRA-TURRAX homogenizer (IKA) in ice-cold Calcum Chloride Buffer (5 mM $CaCl_2$, pH 8), snap frozen on dry ice and sent frozen to the laboratory of Julia Mayerle, MD at the Department of Medicine A, Ernst-Moritz-Arndt-University Greifswald, Geifswald, Germany where they were analyzed. The trypsin activity was measured by fluorometric enzyme kinetic over 1h at 37°C using rhodamine-110-Ile-Pro-Arg (Invitrogen) as a substrate and was related to protein content as assessed by Bradford assay (BioRad).

5.4. Carboxypeptidase activation

Procarboxypeptidase to carboxypeptidase activation was assessed using western blotting and band densitometry as described in 4.1 and 4.2 by measuring the abundance of cleaved form of this enzyme (clCpa1) represented by a band of a higher polyacrylamide gel mobility (35kDa as opposed to 47kDa of a precursor form) immunodetected with rabbit anti-Cpa1 antibody (1810-0006, 1:1000) (AbD Serotec) in

2ug of pancreatic extracts. Levels of the cleaved form were normalized against levels of actin protein and represented as relative fold change over basal levels in control samples.

5.5. Protein content assessment

Protein concentration was quantitated using Bradford assay (BioRad) in lysates prepared for pancreatic amylase activity (see 5.1) and was calculated as mg of protein per mg of DNA which concentration in samples was assessed using Nanodrop (Thermo Scientific).

6. ACINAR CELL ISOLATION AND FUNCTIONAL ANALYSIS

6.1. Mouse pancreatic acini isolation and measurement of amylase release by acinar cells in vitro

Amylase release by isolated mouse acini was analyzed using procedure described by John A. Williams (Williams, 2010b). Briefly, mouse pancreatic acini were isolated by NB8 collagenase (1mg/pancreas) (Serva) digestion in DMEM medium (with high glucose, L-glutamine, pyruvate, 100µg/mL soybean trypsin inhibitor and 1% fetal bovine serum) (Sigma), preincubated for 30min at 37°C and treated with different concentrations of caerulein (Sigma) for 30min at 37°C. After the treatment acini were collected and centrifuged and the amylase activity was measured both in medium and in cell pellets using Phadebas® Amylase Test (Magle AB). Amylase release was quantitated as a percentage of total amylase in acinar cells.

6.2. Measurement of trypsin and cathepsin B activity and cell death in acinar cells in vitro

Trypsin and cathepsin B activities were measured in isolated acinar cells by fluorometric enzyme kinetic as described in Sendler et al., 2012. Briefly, isolated acini were incubated with or without CCK (10nM) for maximum of 60min and were collected prior to the treatment and every 20 min during the treatment. Trypsin activity was measured using 10µM rhodamine-110-Ile-Pro-Arg and cathepsin B activity using 20µM AMC-Arg2 (Invitrogen). Cell death was quantified by propidium iodide (Sigma) exclusion as described in Wartmann et al., 2010. All parameters were normalized by

DNA content of live cells as assessed by propdium iodide exclusion after lysing the cells with 0.1% Triton X-100 for 30min at 37°C.

7. CELL CULTURE

7.1. Cell lines

All the cells used in this work were cultured in Dulbecco´s modified Eagle´s medium, supplemented with 10% fetal bovine serum (Sigma), penicillin/streptomycin and sodium pyruvate (Gibco). The cell lines used were available in the Epithelial Carcinogenesis Group.

7.2. Hydrogen peroxide (H_2O_2) treatment of 266-6 cells

266-6 cells were seeded in 60mm culture dishes and allowed to grow until they reached 70% of confluence. At this point, culture medium was replaced with fresh medium containing 1mM H_2O_2. After 30min the treatment medium was removed and fresh culture medium was added for further cell incubation. Cells were collected for protein analysis before or at different time-points (15min, 30min, 1h, 2h, 4h, 8h or 16h) after adding H_2O_2. Cell collection involved quick wash with PBS and lysis in 300µl of RIPA buffer (50mM TRIS-HCL pH8, 150mM NaCL, 5mM EDTA, 0,5% NP-40, 0,1% SDS) for 5min on culture dishes after which the cells were scraped with 1.8cm-blade Cell Scrapers (BD Falcon) and transferred into 1.5 mL eppendorf tubes.

7.3. Mnk1 interference in 266-6 cells using lentiviral vectors

Mnk1 expression was interfered using MISSION® shRNA lentiviral constructs (Sigma): TRCN0000024433, targeting CAGAAGCGGAAGCACTTCAAT sequence (for the purpose of simplicity called “sh1”) and TRCN0000024430, targeting GAGATGCAAACCCATGTTT sequence (“sh2”). Control cells were transformed using scrambled vector (“shNT”). To prepare lentiviral particles HEK293-FT cells were used. Briefly, HEK cells were seeded in 150mm culture dishes, allowed to reach 70-80% of confluence and transfected with 45µg of shNT, sh1 or sh2 plasmids together with 30µg of psPAX2 (packaging) and 10µg of pCMV-VSVG (envelope) helper plasmids. Transfection was performed using calcium phosphate for 16h, after which the culture medium was replaced with 14mL of fresh medium. After 2 days of virus production this medium was collected, filtered through 0.45µm sterile filter and added to 266-6 cells growing in 10mm culture dishes (at 50-

60% of confluence). After 2 days the infection medium was removed and fresh medium was added. After 1 day of recovery from the infection 266-6 cells were trypsinized and plated in fresh medium containing puromycin (2μg/ml) (Sigma-Aldrich) to select for the infected cells. After 2 days of selection the medium was replaced and the next day cells were trypsinized and divided into 2 tubes – for RNA and protein isolation. Total RNA was isolated and mRNA levels of genes of interest were assessed by quantitative RT-PCR as described in 3.2. Protein extracts were prepared by lysing the cells in RIPA buffer and protein expression was analyzed by western blotting as described in 4.2.

7.4. Ectopic expression of $Kras^{G12V}$ in 266-6 cells using pBabe-puro retroviral vector

266-6 cell retroviral infection was performed similarly to the described above lentiviral infection with the exception that HEK293-FT cells were transfected with 45μg of the pBabe-puro empty vector or pBabe-puro-$Kras^{G12V}$ plasmid together with 45μg of pCL-ECO helper plasmid.

7.5. Induction of *$Kras^{G12V}$* expression in acinar cells of embryonic mouse pancreas

$Kras^{G12V}$ expression from endogenous promoter specifically in embryonic pancreatic acinar cells was induced by intraperitoneal injection of 4-hydroxytamoxifen in pregnant *$Ptf1a^{+/CreERT2}$;$Kras^{+/G12Vgeo}$* mice at E17.5. The females were sacrificed 2 days later and embryonic pancreata were collected and processed for either RNA or protein extraction. RNA was extracted as described in 3.1 and quantitative PCR on genes of interest was performed as describe in 3.2. Protein extraction and western blotting were performed as described in 4.2.

8. STATISTICAL ANALYSES

Data are provided as mean±SEM. Statistical analyses were performed using two tailed Student's test and $p<0.05$ was considered significant. For all statistical analyses VassarStat.net software was used.

Results

1. Mnk1 is a novel acinar-specific stress response kinase in the pancreas

1.1. Identification of Mnk1 as a gene highly expressed in the pancreas

Genes potentially involved in acinar cell biology were identified by screening the Genepaint server (www.genepaint.org) that provides online data of *in situ* hybridization performed on whole mouse embryo sections. After searching for genes expressed at high levels specifically in the embryonic pancreas, several candidates were found from which - after initial analyses - Mnk1 was chosen to be the focus of this thesis (see discussion). *In situ* hybridization images provided by Genepaint showed high expression of Mnk1 in the embryonic pancreas (Fig. 1A). To confirm high-level pancreatic expression, immunohistochemistry using anti-Mnk1 antibody was performed. Fig. 1B shows that, among mouse embryonic tissues, pancreas displayed the strongest Mnk1 immunoreactivity.

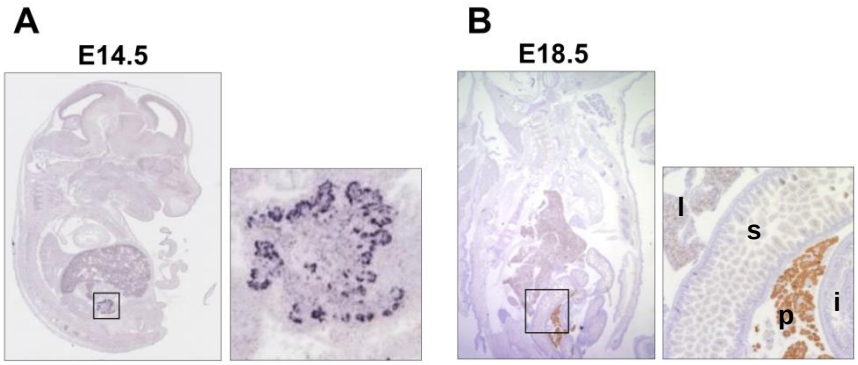


Figure 1. Mnk1 is highly expressed in mouse embryonic pancreas. Mnk1 expression in mouse embryo as shown by *in situ* hybridization at E14.5 stage (A) and immunohistochemistry at E18.5 stage (B). Squares show higher magnification pictures of embryonic pancreas. p, pancreas; s, stomach; i, intestine; l, liver.

Next, Mnk1 expression in adult mouse tissues was investigated using RT-qPCR. Mnk1 mRNA expression was 20-30 fold higher in the pancreas than in any other investigated tissue (Fig. 2A). In agreement with these data, Mnk1 protein was also most abundant in the pancreas among all organs tested (pancreas, lung, liver, lymph node, bladder, kidney, ovary) (Fig. 2B). The second strongest signal intensity was observed in the lymph node, however it was much lower than the signal detected in the pancreas. Transcripts coding for Mnk2 and for the known Mnk1/2 targets (eIF4E, Hnrnpa1, Spry2) were not overexpressed in the pancreas comparing to other tissues. Of interest, Mnk2 mRNA

levels were high in salivary glands and lymph nodes and they correlate with increased expression of eIF4E and Spry2 in these organs (Fig. 2C).

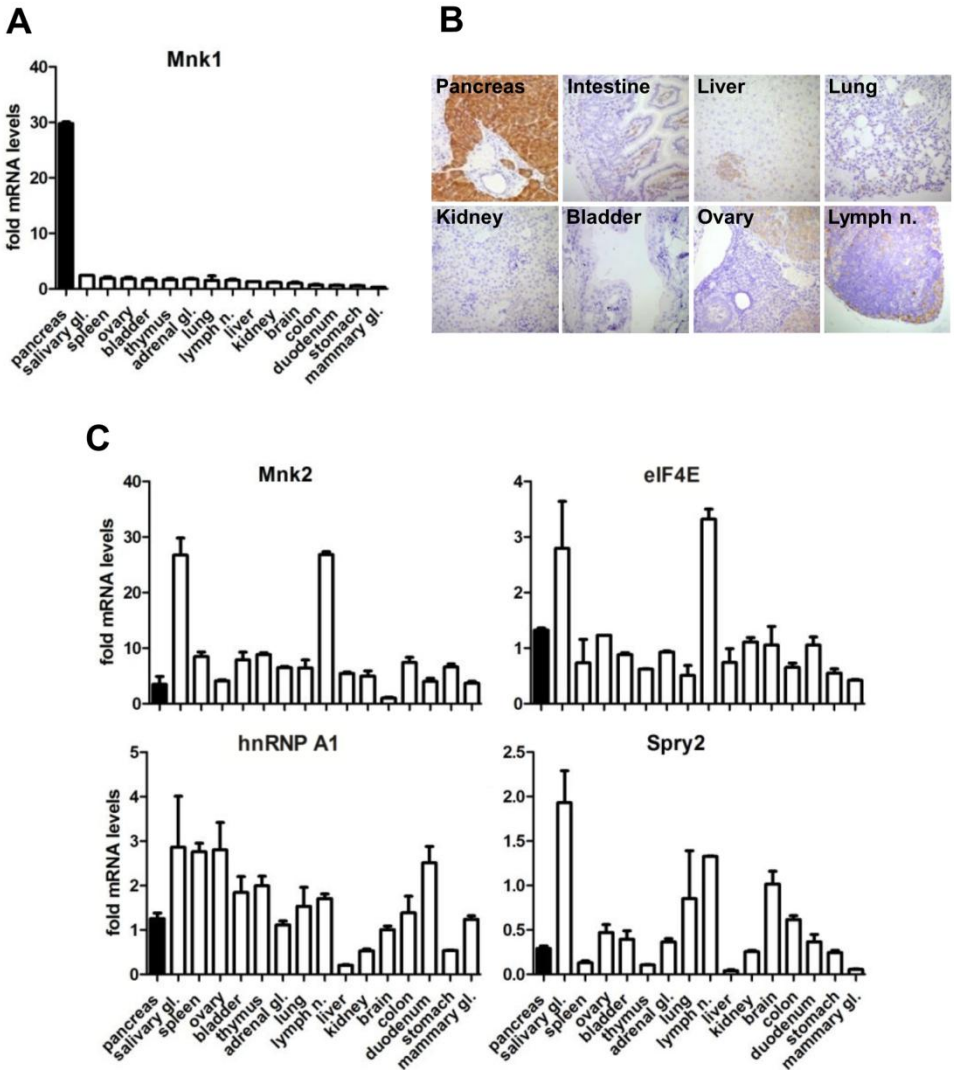


Figure 2. Adult pancreas expresses exceptionally high levels of Mnk1. (A) Mnk1 expression in a panel of adult mouse tissues as assessed by RT-qPCR. (B) Immunohistochemistry showing Mnk1 expression in different mouse organs. (C) mRNA expression of Mnk2, eIF4E, hnRNPA1 and Spry2 in adult mouse tissues as assessed by RT-qPCR. mRNA expression represented as mean±SEM (n=3).

1.2. Mnk1 is embedded in the acinar transcriptional program

Mnk1 expression during mouse pancreas development was investigated in more detail. As shown in Fig. 3A, at E12.5 Mnk1 protein was detected in pancreatic multipotent precursors (Cpa1^{low} and Amy2^{-}); later, at E16.5, the signal was restricted to acinar precursors (Cpa1^{high} and Amy2^{high}). In the adult pancreas Mnk1 was detected exclusively in acinar cells and was absent from islets and ducts. To analyse Mnk1 mRNA expression dynamics during pancreas development, RT-qPCR was performed. Mnk1 mRNA

expression increased progressively following a pattern that was highly similar to that of the digestive enzyme transcripts (Fig. 3B). In contrast, Mnk2 mRNA levels remained essentially unchanged during mouse pancreas development.

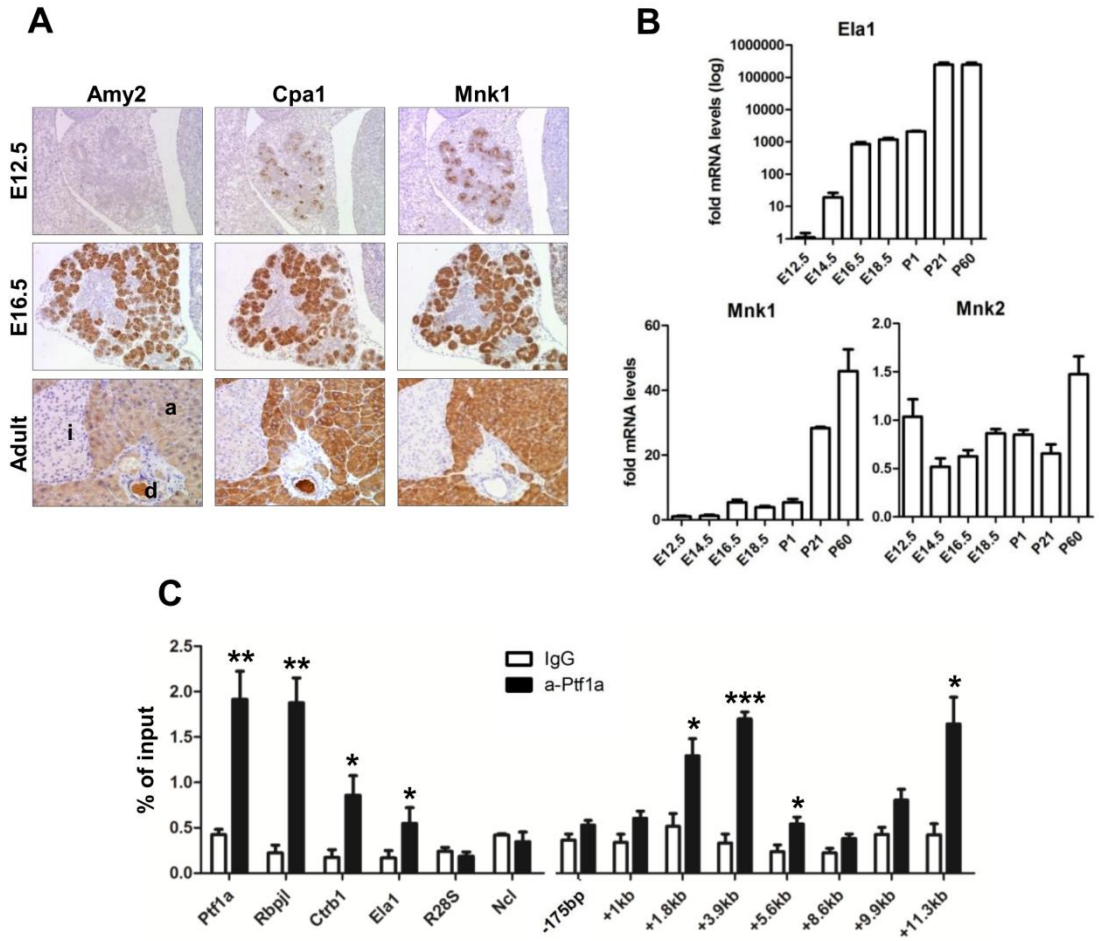


Figure 3. Mnk1 is a novel marker of acinar cells. (A) Amy2, Cpa1 and Mnk1 expression during mouse pancreas development as assessed by immunohistochemistry. a, acinar cells; d, duct;, i, islet of Langerhans. (B) Ela1, Mnk1 and Mnk2 mRNA expression during mouse pancreas development as assessed by RT-qPCR. mRNA expression presented as fold levels related to expression at E12.5 (n=3). (C) Ptf1a binding to E-boxes as assessed by ChIP-qPCR. Ptf1a enrichment was quantified in genomic regions of *Ptf1a*, *Rbpjl, Ctrb1* and *Ela1* (positive controls); *R28S* and *Ncl* (negative controls); and in putative Ptf1a binding sites localized at different positions from TSS of *Mknk1*. All graphs represent mean±SEM (n=3 mice), *p<0.05, **p<0.01, ***p<0.001.

The fact that Mnk1 expression is highly restricted to acinar cells and follows a dynamics similar to that of digestive enzyme transcripts suggested that Mnk1 might be a PTF1 target. To address this possibility, Ptf1a ChIP-seq data obtained by R. MacDonald were mined and several peaks were found within *Mknk1;* 7 high scoring putative PTF1 binding sites (containing an E-box and a proximal TC-box) were located +1kb, +1.8kb,

+3.9kb, +5.6kb, +8.6kb, +9.9kb, and +11.3kb downstream from transcription start site (TSS). As shown in Fig. 3C, Ptf1a occupancy of these sites was confirmed by ChIP-qPCR. The 3 sites with the strongest signal correspond to the 3 top-scoring ChIP-seq peaks. Canonical PTF1 binding sites have been reported to occur proximal to promoter regions up to 300bp upstream from TSS (Beres et al., 2006) and a weak signal at -175bp was also detected by ChIP-qPCR (Fig. 3C).

In summary, Mnk1 is a direct PTF1 transcription target, expressed at exceptionally high levels in the pancreas and – therefore – it is a novel acinar cell marker.

1.3. Mnk1 expression and activation in mouse pancreas upon experimental caerulein-induced acute pancreatitis

Mnk1 has been described to be involved in different types of cellular stress response. However, its role in the pancreas has not been reported. Given its prominent expression in acinar cells, we analyzed expression and activation during acute, self-resolving, caerulein-induced pancreatitis. Consistent with published data, Ptf1a mRNA levels were down-regulated by 80% 8h after the first caerulein injection and were gradually restored during the recovery period (1, 3 and 5 days after treatment) (Fig. 4A). In agreement with this observation and prior reports (Molero et al., 2012), mRNA levels of acinar enzymes (Amy2, Cpa1) were also down-regulated reaching a nadir 1 day after the first caerulein injection. Mnk1 mRNA expression dynamics followed the same pattern as acinar enzyme mRNAs, consistent with the finding that Mnk1 is a PTF1 target.

Mnk1 protein expression dynamics and activation in response to caerulein were assessed by western blotting (Fig. 4B). Total Mnk1 protein was first gradually down-regulated (reaching the lowest level 1 day after treatment) and then completely restored by day 7. The MAPK-Mnk1-eIF4E pathway was activated in response to caerulein treatment: an increase in p-Erk1/2, p-Mnk1 and p-eIF4E was observed as early as 30 min after the first cerulein injection. Phosphorylation of these proteins was highest at 4h and was still relatively very high at 8h. At 24h, p-Erk1/2 signal had reversed to basal levels but increased phosphorylation of both Mnk1 and eIF4E could still be detected (Fig. 4B). Additionally, using antibodies specific for p-Mnk1, a strong increase in signal corresponding to a protein of approximately 39kDa was detected. This signal was highest at 30min and 4h and could still be detected at 8h, possibly reflecting

phosphorylation of the shorter isoform of Mnk1 – Mnk1b (O´Loghlen et al., 2004). Similarly to Mnk1, eIF4E protein levels were rapidly down-regulated and then restored during the recovery phase (Fig. 4B). Although the changes in Mnk1 protein levels can, at least in part, be a consequence of mRNA down-regulation, the same does not apply for eIF4E, since its transcript levels were unchanged during acute pancreatitis (data not shown).

Caerulein administration also led to changes in the intracellular localization of Mnk1 (Fig. 4C). In basal conditions, Mnk1 displayed a uniform cytoplasmic distribution; by contrast, 4h and 8h after the first caerulein injection, Mnk1 predominantly localized to the basolateral region of acinar cells. This localization was not observed 1 day after the treatment, when down-regulation of Mnk1 protein expression occurred. 2 days post-pancreatitis a strong Mnk1 signal was detected in the perinuclear region of acinar cells. At 7 days, Mnk1 distribution in the cells was uniform, as it was prior to the treatment (Fig. 4C).

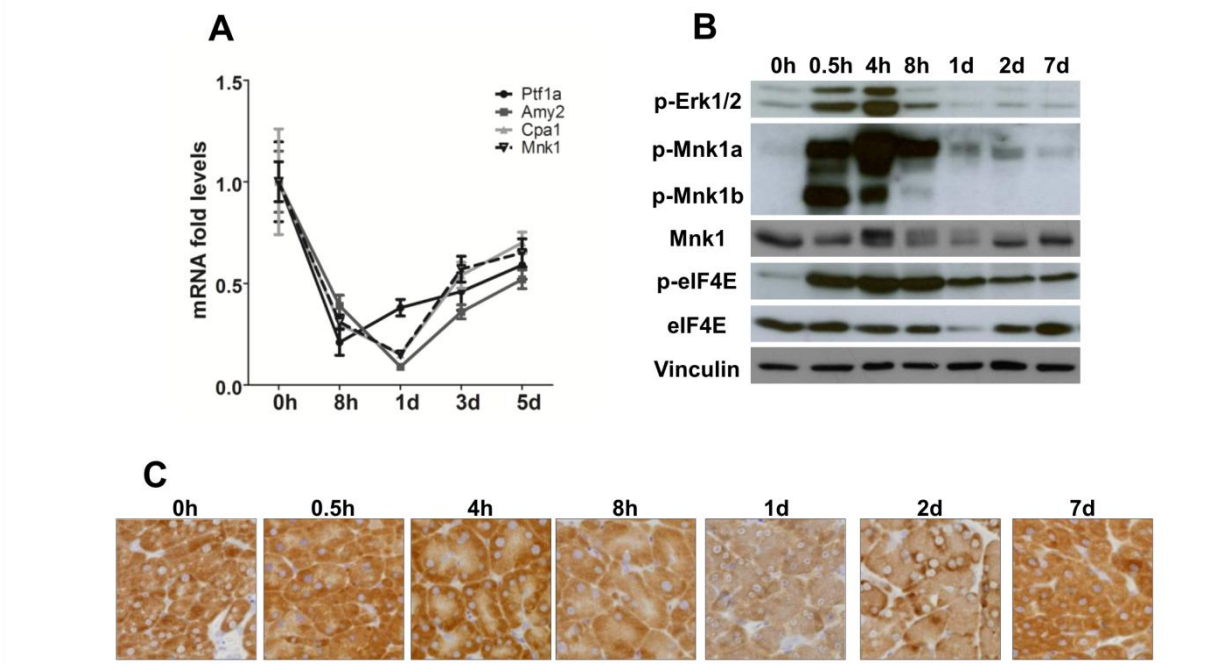


Figure 4. Mnk1 expression and activity are regulated upon caerulein-induced acute pancreatitis. (A) mRNA expression in mouse pancreas during experimental acute pancreatitis – AP as assessed by RT-qPCR. Data represented as mean±SEM (n=3 mice). (B) Immunoblot depicting pancreatic protein expression and phosphorylation upon AP in mouse. (C) Immunohistochemistry showing Mnk1 protein distribution in mouse pancreatic acinar cells during pancreatitis induction. 0h, 0.5h, 4h, 8h, 1d, 2d, 5d, 7d – experimental time-points (h – hours after first injection, d – days after the treatment).

2. Mnk1 is dispensable for pancreas development but plays a protective role in pancreatitis regulating secretory response

2.1. The pancreas of *Mnk1*$^{-/-}$ mice has increased enzymatic content and decreased expression of p-eIF4E targets

In order to investigate the role of Mnk1 in the pancreas, Mnk1$^{-/-}$ mice were analysed. Lack of Mnk1 expression in the pancreas was confirmed by immunohistochemical staing (Fig. 5A) and western blotting (Fig. 6A). In the absence of Mnk1, the pancreas developed normally and was histologically indistinguishable from that of wild type mice (Fig. 5B). However, as assessed by quantitative RT-PCR (Fig. 5C), *Mnk1*$^{-/-}$ pancreata showed increased (1.5 to 2-fold) levels of mRNAs coding for transcription factors Ptf1a and Rbpjl, and Hnf1α and for digestive enzymes Amy2, Cpa1, Cel, Ela1, and Ctrb1. By contrast, the levels of Mist1 and Nr5a2 were unchanged.

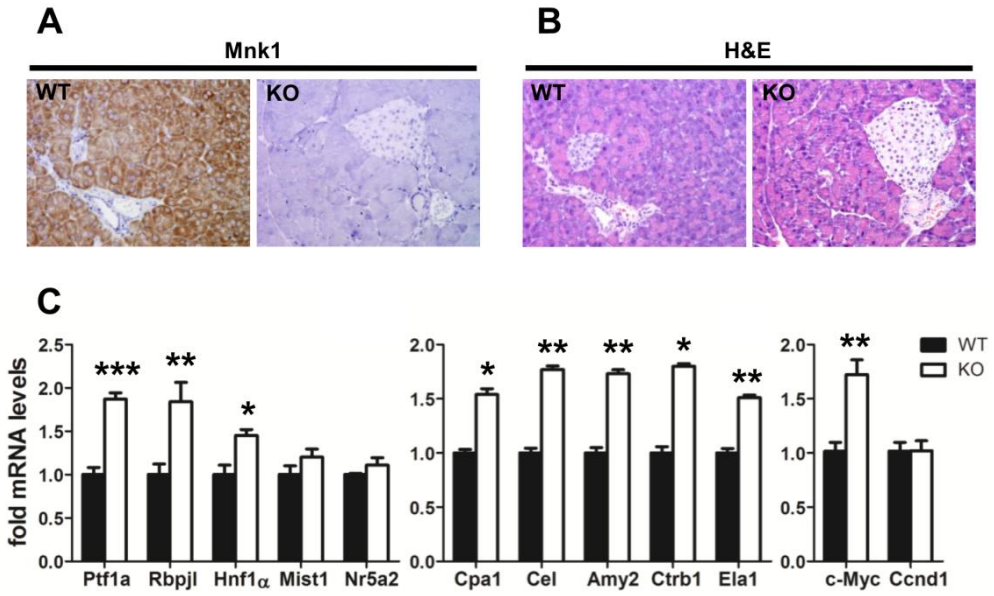


Figure 5. *Mnk1*$^{-/-}$ mouse pancreas has increased levels of acinar gene transcripts. (A) Immunodetection of Mnk1 in pancreatic sections from *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice. (B) Hematoxylin-eosin staining of WT and KO mouse pancreatic sections. (C) mRNA expression of acinar transcription factors, digestive enzymes, c-Myc and Ccnd1 in pancreata of WT and KO mice as assessed by RT-qPCR. Data represented as mean±SEM (n=6 mice), *p<0.05, **p<0.01, ***p<0.001.

Protein levels of selected gene products were assessed by western blotting and densitometry (Fig. 6A and B). Although no significant difference in Ptf1a protein abundance was observed, levels of 3 analysed digestive enzymes were higher in the

pancreas of *Mnk1*$^{-/-}$ *mouse* (Cel, 170%; Cpa1, 140%; and Amy2, 125%). A similar 25% increase in amylase activity was observed in pancreatic lysates from independent group of mice (Fig. 6C). In order to assess whether the above described differences in protein expression affect pancreatic protein content in *Mnk1*$^{-/-}$ mouse pancreas, protein/DNA ratio was calculated. As shown in Fig. 6D, lack of Mnk1 did not affect total protein content levels.

In normal conditions, low levels of pancreatic digestive enzymes are detected in plasma what is thought to reflect both their production in the pancreas and leakage from acinar cells. In order to assess whether basal pancreatic enzyme release to the plasma is affected by the absence of Mnk1, serum amylase activity was measured. As shown in Fig. 6E, serum amylase activity was 15% lower in *Mnk1*$^{-/-}$ mice than in wild type controls, despite the higher amylase content.

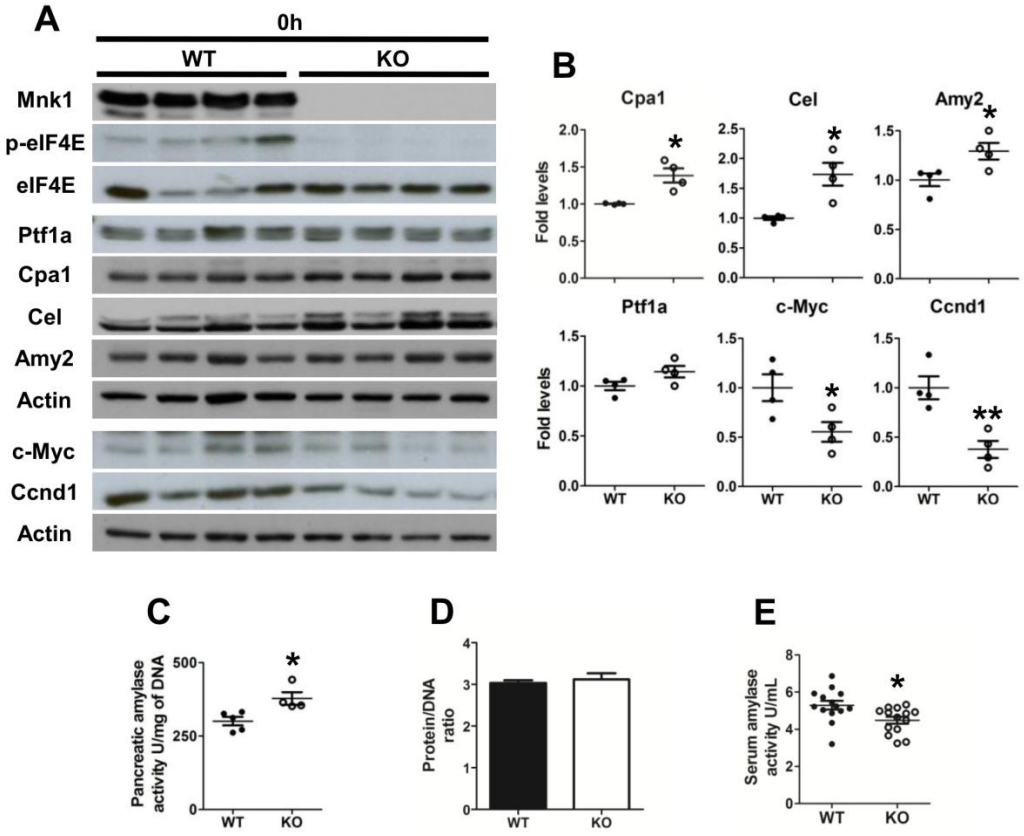


Figure 6. *Mnk1*$^{-/-}$ mouse pancreas has increased enzymatic content and decreased expression of p-Eif4e targets. (A) Immunoblot depicting pancreatic protein expression and phosphorylation in *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice. (B) Cpa1, Cel, Amy2, Ptf1a, c-Myc and Ccnd1 expression in WT and KO mouse pancreata as assessed by western blotting band densitometry; related to WT. (C) Pancreatic amylase activity represented as U/mg of pancreatic DNA. (D) Relation between pancreatic protein and DNA content (n=5 for WT and 6 for KO). (E) Amylase activity in sera from WT and KO mice (n=14). Data represented as mean±SEM, *p<0.05, **p<0.01.

Given, that the best characterized Mnk1 substrate is eIF4E, its expression and phosphorylation in pancreata from *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ mice was compared by western blotting (Fig. 6A). Total eIF4E protein levels were not affected by the lack of Mnk1 but its basal phosphorylation was lower in the KO pancreas. Next, abundance of c-Myc and Ccnd1, two proteins the biosynthesis of which is known to be regulated by p-eIF4E, was analysed. Consistently, the levels of both proteins were lower in the Mnk1 null mouse pancreas (Fig. 6A and B) while the abundance of their transcrips was not: mRNA levels of Ccnd1 were unchanged and of those of c-Myc were higher in *Mnk1*$^{-/-}$ mice.

We conclude that Mnk1 positively regulates prosurvival proteins (p-eIF4E targets) such as c-Myc and Ccnd1 and negatively regulates acinar-specific digestive enzymes.

2.2. Mnk1 is required for eIF4E phosphorylation and participates in the regulation of cell proliferation during acute pancreatitis.

As shown above, pancreatitis-induced phosphorylation of Mnk1 was associated with strong eIF4E phosphorylation and no basal p-eIF4E was decreased in the absence of Mnk1. Thus, we analysed whether stress-related phosphorylation of eIF4E in the pancreas depends on Mnk1. Fig. 7 shows that in *Mnk1*$^{-/-}$ mouse pancreas, pancreatitis induced p-eIF4E increase was completely abrogated.

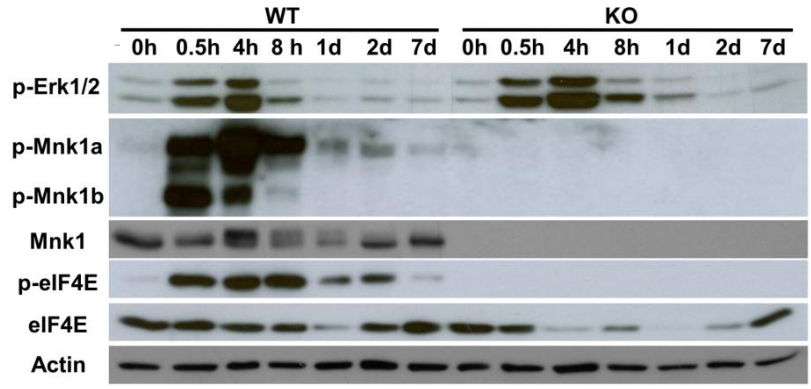


Figure 7. Mnk1 is required for eIF4E phosphorylation upon acute pancreatitis. Immunoblot depicting pancreatic protein expression and phosphorylation in *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice upon experimental acute pancreatitis (AP). 0h, 0.5h, 4h, 8h, 1d, 2d, 7d – experimental time-points (h – hours after first injection, d – days after the treatment).

Next, we assessed pancreatitis-associated expression regulation of two known p-eIF4E target proteins, c-Myc and Ccnd1. As it was shown above, the levels of these proteins were lower in untreated *Mnk1*$^{-/-}$ mice. In wild type mice, acute pancreatitis led to increased c-Myc protein levels in the pancreas (5-fold at 8h and 4-fold at 1day); in

contrast, Ccnd1 protein expression was progressively down-regulated (Fig. 8A and B). In the absence of Mnk1, the induction of c-Myc expression was markedly impaired (2.5-fold at 8h and 2-fold at 1day with respect to untreated). In the case of Ccnd1 there was no significant difference between *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ mice at 8h. After 1day, regardless the strong decrease observed in wild type, Ccnd1 protein levels were significantly lower in pancreas of mice lacking Mnk1. Importantly, *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ mouse pancreata had similar levels of c-Myc and Ccnd1 mRNA at 8h and 24h (Fig. 8C).

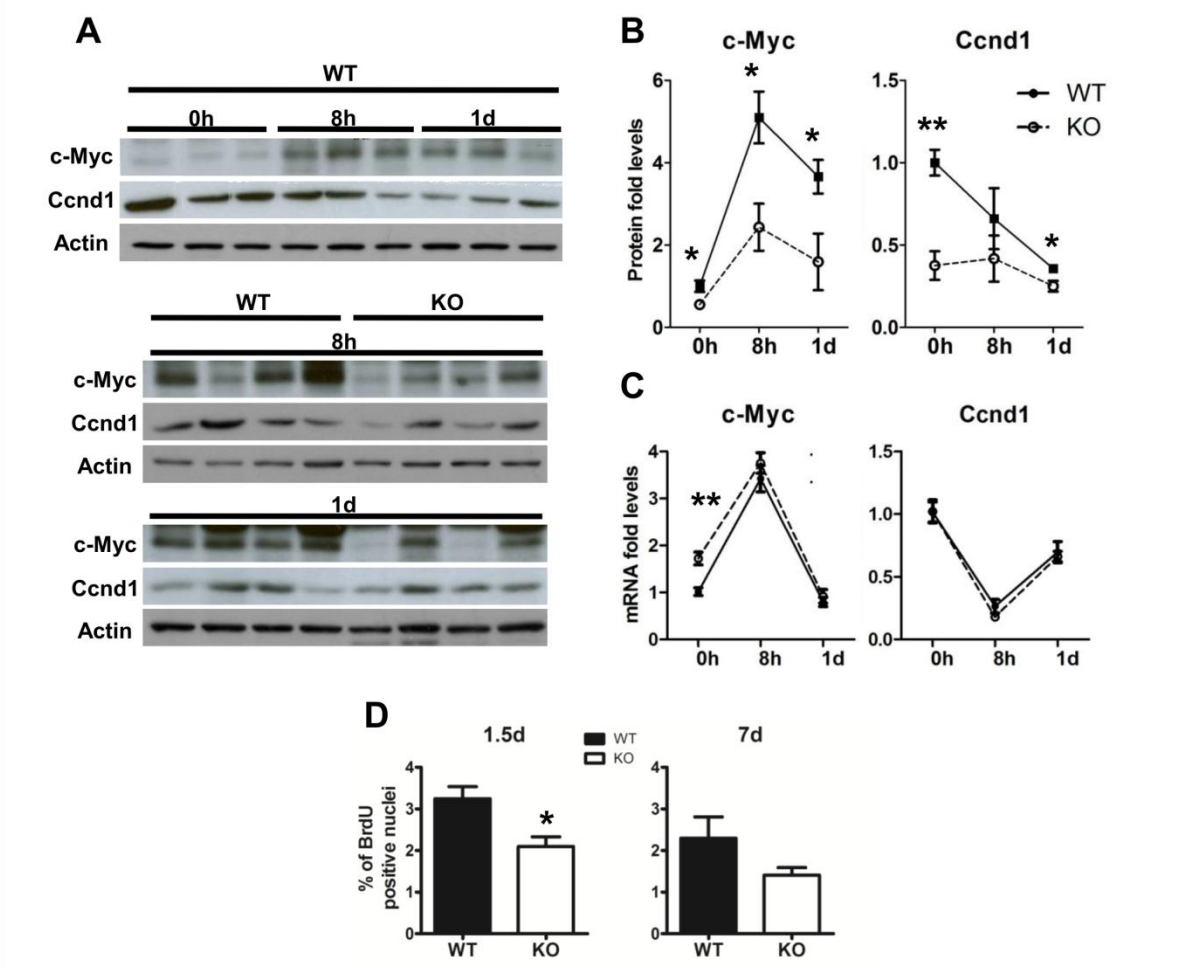


Figure 8. Mnk1 is involved in induction of c-Myc upon upon acute pancreatitis. (A) Immunoblot showing protein levels in *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice upon experimental acute pancreatitis (AP). (C-D) c-Myc and Ccnd1 protein and mRNA levels in WT and KO mice upon AP. (C) Western blotting band densitometry related to expression in WT at 0h. (D) RT-qPCR (n=6). (E) Percentage of BrdU positive nuclei detected by immunohistochemistry in pancreatic sections from WT and KO mice at 1.5 (n=6 for WT, 5 for KO) and 7 days (n=5) after pancreatitis induction. 0h, 8h, 1d, 1.5d, 7d – experimental time-points (h – hours after first injection, d – days after the treatment). Data represented as mean±SEM, *p<0.05, **p<0.01.

It has been described that regeneration from the damage induced during acute pancreatitis is associated with the proliferation of a small fraction of acinar cells (Lechene de la Porte et al., 1991). Because the pancreas of *Mnk1*$^{-/-}$ mice had lower levels of pro-proliferatory proteins upon pancreatitis, we asked whether this could affect acinar cell proliferation rate during the recovery stage. As assessed by BrdU nuclear labeling, acinar cell proliferation was lower in the absence of Mnk1 (Fig. 8D). In wild type mouse pancreas, 3.2%+/-0.3 and 2.2%+/-0.6 BrdU^{+} cells were detected at 1.5 and 7 days post-pancreatitis. Significantly less BrdU^{+} cells were found in *Mnk1*$^{-/-}$ mice pancreata at the same time points: 2.1%+/-0.2 (p=0.015) and 1.4%+/-1.5 (p=0.14) (Fig. 8D).

Thus, during pancreatitis, Mnk1 regulates proliferation through p-eIF4E target protein translation.

2.3. In response to caerulein, *Mnk1*$^{-/-}$ mice display a more severe acute pancreatitis

Given that Mnk1 was highly phosphorylated as a consequence of IP caerulein administration, we investigated the effect of lack of Mnk1 on the severity of caerulein-induced acute pancreatitis.

As it was mentioned before, a common strategy to assess the severity of pancreatitis is measurement of amylase activity in the serum. Consistent with published data (Saluja et al., 2007) serum amylase activity increased with subsequent caerulein injections (Fig. 9A). Wild type mice showed a 6-fold increase in amylase activity 8 h after the first injections, whereas in *Mnk1*$^{-/-}$ mice a much higher (12-fold) increase was observed (p value = 0.0023). After 1 day, amylase serum levels were still significantly higher (2-fold with respect to untreated animals) in *Mnk1*$^{-/-}$ than in *Mnk1*$^{+/+}$ animals (1.3-fold higher than controls) (p value = 0.002). This increase in amylase leakage in *Mnk1*$^{-/-}$ mice was transient and was not detected after 2 days, when amylase activity had returned to basal levels. At this stage, amylase levels were consistently slightly lower in *Mnk1*$^{-/-}$ than in *Mnk1*$^{+/+}$ mice (Fig. 9A).

In parallel, pancreatic injury and regeneration were assessed by histopathology. There were no major histological differences between the pancreata of *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ mice (Fig. 9B and C). As measured by histological scoring, inflammation and edema were

present to similar extents during the acute phase (at 8h and 1 day) and during late recovery stage (7 days) in both mouse strains. However, after 2 days a significant increase in inflammatory cell infiltration (predominantly PMN cells) was detected in pancreata of *Mnk1*$^{-/-}$ mice. This result was confirmed by immunostaining and quantification of MPO^{+} cells (11.8+/- 1.6 cells/field in *Mnk1*$^{-/-}$ vs. 7.9+/-0.9 in *Mnk1*$^{+/+}$ mice; p=0.038) (Fig. 9D).

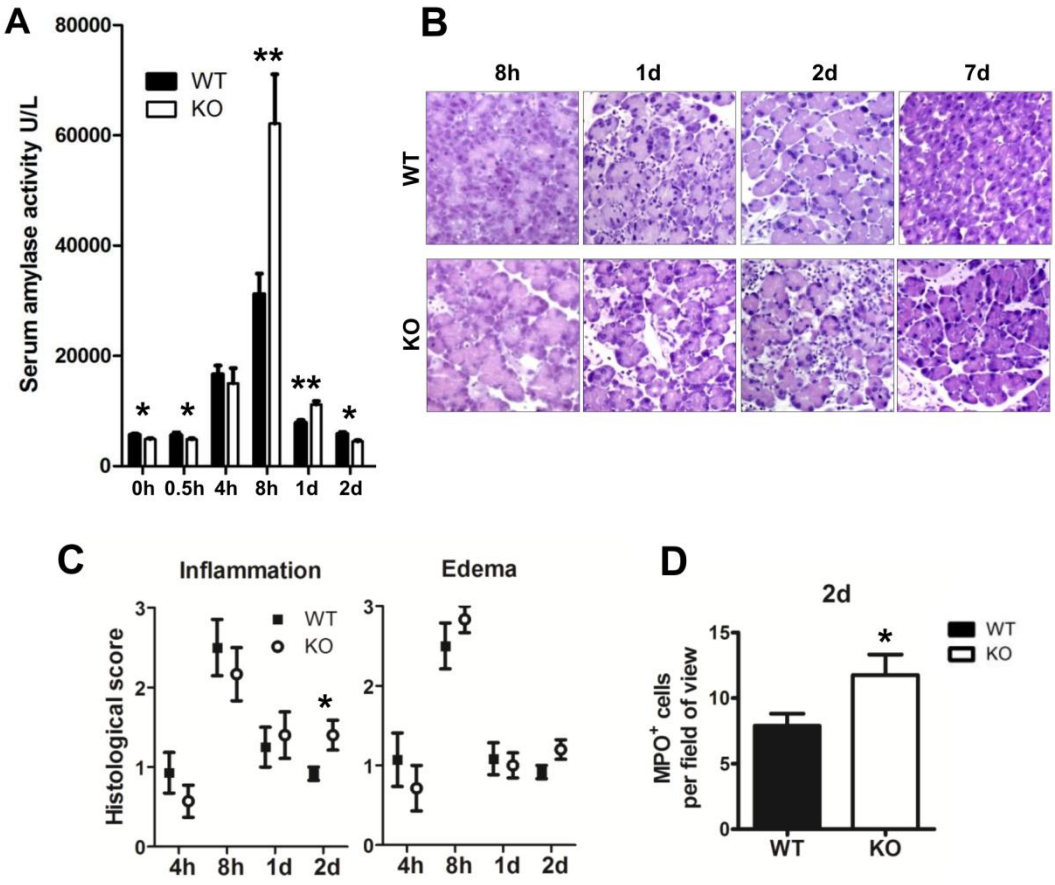


Figure 9. Caerulein-induced acute pancreatitis is more severe in *Mnk1*$^{-/-}$ mice. (A) Serum amylase activity in *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice during the course of experimental acute pancreatitis (AP) represented as U/mL of mouse serum (n=14 at 0h, n=4 at 0.5h, n=6 at 4h, n=6 at 8h, n=6 at 1 day, n= 6 at 2days). (B) Hematoxylin-eosin staining of WT and KO pancreatic sections upon AP. (C) Histological score of inflammation and acinar cell edema in hematoxylin-eosin-stained pancreatic sections from WT and KO mice upon AP (n=6 at 4h, n=3 at 8h, n=6 at 1day, n=5 at 2days). (D) Number of MPO^{+} cells per field of view (100x) in sections of pancreata collected 2 days after AP induction (n=6 for WT and n=5 for KO). 0h, 0.5h, 4h, 8h, 1d, 2d, 7d – experimental time-points (h – hours after first injection, d – days after the treatment). Data represented as mean±SEM, *p<0.05, **p<0.01.

Next, the involvement of Mnk1 in pancreatitis-induced regulation of mRNAs coding for acinar transcription factors (Ptf1a, Rbpjl, Hnf1α, Mist1, Nr5a2) (Fig. 10A) and digestive enzymes (Amy2, Ela1, Cpa1, Ctrb1, and Cel) was analysed (Fig. 10B). Despite the fact

that basal levels of Ptf1a, Rbpjl, Hnf1α, Amy2, Ela1, Cpa1, Ctrb1, and Cel transcripts were higher in *Mnk1*$^{-/-}$ mice, during the early stages of acute pancreatitis (8h) mRNA expression of these genes was more rapidly down-regulated than in wild type mouse pancreas, reaching similar levels. At later time points, a more sluggish overall recovery of levels of these transcripts was observed in *Mnk1*$^{-/-}$ mice; in some cases the differences were statistically significant (Fig. 10).

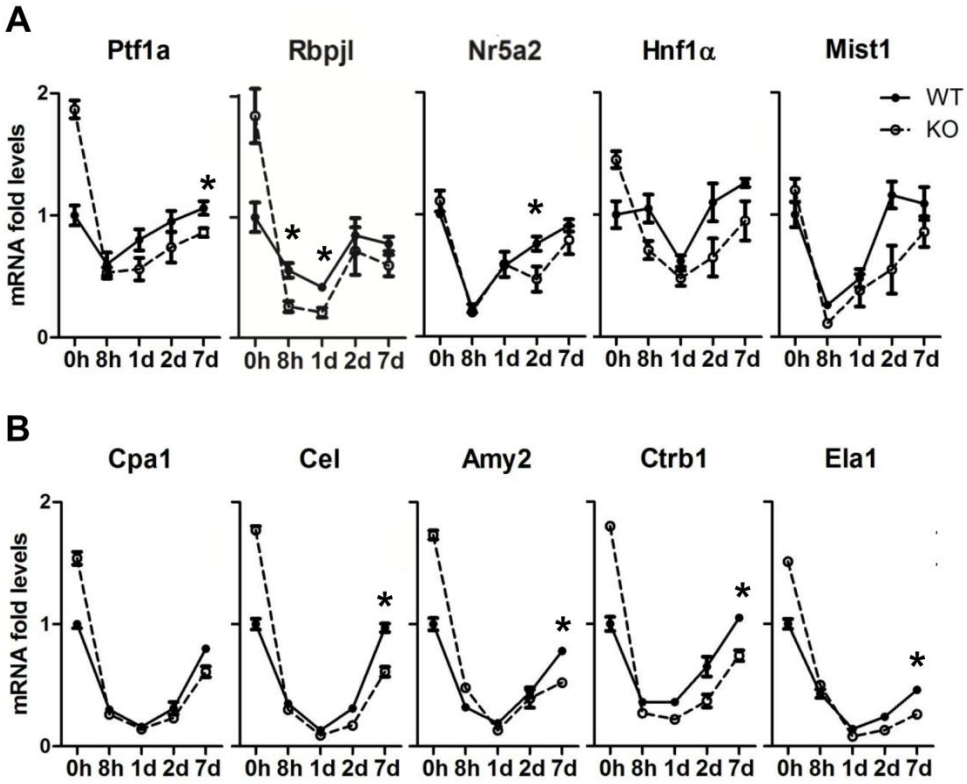


Figure 10. *Mnk1*$^{-/-}$ mice show stronger acute pancreatitis-associated down-regulation of acinar transcription program. Expression dynamics of (A) acinar transcription factors and (B) digestive enzymes in *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mouse pancreata during experimental acute pancreatitis as assessed by RT-qPCR. Data represented as mean±SEM (n=6 at 0h, n=4 at 8h, n=3 at 1day, n= 3 at 2days, n=5 at 7days) *p<0.05.. 0h, 0.5h, 4h, 8h, 1d, 2d, 7d – experimental time-points (h – hours after first injection, d – days after the treatment).

This greater downregulation of the acinar transcription program is not associated with lower levels of Ptf1a transcription factor. Its pancreatic abundance is similar in *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ mice during the course of acute pancreatitis (8h, 2 days and 7 days) (Fig. 11).

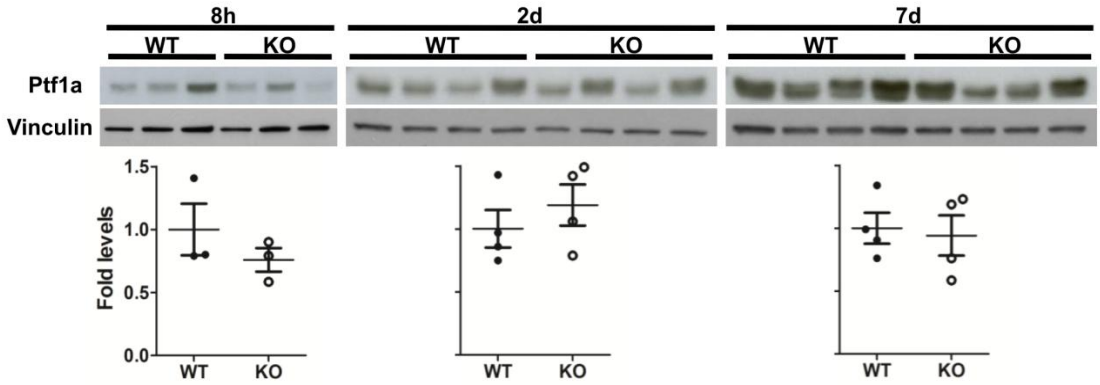


Figure 11. Lack of Mnk1 does not affect Ptf1a protein levels upon acute pancreatitis. Ptf1a expression in pancreata of *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice at different time-points of acute pancreatitis depicted by immunoblot (top panel) and represented as western blotting band densitometry related to expression in WT (graphs). 8h, 2d, 7d – experimental time-points (h – hours after first injection, d – days after the treatment).

Therefore, lack of Mnk1 leads to an increase in the severity of acute caerulein-induced pancreatitis, likely a reason for the stronger down-regulation of acinar specific transcription program.

2.4. Pancreatic enzymatic protein content in *Mnk1*$^{-/-}$ mouse is not properly down-regulated during acute pancreatitis

It has been described (Molero et al., 2012) that repeated IP caerulein administration leads to rapid down-regulation of amylase content in the mouse pancreas. Considering this and the greater decrease in levels of mRNA coding for the digestive enzymes in mice lacking Mnk1 during pancreatitis, we investigated the changes in pancreatic enzyme protein content upon caerulein treatment. Consistent with published results, Amy2, Cpa1 and Cel (0h, 8h and 1 day) were down-regulated by 30-40% in *Mnk1*$^{+/+}$ mice upon induction of pancreatitis (Fig. 12A, B); similarly, intrapancreatic amylase activity was reduced (0h and 8h) (Fig. 12C). In contrast, this down-regulation was not observed at 8h in *Mnk1*$^{-/-}$ mice, both using western blotting and amylase activity measurement. At 1 day both, wild type and *Mnk1*$^{-/-}$, mice showed highly reduced intrapancreatic enyzme levels. As shown in Fig. 12D the impaired reduction of digestive enzyme abundance in mice lacking Mnk1 at 8h was associated with higher protein/DNA ratio as compared to *Mnk1*$^{+/+}$ animals.

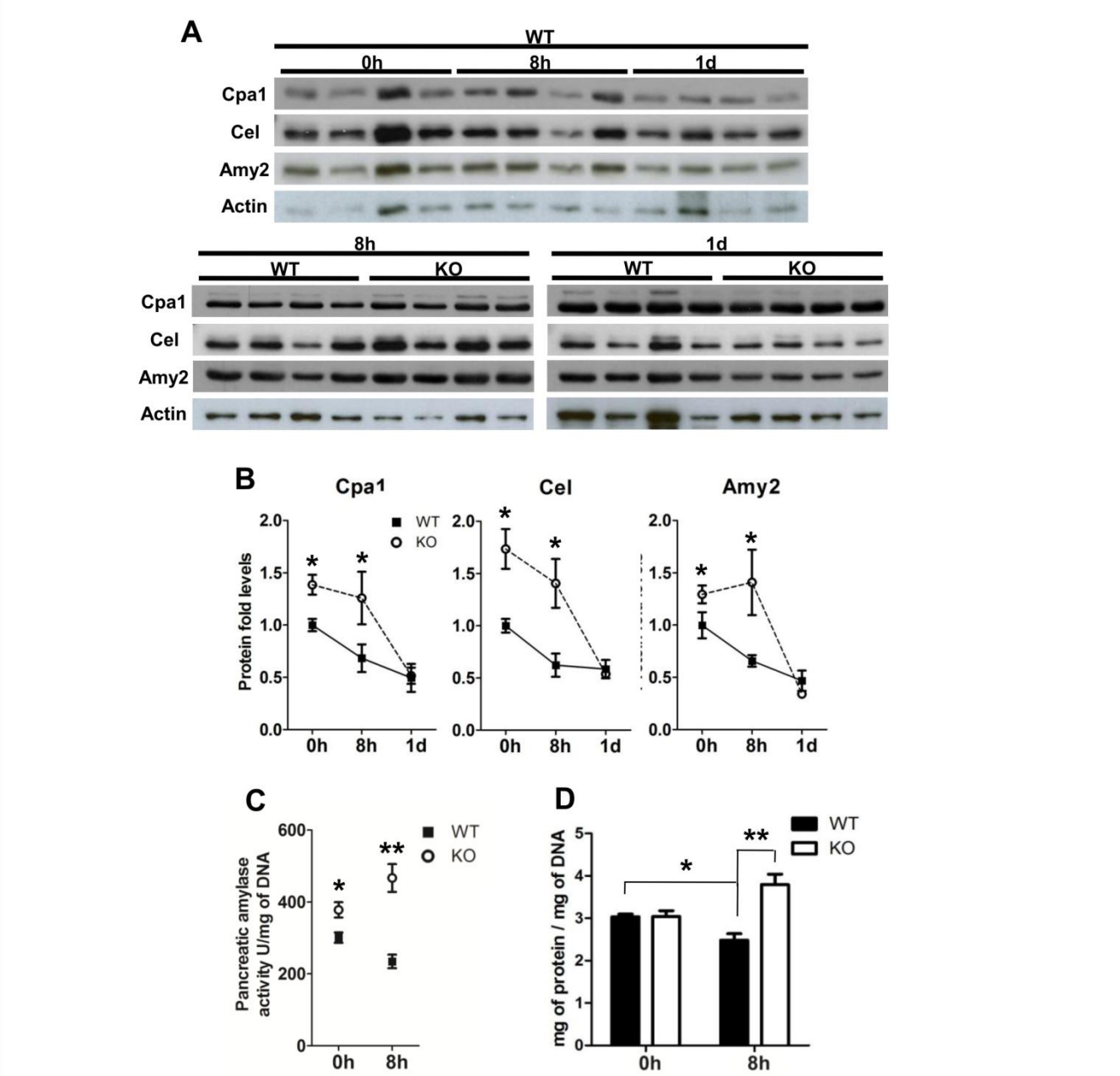


Figure 12. Down-regulation of enzymatic content upon acute pancreatitis is impaired in *Mnk1*$^{-/-}$ mouse. (A) Immunoblot depicting Cpa1, Cel, Amy2 and Actin protein levels in *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice upon acute pancreatitis (AP). (B) Cpa1, Cel, Amy2 expression upon AP as assessed by western blotting band densitometry. (C) Pancreatic amylase activity upon AP represented as U/mg of pancreatic DNA. (D) Pancreatic protein content upon AP related to DNA content (n=5 for WT and n=6 for KO). 0h, 0.5h, 4h, 8h, 1d, 2d, 7d – experimental time-points (h – hours after first injection, d – days after the treatment). Data represented as mean±SEM, *p<0.05, **p<0.01.

Next, we analysed whether impaired down-regulation of enzymatic content in the absence of Mnk1 affected zymogen activation. Both *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ showed similar pancreatic trypsinogen activation upon acute pancreatitis induction – elevated at 0.5h

after first injection, lower at 4h and again slightly elevated at 8h (Fig. 13A). Zymogen activation can also be measured by analyzing processing of procarboxypeptidase A1 that is cleaved by trypsin yielding a product of around 35kDa (clCpa1) (Grady et al., 1998). In wild type mice, the levels of clCpa1 increased 30 min after the first administration of caerulein and then progressively decreased (Fig. 13B and C). By contrast, in *Mnk1*$^{-/-}$ mice elevated clCpa1 levels were detected until 8h (Fig. 13B and C). By 1 day both in *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ pancreatic clCpa1 levels reached the baseline (data not shown).

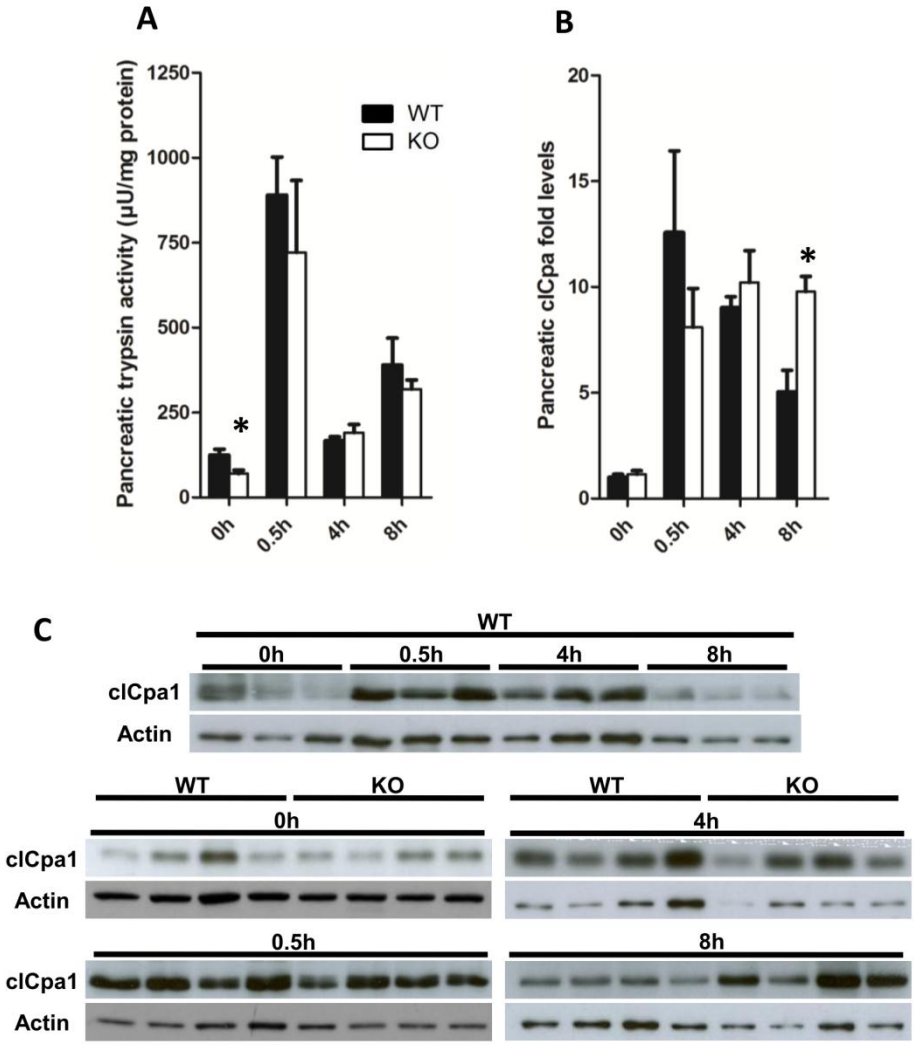


Figure 13. Caerulein induces pancreatic Cpa1 cleavage in mouse that is prolonged in the absence of Mnk1. (A) Trypsin activity in pancreata of *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice upon acute pancreatitis (AP) represented as µU/mg of pancreatic protein (n=5 at 0h, n=4 at 0.5h, n=3 at 4h, n=5 8h). (B) Pancreatic clCpa1 levels in WT and KO mice upon AP as assessed by western blotting band densitometry. (C) Immunoblot depicting clCpa1and Actin protein levels. 0h, 0.5h, 4h, 8h – experimental time-points (h – hours after first injection). Data represented as mean±SEM, *p<0.05.

In summary, Mnk1 negatively regulates enzymatic protein content and in *Mnk1*$^{-/-}$ mice pancreata zymogen abundance reduction in response to serial caerulein injections is impaired. This might be a reason for the increased severity of acute pancreatitis, possibly in part caused by elevated Cpa1 cleavage.

2.5. Mnk1 is required for subcellular redistribution of digestive enzymes upon caerulein-induced acute pancreatitis

As it was described above, Mnk1 is redistributed to the basolateral membranes of acinar cells upon caerulein administration (Fig. 4C). As shown by double immunofluorescence, Mnk1 and Cpa1 had mutually exclusive expression patterns in wild type mice (Fig. 14A). Serial caerulein administration led to the accumulation of Cpa1 in larger granules located towards the lumen of acinar cells at 4h and 8h; a similar distribution of trypsinogen (Try) was observed at 8h. By contrast, the subcellular redistribution of these enzymes did not occur in *Mnk1*$^{-/-}$ mice (Fig. 14B).

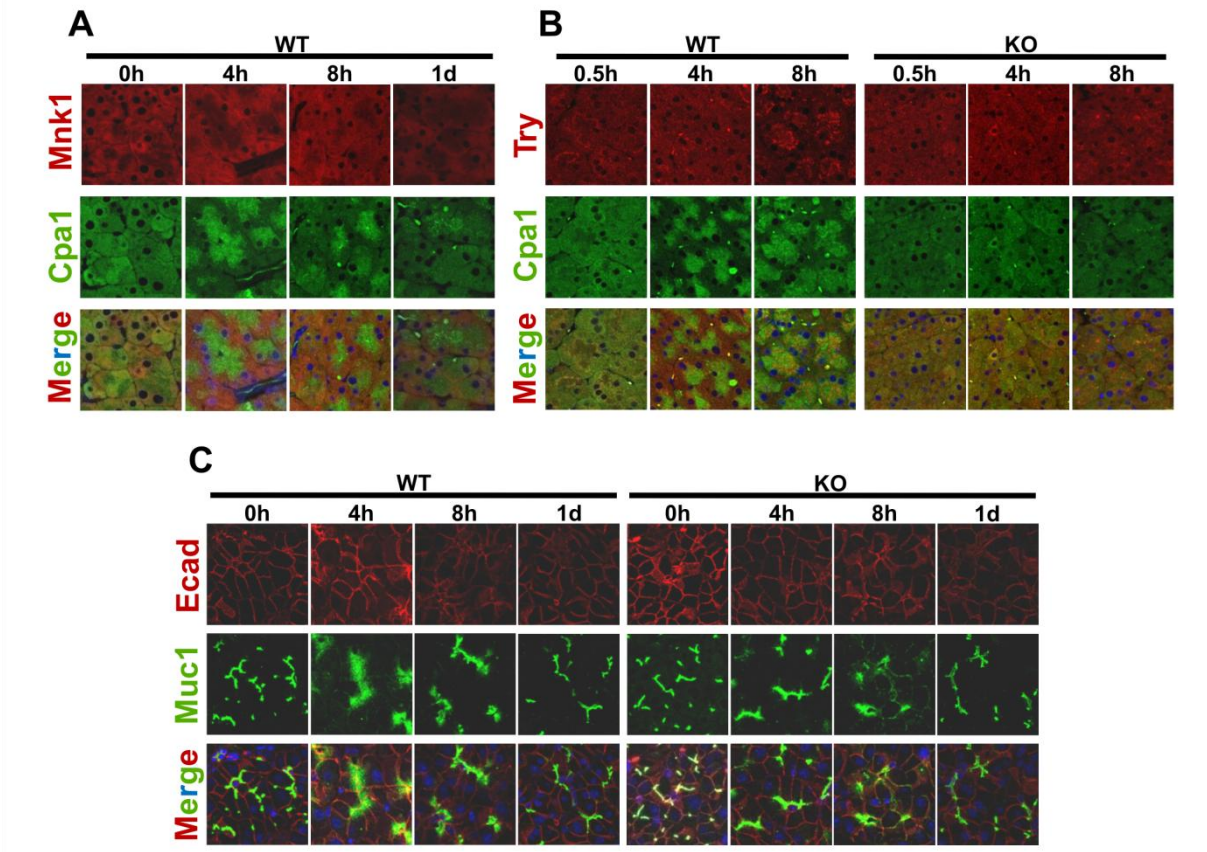


Figure 14. Pancreatitis associated subcellular distribution of pancreatic acinar enzymes is impaired in the absence of Mnk1. (A-C) Double immunofluorescence staining performed in *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice pancreatic sections. Images show immunolocalisation of: (A) Mnk1 (red) and Cpa1 (green) in WT, (B) Try (red) and Cpa1 (green) in WT and KO and (C) Ecad (red) and Muc1 (green) (C) in WT and KO mouse acinar cells.

The differences between wild type and *Mnk1*$^{-/-}$ mice described above suggest that Mnk1 is required for a physiological polarity response in acinar cells. Apical Muc1 and basolateral E-cad showed the expected distribution in untreated mice of both strains. In response to caerulein administration to wild type mice, Muc1 acquired a transient diffuse pattern (4h) with recovery of the normal apical distribution by 24h (Fig. 14C). In *Mnk1*$^{-/-}$ mice, these changes were less pronounced and showed a different kinetics: at 4h Muc1 staining pattern was less diffuse while at 8h it was more disorganized and partially associated with basolateral membranes marked by Ecad. By 24h, Muc1 displayed the normal apical distribution in both mouse strains (Fig. 14C). We did not observe any difference in E-cadherin distribution between *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ mice upon acute pancreatitis (Fig. 14C).

Therefore, in the absence of Mnk1, regulation of acinar cell polarity in response to caerulein-induced acute pancreatitis is altered, what may be a reason for different distribution of zymogens.

2.6. *Mnk1*$^{-/-}$ mouse acinar cells are less responsive to secretagogue stimulation in vitro

The increased enzymatic content in the pancreas of *Mnk1*$^{-/-}$ mice and the decreased amylase serum levels and the abnormal pattern of expression of membrane proteins upon caerulein administration suggested a defect in secretion. We therefore assessed the response of acinar cells isolated from wild type and *Mnk1*$^{-/-}$ mice to caerulein.

Isolation of acinar cells from *Mnk1*$^{+/+}$ mice was associated with hyper-phosphorylation of Mnk1 and eIF4E (Fig. 15A). The increase in p-eIF4E levels did not occur in cells from *Mnk1*$^{-/-}$ mouse.

In wild type cells, increasing caerulein concentrations resulted in a typical biphasic dose response curve with basal amylase release of approximatley 3.5%/30 min, optimal release for 100pM concentration (8%/30 min), and a fall of amylase secretion at supramaximal (1nM and 10nM) concentrations (Fig. 15B). Secretion by *Mnk1*$^{-/-}$ acinar cells yielded a similar dose-response but both basal (2.7%/30 min) and caerulein-induced (6.5%/30 min) secretion were significantly impaired (p value = 0.0303 and 0.0201 respectively).

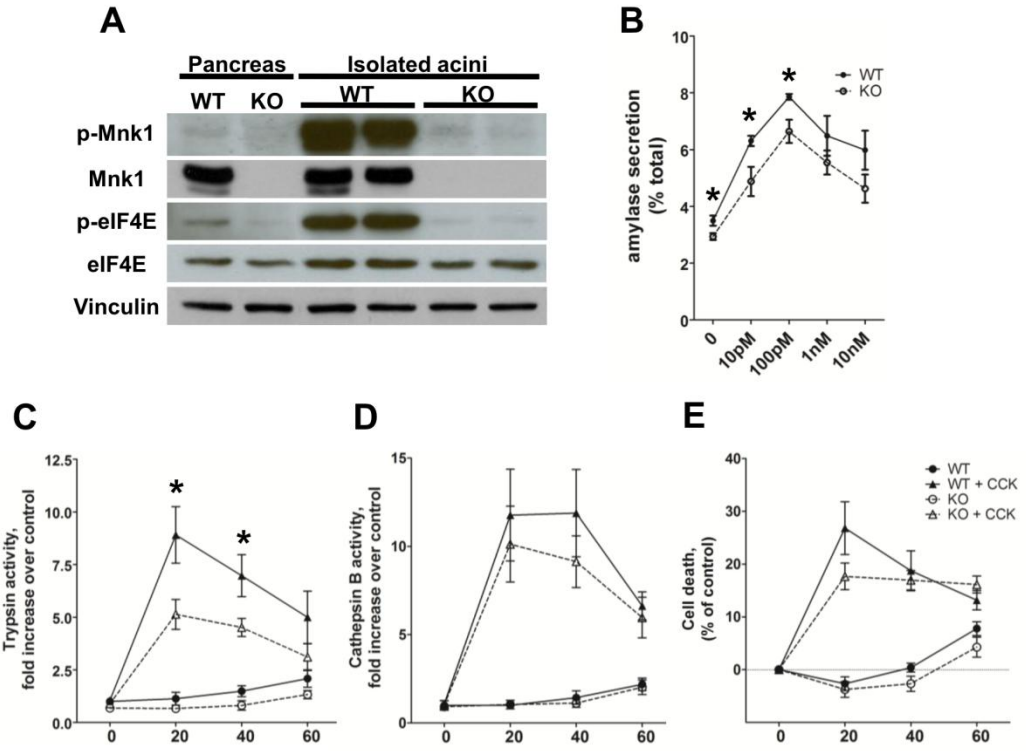


Figure 15. *Mnk1-/-* mouse acinar cells display suboptimal secretion capacity. (A) Immunoblot depicting protein levels and phosphorylation in pancreas or isolated acini from *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice. (B) Amylase release by isolated WT and KO mouse acinar cells upon stimulation with increasing concentrations of caerulein (0, 10pM, 100pM, 1nM and 10nM) represented as percentage of total amylase. (C-E) Trypsin activity, cathepsin B activity and cell death in isolated WT and KO mouse acinar cells upon stimulation with 10nM CCK for different time intervals (0, 20, 40 and 60 minutes). Trypsin and cathepsin B activity represented as fold increase over non-treated cells. Cell death represented as percentage of all cells. All data represented as mean±SEM (n=5), *p<0.05

Next, we analyzed whether lack of Mnk1 affected trypsinogen activation upon supramaximal secretagogue treatment (Fig. 15C). As reported (Sendler et al., 2013), incubation of wild type acini with 10nM CCK resulted in strong intracellular trypsinogen activation at 20 min which was gradually reduced at later time points. In *Mnk1*$^{-/-}$ acini, trypsinogen activation was significantly lower at 20 min (by 40%) and 40 min (by 30%); a similar trend was observed at 60 min. During the incubation there was no significant difference in trypsin activity between wild type and KO in non-stimulated cells. Trypsinogen activation in cultured acini is predominantly catalyzed by Cathepsin B, the activity of which increases upon treatment, but it is partially also a consequence of cell death. The lower trypsin activity in cells lacking Mnk1 cannot be explained by impaired intracellular CatB activation nor by decreased cell death because these parameters where similar in *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ acini (Fig. 15D and E).

In, summary acinar cells lacking Mnk1 respond worse to secretagogues: they release less amylase and display lower intracellular trypsinogen activation. It is possible that the increased severity of acute pancreatitis after serial caerulein administration in vivo (showed in Fig. 6) is a consequence of improper response of acinar cells at the beginning of the treatment. For instance, the enzymatic protein accumulation inside the cells during caerulein injection (showed in Fig. 9) may result from secretory defects suggested by the result of amylase release experiment.

2.7. *Mnk1*$^{-/-}$ mice display less ductal atrophy but reduced Ptf1a levels upon multiple episodes of acute pancreatitis

The observed impaired recovery of acinar specific gene expression in pancreata of *Mnk1*$^{-/-}$ mice after acute pancreatitis led us to analyse the outcome of a repetitive (once a week for 8 weeks) induction of acute pancreatitis. The recurrent treatment led to development of a mild chronic pancreatitis (CP) characterized by chronic inflammation and presence of metaplastic tubular complexes (TCs).

In *Mnk1*$^{-/-}$ mice, edema and inflammatory cell infiltration were not significantly different from *Mnk1*$^{+/+}$ mice upon chronic damage (Fig. 16A). However, pancreata from animals lacking Mnk1 contained a lower number of TCs as assessed by histological score (Fig. 15A).

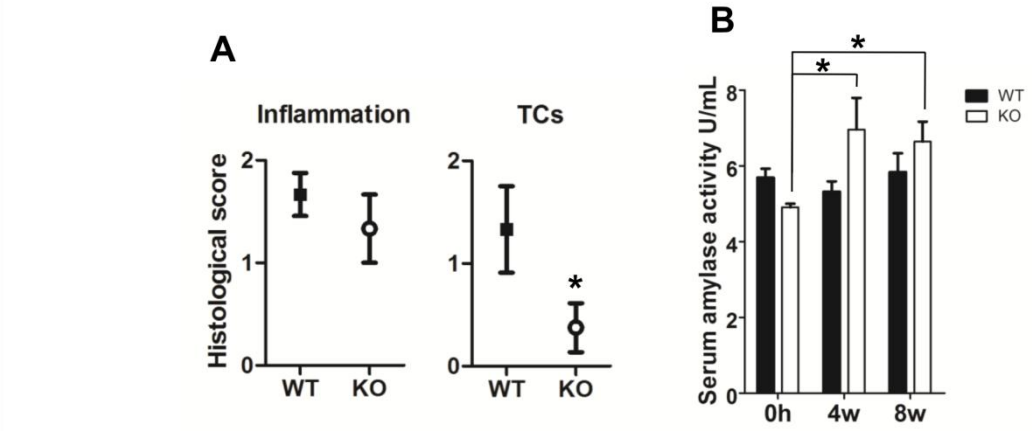


Figure 16. Mice lacking Mnk1 develop less tubular complexes after multiple episodes of acute pancreatitis. (A) Histological score of inflammation and tubular complexes (TCs) in pancreatic sections from *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice upon chronic pancreatitis (CP) stained with hematoxylin-eosin. (B) Serum amylase activity in WT and KO mice upon CP represented as U/mL. Data represented as mean±SEM (n=5 for WT and n=4 for KO), *p<0.05

Serum was collected after 4 weeks of treatment and at the time of sacrifice (ca. 9 weeks). In wild type animals no increase in serum amylase was observed (Fig. 16B). On the other hand, *Mnk1*$^{-/-}$ mice showed elevated (by 20%) levels of serum amylase with respect to untreated controls both at 4 and 8 weeks.

Upon chronic stress induction in *Mnk1*$^{+/+}$ mice, both Mnk1 and eIF4E were phosphorylated, unlike in *Mnk1*$^{-/-}$ mice (Fig. 17A). Interestingly, total eIF4E protein levels were 50% lower in pancreata of mice lacking Mnk1 than in those of wild type animals (Fig. 17A and B).

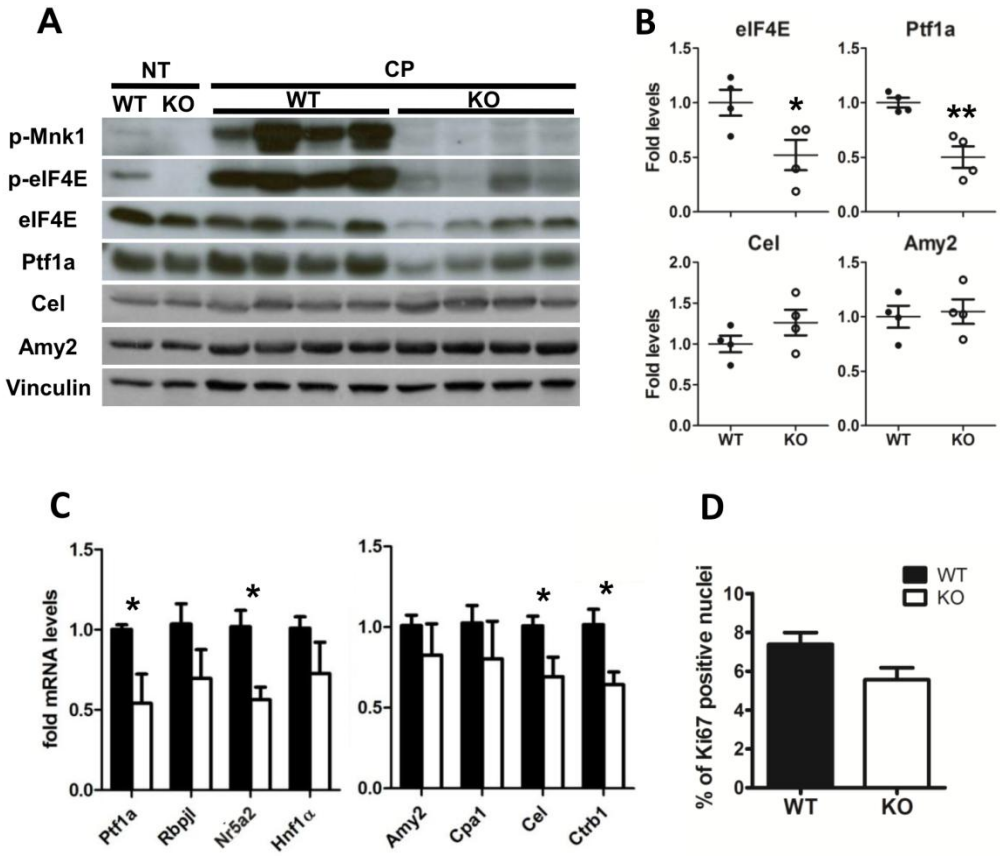


Figure 17. *Mnk1*$^{-/-}$ mice express less Ptf1a protein upon mild chronic pancreatitis. (A) Immunoblot depicting protein expression and phosphorylation in pancreata *Mnk1*$^{+/+}$ (WT) and *Mnk1*$^{-/-}$ (KO) mice upon mild chronic pancreatitis (CP). (B) Protein expression of eIF4E, Ptf1a, Cel and Amy2 in WT and KO mice upon CP as assessed by western blotting band densitometry related to WT. (C) mRNA levels of acinar transcription factors and digestive enzymes in WT and KO mouse pancreata upon CP as assessed by RT-qPCR related to WT. (D) Percentage of Ki67 positive nuclei in pancreatic sections from WT and KO mice upon CP. Data represented as mean±SEM (n=5 for WT and n=4 for KO), *p<0.05, **p<0.01.

Next we assessed the pancreatic expression of acinar specific markers in *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ mice upon CP. Importantly the levels of Ptf1a protein were 50% lower in the latter than in the former (Fig. 17A and B). This difference was probably due to lower abundance of Ptf1a transcripts (by 50%) (Fig. 17C). mRNA levels of some other acinar specific genes were also lower in *Mnk1*$^{-/-}$ mice with most apparent, statistically significant differences observed for Nr5a2, Cel and Ctrb1 (by 30% to 40%) (Fig. 17C). Interestingly protein levels of Cel and Amy2 (Fig. 17A and B) and Cpa1 (not shown) were unchanged (see discussion).

In contrast to untreated animals and to mice challenged with acute pancreatitis in which Mnk1 loss was associated with lower expression of c-Myc and Ccnd1, upon CP no difference in protein levels of these genes was observed (data not shown). This is in agreement with a lack of differences in acinar cell proliferation (Fig. 17D).

To conclude, upon multiple episodes of acute pancreatitis acinar cells from *Mnk1*$^{-/-}$ mice on one hand undergo less ductal atrophy, but on the other show stronger down-regulation of acinar phenotype hallmarked by a reduction of Ptf1a protein.

3. Mnk1 modulates acinar phenotype and pancreatic carcinogenesis upon oncogenic Kras

3.1. Mnk1 interference destabilizes acinar phenotype of 266-6 cells

To analyze in more detail whether Mnk1 is not only part of the acinar program but can also be involved in its regulation, we knocked-down Mnk1 in 266-6 cells – cancer cells that retain acinar features. We used two lentiviral shRNA constructs targeting Mnk1 and achieved 50% and 90% down-regulation of Mnk1 transcript levels without affecting Mnk2 expression (Fig. 18A). As a consequence, total Mnk1 protein levels as well as basal Mnk1 phosphorylation were decreased (Fig. 18B). The most effective construct (sh2) also led to a reduced basal eIF4E phosphorylation. Upon Mnk1 knockdown, 266-6 cells underwent morphological changes acquiring a flatter shape and abundant filopodia (Fig. 18C). Moreover, some extent of cell death (not quantified) was observed with both hairpins.

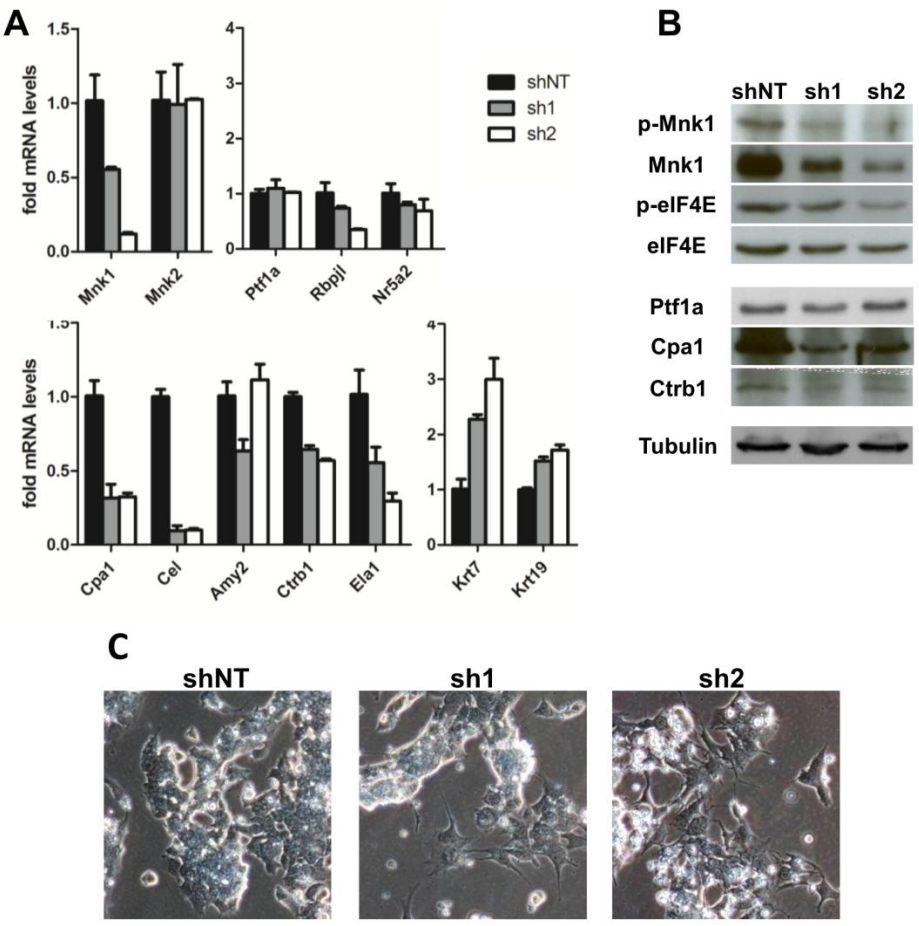


Figure 18. Mnk1 knock-down in 266-6 cells reduces acinar gene expression. (A) mRNA expression of Mnks, acinar transcription factors, digestive enzymes and ductal keratins in control (shNT) and Mnk1-interfered (sh1, sh2) 266-6 cells as assessed by RT-qPCR; data represented as fold levels over expression in shNT cells (mean±SEM, n=3). (B) Immunoblot showing protein expression and phosphorylation in control and interfered 266-6 cells. (C) Microscopic pictures of control and interfered 266-6 cells.

Both hairpins caused a reduction of mRNAs coding for Rbpjl and acinar enzymes (Fig. 18A). Consistently, Cpa1 and Ctrb1 protein levels were down-regulated (Fig. 18B). Interestingly, no changes in Ptf1a mRNA or protein levels were observed. On the other hand, Mnk1 knock-down led to an up-regulation of ductal keratins (Krt7 and Krt19) (Fig. 18A). The above results were reproduced in an independent experiment (data not shown).

Next we analyzed the effect of Mnk1 interference on stress-induced down-regulation of acinar phenotype in 266-6 cells. Hydrogen peroxide (H_2O_2) treatment of 266-6 cells led to Mnk1 phosphorylation and concomitant down-regulation of Ptf1a and Cpa1 (Fig. 19A and B). Interestingly, it was not associated with p-eIF4E increase. To address whether

Mnk1 activation upon stress is also important for acinar phenotype regulation, cells were treated with H_2O_2 after Mnk1 interference using sh2 construct. In Mnk1-interfered cells, H_2O_2 treatment induced only a mild Mnk1 phosphorylation and the decrease of Ptf1a and Cpa1 proteins was stronger than in control cells (Fig. 19A and B). This result was also reproduced in a separate experiment (data not shown).

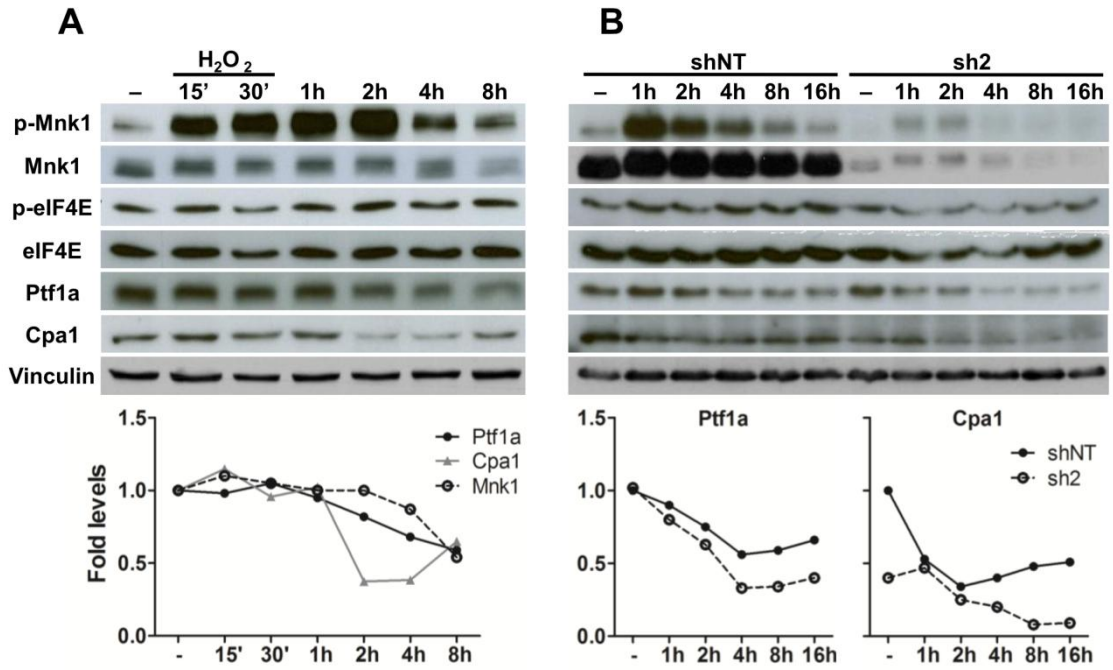


Figure 19. Mnk1 interefered 266-6 cells respond with stronger reduction of Ptf1a and Cpa1 upon H_2O_2 treatment (A and B) Mnk1, eIF4e, Ptf1a, Cpa1 and Vinculin protein expression and Mnk1 and eIF4E phosphorylation upon hydrogen peroxide (H_2O_2) treatment of 266-6 cells in normal culture conditions (A) and of control (shNT) and Mnk1-interfered (sh2) 266-6 cells (B). Top panels are immunoblots. Bottom panels are graphic representations of western blotting band densitometry for Mnk1, Ptf1a and Cpa1. -, 15´, 30´, 1h, 2h, 4h, 8h, 16h – experimental time-points after H_2O_2 treatment, where "-" represents untreated cells

Thus, acute depletion of Mnk1 in 266-6 cells leads to down-regulation of the acinar transcription program both in basal conditions and upon stress.

3.2. Mnk1 activation by Kras in acinar cells modulates Mnk1-eIF4E pathway

In order to determine whether mutant Kras is able to activate Mnk1 in acinar cells, $Kras^{G12V}$ was ectopically expressed in 266-6 cells (Fig. 20A). As expected, overexpression of an activated form of Kras led to very strong phosphorylation of Erk1 and Erk2 MAP kinases. By contrast there was only a minor increase of p-Mnk1 but antibodies against both p-Mnk1 and total Mnk1 detected additional higher band which presence has been associated with Mnk1 activation (Fukunaga and Hunter, 1997). Interestingly using

phosphospecific antibodies an increased signal for a 39 kDa band which might represent p-Mnk1b was detected. In addition modest phosphorylation of eIF4E, Mnk1 substrate, was increased (with no changes in total eIF4E abundance) (Fig. 20A).

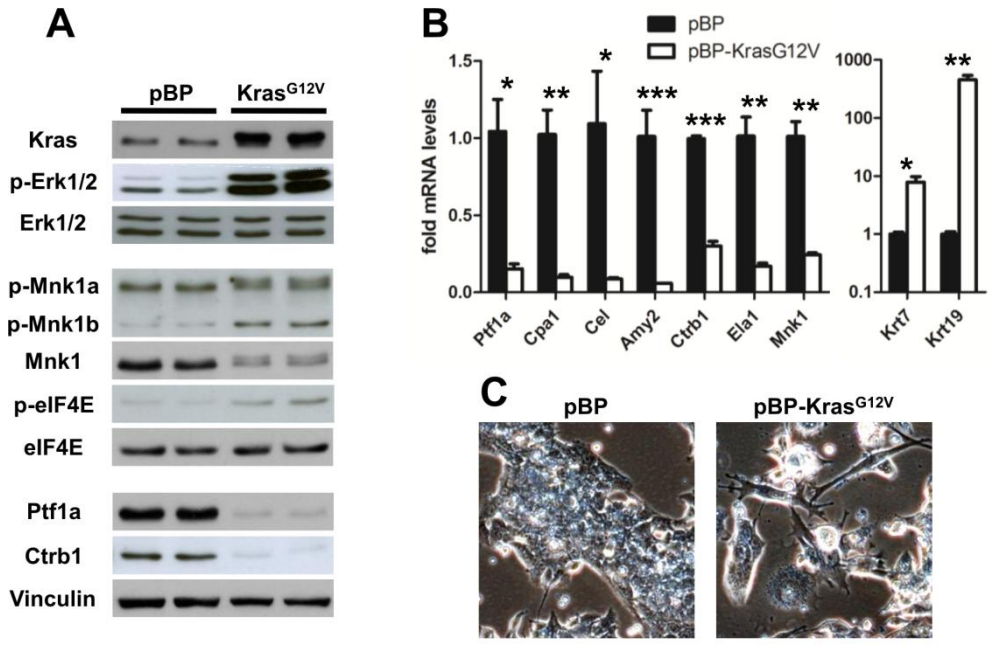


Fig. 20 Ectopic expression of Kras^{G12V} in 266-6 acinar cancer cells represses acinar program and activates Mnk1-Eif4e pathway (A) Immunoblot depicting Kras, Erk1/2, Mnk1, eIF4E, Ptf1a, Ctrb1 and Vinculin protein expression and Erk, Mnk1 and eIF4E phosphorylation in 266-6 cells transfected with empty vector (pBP) and with vector containing KrasG12V cDNA. (B) mRNA levels of Ptf1a, Cpa1, Cel, Amy2, Ctrb1, Ela1, Mnk1, Krt7 and Krt19 in 266-6 cells transfected with pBP or pBP-Kras G12V vectors as assessed by RT-qPCR; data represented as fold levels over expression in pBP transfected cells (mean±SEM, n=3). (C) Microscopic pictures of control and Kras G12V overexpressing 266-6 cells.

Interestingly, mutant Kras over-expression led to a loss of the epithelial morphology with acquisition of a more spindle-like or flattened cell shape (Fig. 20C) and to a strong repression of the acinar transcription program (Fig. 20B) manifested by marked down-regulation of mRNA levels of Ptf1a and its target genes (Ela1, Ctrb1, Cpa1, Cel and Mnk1) and up-regulation of ductal markers – Krt7 (by 7-fold) and Krt19 (by 500-fold). Down-regulation of expression seen on mRNA level for Ptf1a, Ctrb1 and Mnk1 was also confirmed at the protein level (Fig. 20A).

To determine whether the effects observed in 266-6 cells also occur in vivo, activation of Mnk1 was investigated using *Ptf1a$^{+/CreERT2}$;Kras $^{+/LSLG12Vgeo}$* mouse. In this model, expression of mutant Kras from its endogenous promoter was induced upon tamoxifen administration to pregnant females in E15.5 mouse embryos. The pancreas was

collected at E18.5 and protein expression was analysed (Fig. 21A). The recombination efficiency was estimated by western blotting using anti-β-galactosidase antibodies (see Materials and Methods). β-galactosidase was undetectable in lysates from tamoxifen-treated control *Ptf1a*$^{+/CreERT2}$;*Kras* $^{+/+}$ a weak band in lysates from tamoxifen-treated *Ptf1a*$^{+/CreERT2}$;*Kras* $^{+/LSLG12Vgeo}$ embryos was detected. In these experiments, we obtained no evidence of Mnk1 phosphorylation or mobility shift (Fig. 21A); on the contrary, the levels of p-Mnk1 were down-regulated. Moreover no clear-cut change in phosphorylation status of eIF4E was observed. In this setting, neither mRNA nor protein levels of Ptf1a were affected however mRNA expression of some acinar specific genes was down-regulated (Mnk1, Amy2, Ctrb1, Cpa1, Cel) (Fig. 21B).

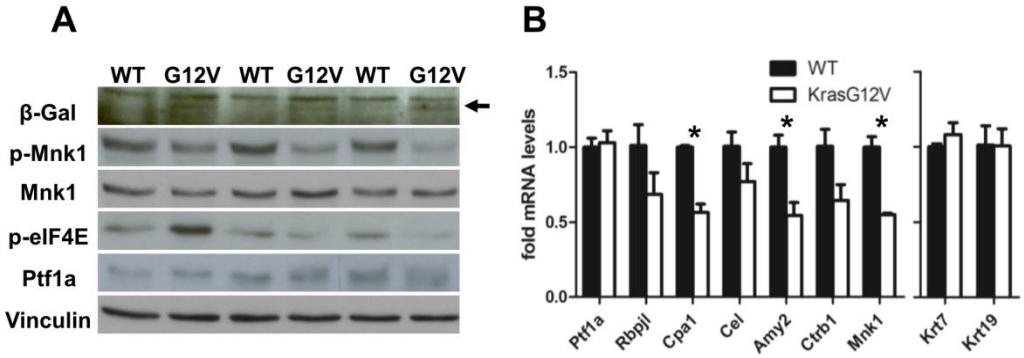
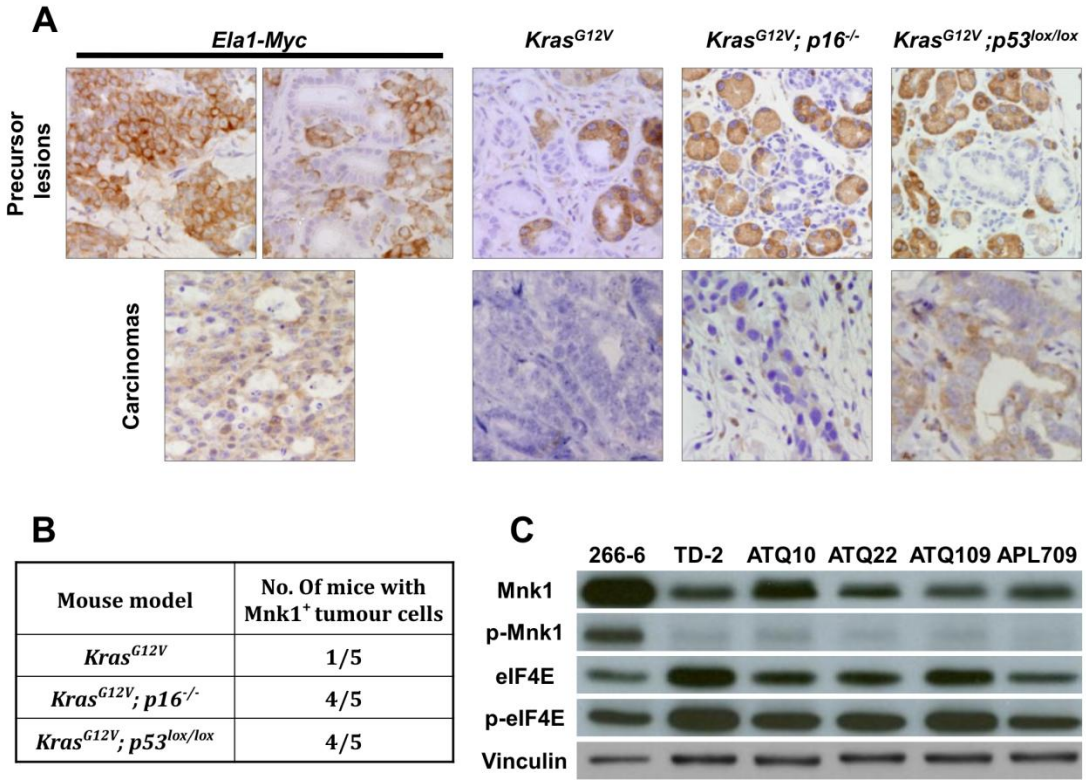


Fig. 21 Mnk1 phosphorylation is downregulated upon *K-Ras*^{G12V} activation in mouse embryonic pancreas. Immunoblot depicting protein levels of β-Gal, Mnk1, Ptf1a and Vinculin and phosphorylation of Mnk1 and eIF4E in pancreatic lysates from tamoxifen treated *Ptf1a*$^{+/CreERT2}$; *Kras* $^{+/+}$ (WT) and *Ptf1a*$^{+/CreERT2}$;*Kras* $^{+/LSLG12Vgeo}$ (G12V) mouse E18.5 embryos; arrow indicates band corresponding to β-Gal. (B) mRNA expression of Ptf1a, Rbpjl, Cpa1, Cel, Amy2, Ctrb1, Mnk1, Krt7 and Krt19 in pancreata from WT and G12V mouse embryos as assessed by RT-qPCR; data represented as fold levels over expression in WT embryos (mean±SEM, n=2).

To conclude, Kras^{G12V} expression leads to Mnk1 pathway activation in 266-6 cells but to lower Mnk1 phosphorylation in embryonic pancreas. Although these results may seem contradictory, together they suggest that Mnk1 may be involved in Kras signaling in acinar cells.

3.3. During PDAC progression Mnk1 protein is downregulated in precursor lesions but detected in carcinomas

Mnk1 has been reported to be involved in cancer progression in several mouse models. Therefore, we analyzed Mnk1 expression in different mouse models of pancreatic cancer (Fig. 22) and in samples from human PDAC (Fig. 23).



Mouse model	No. Of mice with Mnk1^{+} tumour cells
Kras^{G12V}	1/5
Kras^{G12V}; p16$^{-/-}$	4/5
Kras^{G12V}; p53$^{lox/lox}$	4/5

Figure 22. Mnk1 expression is downregulated upon metaplasia but detected in some tumor cells in mouse models of PDAC. (A) Immunohistochemistry showing Mnk1 expression in precursor lesions and carcinomas in pancreatic sections from different mouse models of pancreatic cancer (*Ela1-Myc*, *Ptf1a$^{+/Cre}$;Kras$^{+/LSLG12Vgeo}$*, *Ela1-tTa/tetO-Cre;Kras$^{+/LSLG12Vgeo}$;p16$^{-/-}$* and *Ela1-tTa/tetO-Cre;Kras$^{+/LSLG12Vgeo}$;p53$^{lox/lox}$*). (B) Table showing frequency of mice with Mnk1 positive tumour cells in different Kras driven mouse tumours. (C) Immunoblot depicting protein expression and phosphorylation in different mouse cell lines: 266-6 (derived from acinar cancer), TD-2 (derived from TGFα driven ductal tumour), ATQ10, ATQ22 and ATQ109 (derived from ductal tumours from *Ela1-tTa/tetO-Cre*;Kras$^{+/LSLG12Vgeo}$;p53$^{lox/lox}$ mice) and APL709 (derived from ductal tumour from *Ela1-tTa/tetO-Cre;Kras$^{+/LSLG12Vgeo}$;p16$^{-/-}$* mouse).

In Ela1-Myc mice, Mnk1 was detected in tumors with acinar differentiation but not in metaplastic ductal cells (n=6) (Fig. 22A). In *Ptf1a$^{+/Cre}$;Kras$^{+/LSLG12Vgeo}$* mice, Mnk1 was undetectable in cells that underwent acinar-to-ductal metaplasia, in PanINs and very seldom detected in tumours displaying ductal differentiation (1 out of 5 mice). Similarly in *Ela1-tTa/tetO-Cre;Kras$^{+/LSLG12Vgeo}$;p16$^{-/-}$* and *Ela1-tTa/tetO-Cre;Kras$^{+/LSLG12Vgeo}$; p53$^{lox/lox}$* models Mnk1 was absent from metaplastic ductal cells and PanINs. However, in these models the protein was detected at low to moderate level in tumour cell of ductal phenotype (in both cases 4 mice out of 5) (Fig. 22A and B).

We used tissue microarrays (TMAs) to assess MNK1 expression in human PDAC. As shown in Fig. 23A, MNK1 expression in preneoplastic and neoplastic lesions from patients with PDAC was similar to that observed in mouse models: low-level expression in metaplastic ductal cells, mainly undetectable in PanINs, and variably expressed at low levels in tumour samples (in 10 out of 29 patients) with different levels of expression (weak in 4 and moderate in 6 patients) but always lower than in acinar cells (Fig. 23B). The only difference between human PDAC specimens and samples from mouse models of pancreatic cancer concerns the fact that in 4/29 samples, weak staining for MNK1 was observed in precursor lesions (Fig. 23A and B).

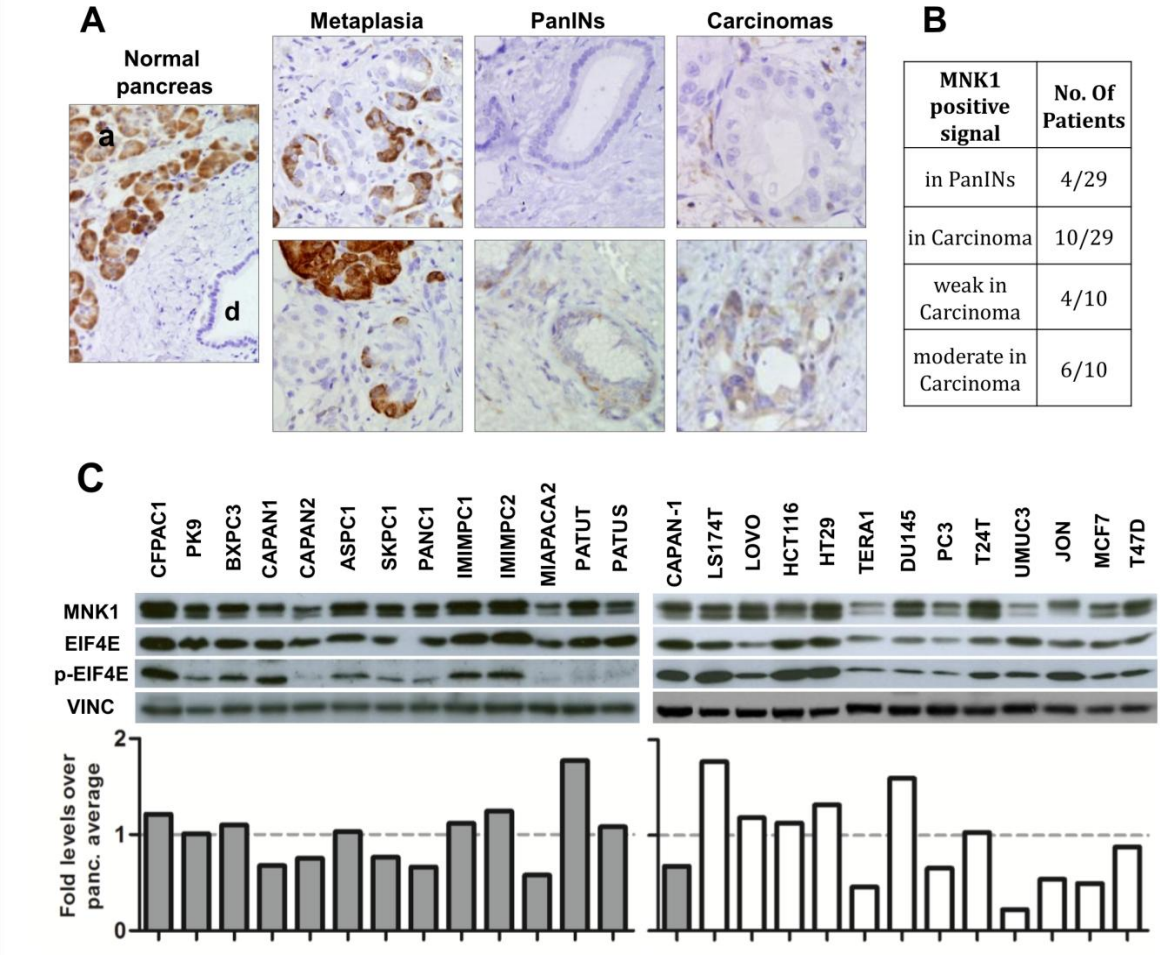


Figure 23. Mnk1 protein is downregulated in precursor lesions but detected in some carcinoma cells of human PDAC. (A) Representative pictures showing Mnk1 expression in metaplastic events, PanINs and carcinomas from human PDAC TMA. (B) Table presenting frequency of Patients with Mnk1 positive PanINs and Carcinomas as well as distribution of expression intensity among carcinomas. (C) (top panel) Immunoblot depicting protein levels and phosphorylation in human PDAC (left) and colon (LS-174T, LOVO, HCT-116, HT-29), teratoma (TERRA-1), prostate (DU-145, PC-3), bladder (T24T, UM-UC-3, JON) and breast (MCF-7, T47D) (right); (bottom panel) Densitometry of bands from top panel western blotting presented as fold levels over average expression in PDAC cells (dashed line).

Subsequently, Mnk1 protein expression and activation in pancreatic cancer cell lines was investigated. Among murine lines, Mnk1 was expressed at highest levels in 266-6 acinar cells and at lower - but detectable - levels in PDAC lines derived from acinar-initiated tumors (Fig. 23C). Basal Mnk1 phosphorylation mirrored the total protein levels in these lines. Unlike Mnk1, its substrate – eIF4E was expressed at similar levels in all cell lines regardless of their differentiation phenotype; similar findings were made with p-eIF4E. Interestingly MNK1 protein was detected in all human PDAC cells and its levels were comparable to those in colon cancer cells (Fig. 23C). The same applies to EIF4E expression, while the levels of p-EIF4E are more variable among pancreatic and other cancer cell lines (Fig. 23C).

Thus, during PDAC development Mnk1 expression is down-regulated along with the acinar transcription program but is expressed at lower levels in some ductal tumor cells, possibly in a PTF1-independent manner (see discussion).

3.4. *Mnk1*$^{-/-}$ mice develop more PanINs and less tubular complexes in Kras^{G12V} PDAC model

To investigate the role of Mnk1 in Kras-driven PDAC development/progression, we crossed *Mnk1*$^{-/-}$ and *Ptf1a*$^{+/Cre}$;*Kras*$^{+/LSLG12Vgeo}$ mice and analyzed precursor lesion abundance in this animals (Fig. 24). By 6 months, all control animals (n=11) (*Ptf1a*$^{+/Cre}$;*Kras*$^{+/LSLG12Vgeo}$;*Mnk1*$^{+/+}$) had developed PanIN-1 lesions (9 lesions per mouse) (Fig. 24A). Few PanIN-2 lesions were observed at this time (4/11, 0.5 lesion per mouse) and only one mouse had PanIN-3. By contrast *Ptf1a-Cre*$^{+/KI}$;*Kras* $^{+/LSLG12Vgeo}$;*Mnk1*$^{-/-}$ mice (n=8) had a higher total number of lesions (p=0.016). They had significantly more 1, 2 and 3 PanINs (17, 2.5 and 0.75 lesions per mice respectively). Importantly, more animals contained PanIN-2 (7/8) and PanIN-3 (4/8) (Fig. 24A). Apart from PanINs wild type controls developed other ductal lesions – tubular complexes (TCs) and metaplastic mucinous metaplasias (MMLs). Interestingly although pancreata of mice lacking Mnk1 showed similar number of MMLs they contained much less tubular complexes (Fig. 24B and C).

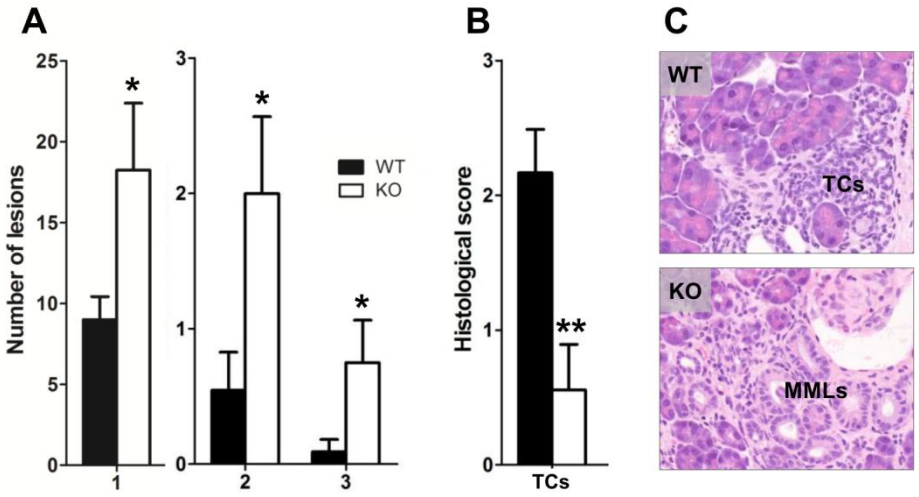


Figure 24. Mnk1$^{-/-}$ mice develop more PanINs and less TCs in Kras-driven PDAC model. (A) Number of different types of PanIN lesions (1A, 1B, 2 and 3 found in pancreatic sections from 6 months old *Ptf1a$^{+/Cre}$;Kras$^{+/LSLG12Vgeo}$;Mnk1$^{+/+}$* (WT) and *Ptf1a$^{+/Cre}$;Kras$^{+/LSLG12Vgeo}$;Mnk1$^{-/-}$* (KO) mice. (B) Histological score of tubular complexes (TCs) abundance in 6 month old WT and KO mice. (C) Hematoxylin-eosin staining of WT (top) and KO (bottom) sections showing TCs, more prominent in WT pancreata, and mucinous metaplastic lesions (MMLs) that are more prominent in KO pancreata.

Discussion

Mortality rate of PDAC has not changed in 50 years, despite the progress that has been made over past 2 decades in understanding molecular mechanisms underlying its development (Siegel et al., 2013). One of the reasons for this is that we do not understand several important aspects of PDAC development. It is still under discussion what is the cell of origin of this cancer and even whether all PDACs develop from the same cell type or different tumors arise from different pancreatic cells. Evidences from mouse models show that acinar cells may acquire ductal characteristics and can give rise to adenocarcinomas and that it is facilitated by pancreatitis – a well established risk factor for this cancer in human (Guerra et al., 2007). However, molecular mechanisms triggering the phenotypic switch, from acinar to ductal, and underlying higher sensitivity to transdifferentiation upon stress are poorly understood.

1. Identification of novel genes involved in pancreas biology

In order to get more insight into biology of acinar cells we initially set out to describe novel genes that are expressed in developing mouse pancreas and could be involved in exocrine differentiation. We reasoned that functional characterization of such genes could shed more light on processes that regulate exocrine cell biology. In search for gene candidates we screened Genepaint database that provides *in situ* hybridization images for different genes in E14.5 whole mouse embryo sections, with annotations of the genes to the tissues in which they are expressed. The search criteria were: 1) selective expression in developing pancreas; 2) scattered or regional expression pattern in the pancreas that suggested lineage specificity; 3) known important function in controlling cellular processes i.e. signal transduction or gene expression regulation; 4) non-described function in the pancreas. As a result of this approach, 5 genes candidates were chosen and subjected for initial analysis of their expression in the pancreas. The candidates were: *Mknk1, c-Myb, Lhx1, Mdk, Nr2f6* (Fig. 25). Analyzing expression pattern in images provided by Genepaint and further expression analysis allowed us to draw conclusions about the specificity of these gene products for different pancreatic cell lineages (Fig. 25): Mnk1 (*Mknk1* product) is expressed in MPCs, acinar precursors and adult acinar cells; c-Myb is detected transiently in acinar precursors; Lhx1 is present transiently in endocrine precursors; Mdk is expressed in bipotent precursors and in adult ductal cells; analysis of Nr2f6 expression did not yield any conclusion but basing

on similar to Mdk pattern in Genepaint image it is possible that it is involved in ductal or endocrine cell differentiation.

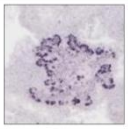
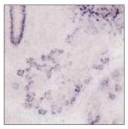
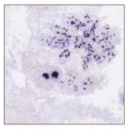
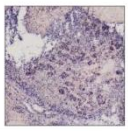
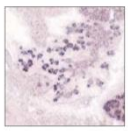
Candidate	Cellular function	Genepaint image	Genepaint expression pattern	Additional analysis of expression in the pancreas
Mnk1	Kinase		Strong in the "tip" domain	In MPCs and acinar cells (E10.5 - adult)
c-Myb	Transcription factor		Weak in the "tip" domain	Transient in acinar precursors (E14.5 - E18.5)
Lhx1	Homeobox transcription factor		Scattered in the "trunk" domain	Transient in endocrine precursors (E14.5 - E18.5)
Mdk	Growth factor		Focal in the "trunk" domain	In bipotent precursors and adult ductal cells (E12.5 - Adult)
Nr2f6	Orphan nuclear receptor		Focal in the "trunk" domain	No conclusive data

Figure 25. Candidate genes identified after screening the Genepaint server.

2. Mnk1 is a novel acinar cell-specific stress response kinase in the pancreas

For further analysis we chose Mnk1. The rationale was based on several facts: a) We found it to be highly expressed in pancreatic acinar cells and in their precursors, b) It is a stress kinase activated by Erk and p38 MAP kinases (Fukunaga and Hunter, 1997; Waskiewicz et al., 1997) which are implicated in two important aspects of pancreatic tumorigenesis: Kras signaling and inflammation, c) Its function has not been described in the pancreas, d) Proper tools for studying Mnk1 were available – good quality antibodies recognizing total and phosphorylated protein, Mnk1 knock-out mouse and lentiviral vectors with Mnk1 targeting shRNAs.

There is only one report that mentions Mnk1 in the pancreas (O'Loghlen et al., 2004). In this work O'Loghlen and colleagues discovered the "b" isoform of Mnk1 and performed a simple semi-quantitative PCR-based analysis of Mnk1a and Mnk1b expression among different human tissues. They detected both isoforms in the pancreas, however, as the panel of tissues in this study was rather small and the technique used was not very accurate no reliable comparison of expression levels could be performed. In contrast, our data undoubtedly show that in mouse pancreas Mnk1 is expressed at very high level comparing to other tissues. Mnk1 expression pattern during pancreas development is similar to that of Cpa1 and Ptf1a (Zhou et al., 2007) as it is present in the whole pancreatic epithelium before secondary transition (in MPCs) and is restricted to acinar precursors in later stages of pancreas development. This suggested that Mnk1 might be a Ptf1a target in the pancreas what we demonstrate by chromatin immunoprecipitation. During mouse pancreas development some acinar specific markers, such as Cpa1, are found already in MPCs (which do not contain PTF1-L), as their expression is activated already by PTF1-J (Masui et al., 2010). It is thus likely that *Mknk1* gene is also a target of both complexes. Although in our experiments we did not analyze binding of neither Rbpj nor Rbpjl to above described sites in *Mknk1*, most of the identified E-boxes were followed by TC-boxes what suggested possible binding of these factors.

Typically, PTF1 binding sites in acinar specific genes are found proximal to TSS within 300bp (Beres et al., 2006). Although in *Mknk1* such putative site (E-box and TC-box) was detected at -175bp, Ptf1a binding to this region was relatively low. In contrast, stronger binding was detected in 7 sites that localize within 1st intron of the gene. Thus, as opposed to known acinar specific enzymes and transcription factors in which PTF1 binding was described to occur upstream from TSS, *Mknk1* gene is bound by this complex in intronic regions, although functional significance of this finding remains unknown. Mnk1 is known to be present in various cell types (among others in macrophages, T-cells, cardiomyocytes, mesangial cells and different cancer cells), however nothing has been reported on its expression levels in different tissues. We conclude that it has an exceptionally high abundance in pancreatic acinar cells and that this can be ascribed to the activity of PTF1 complex. As opposed to Mnk1, Mnk2 is not overrepresented in the pancreas and its expression is not regulated during development of this organ, hence it is not specific for exocrine lineage. In order to get some insight

into putative function of Mnk1 in the pancreas we analyzed the expression of various known Mnk targets in mouse tissues, however none of them was overrepresented in the pancreas.

Results of acute pancreatitis experiment show that Mnk1 is an integral part of acinar transcription program also in the context of stress response. Upon injury its expression is regulated in the same way as that of other acinar markers. Importantly, to our knowledge it is the first acinar cell specific kinase to be described. We provide evidence that Mnk1 is activated in acinar cells upon stress: in acute and chronic pancreatitis, upon acini isolation and in response to oxidative damage in 266-6 cells. There are two hallmarks of Mnk1 activation: T-loop phosphorylation and the appearance of a higher band detected with western blotting (Fukunaga and Hunter, 1997) and both occur in these conditions. Moreover, Mnk1 activation can also be indirectly assessed by measuring the eIF4E phosphorylation which we observe to increase upon pancreatitis and acinar cell isolation in Mnk1 dependent manner. It has not been reported whether the T-loop phosphorylation contributes to observed change in gel mobility of the Mnk1 protein. During the induction of acute pancreatitis in mouse, already first caerulein injection causes Mnk1 phosphorylation but at this time no total protein band shift is yet observed, what suggests an involvement of additional modifications. Apart from its activation caerulein treatment also leads to down-regulation of Mnk1 protein levels. They are the lowest 1 day after the treatment what coincides with the lowest abundance of its mRNA. Although pancreatitis-induced down-regulation of Mnk1 protein can be explained by PTF-1-dependent transcription regulation, it is possible that increased rate of protein degradation is also involved. This notion is suggested by the fact that possibly activated form of the protein (the higher band) is down-regulated faster. It is known that protein modifications can negatively affect their stability (Dasilva et al., 2006) however this issue has never been studied in the case of Mnk1.

While Mnk1 activity has been associated with phosphorylation of this kinase and its substrates, little is known whether it affects cellular distribution of this protein. A recent report has described that Mnk1 in some contexts may localize to centrosomes and spindle microtubules, although this remains to be confirmed (Rannou et al., 2012). We report that activation of this kinase in acinar cells is associated with its transient

localization towards basolateral region. Considering, that the polarization of Mnk1 distribution is the strongest at 4h when the highest p-Mnk1 levels are detected it is conceivable that it reflects its activation.

The discussed above phosphorylation signal refers to Mnk1a isoform, however we also observe an additional lower band corresponding to 39kDa protein that can be detected in pancreata from caerulein treated wild type but not from *Mnk1*$^{-/-}$ mice. This likely reflects Mnk1b – the isoform that so far has only been described in human (O'loghlen et al., 2004). It is believed to be a nuclear protein, however its function is unknown (Buxade et al., 2008). Interestingly phosphorylation of this 39kDa protein occurs very early during the pancreatitis induction as the highest phospho-signal is detected at 30' after first caerulein injection. If this was indeed the Mnk1b isoform this could suggest some early function of Mnk1 in acinar cell nucleus upon caerulein stimulation, however we do not detect any pancreatitis-associated Mnk1 nuclear localization.

3. *Mnk1*$^{-/-}$ mice display altered expression of acinar digestive enzymes and p-eIF4E target proteins in the pancreas

Ueda and colleagues (Eueda et al., 2004) reported that Mnk1 is dispensable for mouse development, however they did not examine in detail pancreata of mice lacking Mnk1. Consistent with accepted concept that this kinase is not essential for normal cell growth we do not observe any macroscopic or microscopic abnormalities in histology of *Mnk1*$^{-/-}$ mouse pancreas. Since we found Mnk1 to be expressed in acinar cells, we focused on whether its absence has an impact on acinar differentiation and found that pancreata of these mice have elevated protein levels of digestive enzymes such as Cpa1, Cel and Amy2. The increase in enzymatic protein in pancreas lacking Mnk1 is modest, however taking into account that protein synthesis rate in this organ is very high we would not expect that enzymatic protein content therein could be much elevated. On the other hand, pancreata of these mice contain less protein of c-Myc and Ccnd1 whose biosynthesis is known to be positively regulated by phosphorylated eIF4E. As in the absence of Mnk1, basal eIF4E phosphorylation is reduced and there is no decrease in c-Myc and Ccnd1 mRNA levels it is likely that posttranscriptional p-eIF4E dependent regulation is involved.

These data suggest that in pancreatic acinar cells Mnk1 negatively affects abundance of digestive enzymes and at the same time positively regulates expression of prosurvival proteins. Although the second observation is not surprising, basing on the known role of Mnk1-eIF4E pathway, the first discovery is novel. Importantly, the above described changes seen in the absence of Mnk1 do not significantly affect total pancreatic protein content, what might be due to opposite effect on abundance of different groups of proteins.

There are several mechanisms through which Mnk1 might regulate digestive enzyme abundance. As we observe elevated levels of their transcripts it might affect transcription or mRNA stability. So far no involvement of Mnk1 in gene transcription has been described. On the other hand this kinase has been shown to regulate transcript stability through activation of ARE binding proteins (Buxade et al., 2008), however mRNAs of acinar digestive enzymes have very short 3´ UTRs that essentially do not contain ARE elements (data not shown). Although it is possible that the increase in acinar gene protein expression is a consequence of elevated mRNA levels we do not prove that this is the case. On the contrary, we do not observe higher abundance of Ptf1a protein despite its elevated mRNA levels and it is possible that the enzymatic protein increase occurs through different mechanisms. For instance Mnk1-eIF4E-mediated translation regulation could also be involved. eIF4E, upon phosphorylation, has more affinity to mRNAs that code for prosurvival proteins, such as cMyc or Ccnd1 (Buxade et al., 2008). Possible scenario could be that binding to this subset of transcripts renders eIF4E less available for global translation. In this way eIF4E phosphorylation would simultaneously positively regulate biosynthesis of ones and negatively of other proteins. This is in line with the proposed hypothesis that eIF4E might be involved in a switch from global to cap-independent translation (Svitkin et al., 2005). Finally, another explanation could be that Mnk1 affects acinar cell exocytosis and that in the absence of this kinase, the enzymes are not properly secreted and as a consequence accumulate in the cytoplasm. This issue will be discussed below.

4. c-Myc expression induction and acinar cell proliferation during caerulein-induced pancreatitis partially depend on Mnk1

Acute pancreatitis is associated with proliferation of a low fraction of acinar cells what is believed to be involved in organ regeneration after injury (Lechene de la Porte et al., 1991) and to be at least partially driven by Insulin growth factor I (IGF-I) secreted by activated fibroblasts (Ludwig et al., 1999). Iovanna and colleagues showed that during taurocholate-induced pancreatitis in rats, acinar cells have increased levels of c-Myc and Hras proteins (Iovanna et al., 1992) however no such analysis was performed regarding caerulein treatment.

We describe, that in mouse pancreas upon caerulein-induced pancreatitis, expression of c-Myc is induced what is primarily due to regulation of its transcript levels. However, the increase of c-Myc protein partially depends on post-transcriptional regulation by Mnk1, as it is lower in mice lacking this kinase with no differences in mRNA expression. It occurs possibly through described IRES-dependent regulation of c-Myc translation by Mnk1-eIF4E pathway (Shi et al., 2012) as pancreata of these mice do not show pancreatitis-induced eIF4E phosphorylation. Regulation of Ccnd1 expression upon pancreatitis has so far not been described. We observe that in contrast to c-Myc, the levels of this cyclin are down-regulated in response to caerulein injection, what is probably due to transient decrease of its mRNA abundance. It is conceivable that it levels the difference in Ccnd1 protein amount between pancreata of untreated *Mnk1*$^{+/+}$ and *Mnk1*$^{-/-}$ mice, as Mnk1-eFI4E-dependent regulation of Ccnd1 mRNA nuclear export might have little effect when the transcript levels are low. This hypothesis is supported by the fact that 1 day post-pancreatitis, when Ccnd1 mRNA levels are reversed, Mnk1$^{+/+}$ mice have significantly more pancreatic Ccnd1 protein.

The impaired induction of c-Myc levels can partially explain lower acinar cell proliferation rate during recovery from AP observed in mice lacking Mnk1, as it is well establilshed that this transcription factor positively regulates cell cycle (Eilers and Eisenman, 2008). However, it is possible that there are also other p-eIF4E targets which induction upon AP is impaired in *Mnk1*$^{-/-}$ mice upon acute pancreatitis.

5. Mnk1 is required for the physiological secretory response of acinar cells and for the homeostatic response to caerulein administration

Based on elevated serum amylase activity, increased neutrophil infiltration and stronger reduction of acinar transcription program we can conclude that lack of Mnk1 results in increased severity of experimentally-induced acute pancreatitis. A number of genes have been described, whose inactivation in mouse induces similar transient increase in pancreatitis severity, among others: Irmap1 and p8 and they are believed to play pancreatitis-protective roles in the acinar cells. Integral membrane-associated protein-1 (Itmap1) is a member of CUB/ZP that regulates zymogen granule formation and Itmap1$^{-/-}$ mice upon caerulein administration display a modest increase in serum enzyme activity and acinar cell apoptosis (Imamura et al., 2002). p8 is a stress induced transcription coactivator and p8$^{-/-}$ mice have transiently elevated serum enzyme levels and pancreas inflammation (Vasseur et al., 2004).

Mnk1 is known to be important for cytokine production and response in inflammatory cells. However, in our experiments the increase in inflammation is not strong and occurs 2 days after the pancreatitis induction when, suggesting that elevated inflammation is rather a consequence of earlier events. Nevertheless it may be one of the reasons for impaired recovery of acinar transcription programme observed in mice lacking Mnk1. Given, that in our model Mnk1 is inactivated in all cells of the organism it is possible that its depletion affects inflammatory response to pancreatitisis. This kinase is known to modulate secretion of cytokines and response to inflammatory mediators in immune cells. We can thus speculate that macrophages and T-cells lacking Mnk1 may respond poorer to damage-induced chemokines and produce less inflammatory mediators or even that acinar cells themselves may release less cytokines in the absence of this protein. In such case *Mnk1*$^{-/-}$ mouse despite elevated acinar cell damage would have inadequate response of the inflammatory compartment. This hypothetic effect compensation could have a considerable impact on acute pancreatitis in this model. In order to rule out such possibility a conditional mouse model should be applied that would allow for Mnk1 inactivation specifically in the pancreas.

There is no consensus on what is the regulation of digestive enzyme protein levels upon caerulein-induced acute pancreatitis. On one hand it has been shown that pancreatic

amylase activity is reduced upon serial caerulein injection (Dembinski et al., 2000; Molero et al. 2012) what is consistent with down-regulation of acinar transcription program. In contrast Halangk and colleagues reported that trypsinogen accumulates in mouse pancreata upon pancreatitis what is in line with the fact that caerulein induces secretion blockade in acinar cells (Halangk et al., 2000). The reason for this discrepancy between studies may be that abundance of different zymogens may be differentially regulated in the pancreas. We report, that caerulein injections lead to protein level down-regulation of Amy2, Cpa1 and Cel and that it is associated with modest reduction of pancreatic protein content observed at 8h of the trearment. Conversely, in mice lacking Mnk1 this downregulation is delayed as at 8h their pancreata have unchanged levels of analyzed digestive enzymes. This impaired reduction of zymogens might be implicated in observed increase in pancreatic protein content in these mice.
It is conceivable that elevated enzyme load in *Mnk1*$^{-/-}$ mouse pancreata is the reason for increased severity of acute pancreatitis, as it is believed that accumulation of enzymes in acinar cells facilitate intracellular zymogen activation (Vonlaufen et al., 2008). As described, early after first caerulein injection two critical events occur, that together induce acinar cell damage: zymogen activation and blockade in secretion that keeps activated enzymes inside of the cells (Saluja and Lerch, 2007). The first zymogen that becomes activated is trypsinogen that is converted into trypsin which can activate other proteases and subsequently lead to acinar cell damage and apoptosis. Interestingly pancreata of *Mnk1*$^{-/-}$ mice do not show more pancreatitis-associated trypsinogen activation than wild type controls. Moreover we do not observe histological changes that would suggest elevated pancreatic cell death in these mice. Consistent with no increase in trypsinogen activation in mouse we also do not observe higher trypsin activity in CCK stimulated isolated acini lacking Mnk1. In fact, the in vitro activation in cells from *Mnk1*$^{-/-}$ mice is even lower than in wild type mouse cells. As it has been shown, the increase in intracellular trypsinogen activation is not necessarily associated with greater acinar damage (Meister et al., 2010) and in Irmap1 defficient mice, acute pancreatitis is stronger despite lower pancreatic trypsin activity (Imamura et al., 2002).

Among the enzymes that accumulate in the pancreas of *Mnk1*$^{-/-}$ mouse at 8h of pancreatitis is procarboxypeptidase A1 (Cpa1). As all acinar proteases, it is produced in an inactive form in which it is stored in zymogen granules. It has been described that

caerulein stimulation of acinar cells in vitro induces its intracellular conversion to active carboxypeptidase (clCpa1) what is associated with cellular injury (Grady et al., 1998). Moreover elevated levels of clCpa1 have been shown to increase during pancreatic damage caused by acinar specific disruption of *Xbp1* gene (Hess et al., 2011). Importantly, we show the dynamics of procarboxypeptidase activation upon AP in mouse. Cpa1 conversion in pancreata of wild-type mice occurs already 0.5h after first caerulein injection, what coincides with trypsinogen activation, after which the levels of cleaved form gradually decrease. This downregulation is however impaired in *Mnk1*$^{-/-}$ mouse in which the elevated levels of clCpa1 are stable between 0.5h and 8h. The higher levels of cleaved Cpa1 in mice lacking Mnk1 can be explained twofold: either it reflects prolonged trypsin dependent procarboxypeptidase conversion, or improper release of cleaved form from acinar cells. Although we do not observe more trypsin activity upon caerulein treatment, the higher availability of the substrate (procarboxypeptidase) may increase the efficiency of the reaction. On the other hand higher abundance of Cpa1 may be partially a result of secretory defects of *Mnk1*$^{-/-}$ mice pancrata (discussed below) which could also lead to accumulation of its cleaved form. Both mechanisms are probable and they may occur simultaneously.

Some pancreatic proteases (as trypsinogen) have damaging potential for the acinar cells but for instance chymotrypsinogen C is known to be protective against pancreatitis (Szmola and Sahin-Toth, 2007). There are no reports explaining the role of carboxypeptidase in this disease, however as its activation has been show to coincide with injury and in our experiments it is associated with elevated pancreatitis severity, we conclude that it might have a damaging effect on the pancreas. Thus, it is likely that in *Mnk1*$^{-/-}$ mice pancreata, accumulation of zymogens such as procarboxypeptidase leads to increase in concentration of their active forms what underlies elevated pancreatitis severity.

While there are no consistent reports on zymogen subcellular localization upon stress, we observe apical localization of Cpa1 and Try proteins upon caerulein injections. These changes are associated with a transient relocalization of Muc1 that in polarized epithelial cells localizes to apical membranes (Pemberton, 1992). As both, enzyme and Muc1 redistribution are impaired in Mnk1$^{-/-}$ mice, we conclude that Mnk1 regulates

intracellular zymogen localization in response to caerulein injection probably through modulation of acinar cell polarity. Thus, higher digestive enzyme abundance in pancreata of *Mnk1*$^{-/-}$ mice upon pancreatitis could be a result of abnormal regulation of acinar cell polarity what can negatively affect exocytosis leading to zymogen accumulation. To address this hypothesis we performed functional studies in freshly isolated acinar cells. Consistent with other reports, treatment of wild type cells with different concentrations of caerulein yields a biphasic dose-response curve of amylase release (Halangk et al., 2000; Williams., 2010b). The hypothesis of secretory alterations in acini lacking Mnk1 is supported by the fact that these cells release less amylase into the medium, both without the stimulant and upon various concentrations of caerulein.

To sum up, Mnk1 is involved in regulation of zymogen secretion and acinar cells lacking Mnk1 display a suboptimal capacity to secrete digestive enzymes. Likely this is at least partially responsible for the increased response to serial caerulein administration in vivo, as it leads to accumulation of digestive enzymes in the cells. However, as discussed above it is also possible that Mnk1 affects the acinar enzyme abundance through p-eIF4E-mediated translation regulation. eIF4E has been reported to be phosphorylated in the pancreas upon stimulation with CCK that increases pancreatic mRNA translation, but it has not been proven to be responsible for this process (Bragado et al., 2000). On the other hand cytoplasmic phospholipase A2 (cPLA$_2$) that is a putative Mnk substrate has been shown to regulate acinar cell polarity and zymogen secretion (Tsuonda and Owyang, 1995; Mizuno et al., 2000). Thus, this protein is a strong candidate to mediate Mnk1 dependent regulation of zymogen secretion. Moreover, Mnk homologue in drosophila – LK6 has been identified as a protein associated with microtubules (Kidd and Raff, 1997), hence Mnk1 may be also involved in regulation of microtubule organization that is known to be important for acinar cell exocytosis (Schnekenburger et al., 2009). We did not however address these hypotheses and they remain to be investigated.

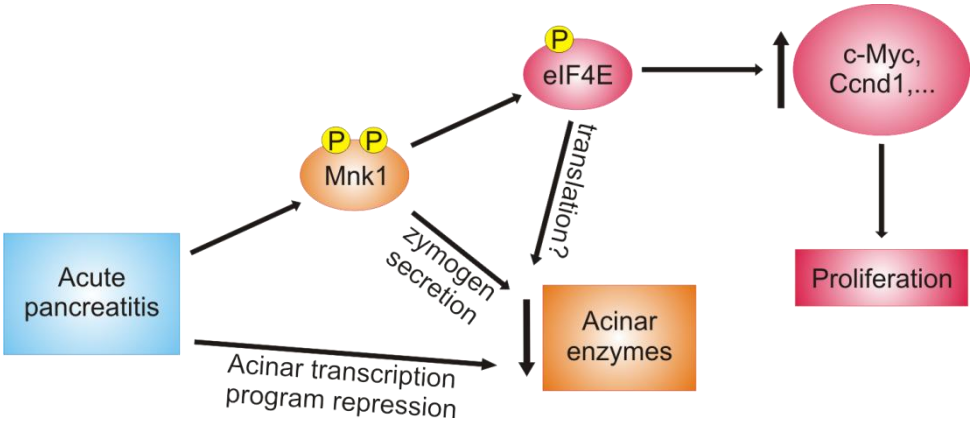


Figure 26. A model summarizing involvement of Mnk1 in regulation of acinar enzyme protein abundance and cell proliferation.

Final conclusion is that in the pancreas Mnk1 is an acinar specific, stress-activated kinase that is invlolved in acute pancreatitis response through regulation of acinar cell proliferation, and protein content (Fig. 26). It mediates biosynthesis of prosurvival proteins, most likely via eIF4E phosphorylation and zymogen load at least partially through its effect on exocrine secretion.

6. Depletion of Mnk2 does not enhance the phenotype of *Mnk1*$^{-/-}$ mouse

Although Mnk2 can also activate eIF4E, it has been proven to be responsible only for basal phosphorylation of this translation factor and we observe that in the pancreas, stimuli-induced eIF4E phosphorylation fully depends on Mnk1. These kinases share some other downstream targets and in the case of cell proliferation and survival have additive functions (Buxade et al., 2008), although Mnk2 has additional role in regulation of Pi3K-mTOR signaling (Hu et al., 2012). We show that Mnk2 is not over-expressed in mouse pancreas and that its expression does not change in this organ in the absence of Mnk1 (data not shown). Nevertheless, we investigated whether depletion of Mnk2 could enhance the effect of Mnk1 loss in the pancreas (data not shown). *Mnk1*$^{-/-}$*;Mnk2*$^{-/-}$ mouse pancreas develops normally and has no apparent abnormalities. p-eIF4E that is barely detected in *Mnk1*$^{-/-}$ animals is further down-regulated, below detection, in *Mnk1*$^{-/-}$*;Mnk2*$^{-/-}$ mouse pancreas. Similarly to *Mnk1*$^{-/-}$ mice, animals deficient for both kinases have elevated pancreatic and reduced serum amylase activity in normal condition. They also show an excessive amylase leakage at 8h of caerulein-induced acute pancreatitis and increased inflammation at 2 days post-treatment.

In summary *Mnk1*$^{-/-}$;*Mnk2*$^{-/-}$ mouse recapitulate most of the effects seen in *Mnk1*$^{-/-}$, both in normal pancreas and upon pancreatitis, thus we can conclude that Mnk1 has pancreas-specific functions that are not shared by Mnk2.

7. Mnk1 loss-of-function has a negative effect on acinar transcription program upon stress

Both, Mnk1 deficiency in mouse pancreas as well as its knock-down in 266-6 cells lead to stronger down-regulation of acinar transcription program upon stress. Pancreata of *Mnk1*$^{-/-}$ mice have reduced transcript levels of some acinar-specific genes during the recovery from acute pancreatitis (AP) and after multiple episodes of injury that lead to chronic pancreatitis (CP). In contrast to AP, the chronic treatment leads to marked reduction of Ptf1a levels what is not due to increased tissue damage as we do not observe elevated CP severity in mice lacking Mnk1.

To address the hypothesis that Mnk1 might be important for maintenance of acinar cell phenotype we used 266-6 cell line that has been shown to be useful to analyze digestive enzyme gene promoter regions (Kruse et al., 1988) or to assess the effect of Notch signaling on acinar cell differentiation (Siveke et al., 2008). These cells have been derived from an acinar tumour, driven by SV40 T antigen and although they are rather poorly differentiated they retain acinar features, such as expression of Ptf1a and digestive enzymes (Ornitz et al., 1985). We show that they express Mnk1 at higher levels than mouse ductal cancer cells and that this kinase is highly phosphorylated in these cells upon treatment with hydrogen peroxide (H_2O_2) that induces oxidative stress known to be involved in acinar damage during pancreatitis (Gukovskaya and Gukovsky, 2011).

Mnk1 interference in the 266-6 cells leads to down-regulation of expression of many acinar specific genes. Interestingly, it does not affect Ptf1a expression in normal culture conditions, however protein levels of this transcription factor upon H_2O_2 treatment are reduced to greater extent in interfered than in control cells. In contrast to normal pancreatic acinar cells, the 266-6 are cancer cells that are subjected to culture stress. Thus it is possible that observed decrease in acinar gene expression upon Mnk1 knock-

down reflects partial requirement of this kinase for maintaining acinar phenotype in culture conditions.

Altogether the above discussed data show, that inactivation of Mnk1 results in higher susceptibility of acinar cells to stress-related acinar program repression and depending on the context it may or may not involve reduction of Ptf1a levels. The reason for this might be that, as discussed in previous sections, Mnk1 takes part in acinar cell response to stress by controlling zymogen load, cell polarity and eIF4E activity and improper regulation of these processes upon Mnk1 loss of function may lead to stronger repression of acinar transcription program.

8. Mnk1 may suppress PDAC development through regulation of acinar-to-ductal transdifferentiation upon Kras signaling

KRAS mutations are found in more than 90% of PDAC cases (Maitra and Hruban, 2008). In cancer cells, KRAS signals mainly via MAPK and Pi3K signaling pathways, however it has been proposed that in PDAC, ERK signaling is the most crucial (Neuzillet et al., 2012 Hoffman et al.). Less is known, whether this oncogene activates p38 MAP kinase pathway in PDAC cells. In vitro studies using PANC-1 cell line showed that $KRAS^{G12V}$ over-expression enhanced the activity of p38 what was crucial for cell migration (Dreissigacker et al., 2006) but nothing has been described in this regard in acinar cells. Although Mnk kinases are activated by Erk1/2 and p38 MAP kinases, very few studies have linked these kinases to particular oncogenes. Mnk activity has been shown to mediate $Kras^{G12V}$-induced IRES-dependent translation in rat RIE-1 cells, however this study was based on pharmacologic inhibition of both Mnks and did not provide evidence of phosphorylation of either of them (Origanti and Schantz, 2007). Thus it is essentially not proven that mutant Kras activates Mnk1.

In order to analyze whether in pancreatic acinar cells Kras signaling leads to activation of Mnk1 we ectopically expressed $Kras^{G12V}$ in 266-6 cells what induced strong phosphorylation of Erk1/2. Interestingly, although we observe that Kras signaling leads to phosphorylation of the 39kDa protein that, as discussed above (2), is likely the Mnk1b isoform we do not detect an increase in p-Mnk1a phosphorylation. However, it is possible that Mnk1a phosphorylation in response to $Kras^{G12V}$ is an early event, as it

happens upon H_2O_2 treatment. As analyzed cells were collected 3 days after retroviral infection that mediated Kras overexpression, it is possible that it was too late to see the effect. Nevertheless, appearance of a higher band, that is also a halmark of Mnk1 activation and increase in eIF4E phosphorylation in transfected cells suggests that the Mnk1-eIF4E pathway is activited by $Kras^{G12V}$ in these cells. Although eIF4E phoshporylation can also be mediated by Mnk2, we did not observe increase in p-Mnk2 (data not shown). Interestingly, the $Kras^{G12V}$-induced Mnk-eIF4E activation is associated with strong repression of acinar transcription programe and concomitant up-regulation of ductal keratins what suggests partial acinar-to-ductal metaplasia.

In contrast to what occurs in 266-6 cells, we do not observe Mnk1 activation in mouse embryonic pancreas after tamoxifen-induced activation of $Kras^{G12V}$ expression. As assessed by Taqman based quantitative PCR assay tamoxifen injection led to recombination only in around 20% of embryonic pancreatic cells (data not shown), nevertheless we observed modest down-regulation of mRNA levels of several acinar markers including Mnk1. Surprisingly Mnk1 phosphorylation in embryo pancreata upon Kras signaling induction is reduced and there is no consistent change in eIF4E phosphorylation status. The discrepancy between the results obtained using cell line and the ones from in vivo experiments might be due to fact that the dynamics of events that lead to acinar-to-ductal metaplasia in mouse pancreas is different from that in 266-6 cells.

As Mnk1 has been shown to be crucial for oncogenic transformation (Wendel et al., 2007) and to promote tumorigenesis in mouse (Ueda et al., 2010; Furic et al., 2010), we set out to analyze its role in PDAC formation. It was particularly important to investigate, given the unusually high expression of Mnk1 in mouse acinar cells that may be at the origin of this tumour. Consistent with the fact that Mnk1 is an acinar specific gene in the pancreas, we observe its rapid downregulation during acinar-to-ductal metaplasia both in mouse and in human samples. Moreover it is essentially undetected in tubular complexes and PanINs, however it is found in some tumour cells at lower levels than in acini. There is a general acceptance in PDAC research that these tumours as opposed to acinar cell carcinomas (ACC) do not express Ptf1a and digestive enzymes (Adell et al., 2000). Consistent, in our study the tumour cells in which we detected Mnk1

did not express Ptf1a or other acinar specific genes (data not shown). Furthermore, Mnk1 is present in all PDAC cell lines, which do not express detectable levels of acinar markers (Adell et al., 2000; Collisson et al., 2011). Hence, Mnk1 expression in these tumor cells is driven by a PTF1-independent mechanism which is not surprising, given the fact that this kinase is found at relatively low levels in a variety of non-pancreatic cells.

Based on the observations from mouse and human samples we can propose 2 alternative theories regarding Mnk1 expression during PDAC development : 1) Mnk1 is down-regulated in neoplastic cells along with the whole acinar transcription program and is not necessary for PanIN cell growth, however it is reexpressed in carcinoma cells when tumour specific transcription mechanisms are activated; 2) Despite the disappearance of the acinar phenotype, Mnk1 expression is maintained at low levels in some metaplastic cells, which give rise to Mnk1 positive PanINs and carcinomas. Both scenarios are probable and it is possible that both may occur.

Since during PDAC development Mnk1 is expressed in acinar cells and in developed tumours, it is possible that it might play two roles: early – in response of acinar cells to oncogenic signalling and late – in adencarcinoma cell proliferation and survival. We decided to address its role in acinar cells. In this regard, we investigated the effect of Mnk1 depletion on precursor lesion formation in *Ptf1a*$^{+/Cre}$;*Kras*$^{+/LSLG12Vgeo}$ mouse model, analyzing mice of age of 6 months when, as we observe, tumours are not yet formed. Surprisingly, mice lacking Mnk1 develop more lesions than wild type controls. They not only have higher amount of PanIN-1 and PanIN-2 lesions but they also very often (50% of animals) present PanIN3 which is carcinoma in situ.

Ptf1a$^{+/Cre}$;*Kras*$^{+/LSLG12Vgeo}$ mice, apart from the whole spectrum of PanINs, develop other ductal lesions: mucinous metaplastic lesions (MMLs) and tubular complexes (TCs). *Ptf1a*$^{+/Cre}$;*Kras*$^{+/LSLG12Vgeo}$;*Mnk1*$^{-/-}$ animals also present MMLs, but interestingly have very few TCs. Thus, on one hand lack of Mnk1 increases number of PanINs and on the other decreases tubular complex formation. It is consistent with the finding that upon chronic pancreatitis (CP) *Mnk1*$^{-/-}$ mice develop less TCs while they express less pancreatic Ptf1a. It is therefore possible that upon Kras signaling Mnk1 favours development of tubular complexes and suppresses PanIN formation. What exact molecular mechanisms underlie

tumour suppressing function of Mnk1 remains unknown. We can speculate that Kras signaling induces stress in acinar cells which turns on mechanisms of acinar phenotype repression but in the absence of Mnk1 their response is altered what leads to preferential PanIN formation over tubular complexes. It is conceivable that the impact of Mnk1 deficiency in acinar cells on PanIN formation could be even stronger upon concomitant induction of CP. However, this should be investigated using mice in which Mnk1 is inactivated specifically in acinar cells to avoid the effect of its deficiency on inflammatory compartment.

Our finding that lack of Mnk1 increases PanIN formation in mouse is surprising, given that this kinase has so far been regarded as pro-oncogenic. In mouse, concomitant depletion of Mnk1 and Mnk2 delays lymphoma formation (Ueda et al., 2010) and similar impact on prostate cancer development has abrogation of eIF4E phosphorylation (Furic et al., 2010). However, the effect seen in these models is due to lack of eIF4E phosphorylation in neoplastic cells. In contrast, our observations concern the effect of Mnk1 depletion in acinar cells which first have to transdifferentiate in order to initiate PDAC formation. Nevertheless, it remains to be investigated whether observed increased amount of precursor lesions in the absence of Mnk1 accelerates adenocarcinoma formation and leads to elevated tumor burden in older mice.

Conclusions

Conclusions

Conclusions for objective #1

1. Mnk1, but not Mnk2, is expressed at exceptionally high levels in the pancreas where it is a novel marker of acinar cell lineage, being a direct target of pancreatic transcription factor 1 complex (PTF1).
2. In pancreatic acinar cells, the Mnk1-eIF4E pathway is activated while Mnk1 expression is down-regulated upon caerulein-induced pancreatitis and stress in vitro.

Conclusions for objective #2

3. In the absence of Mnk1, mouse pancreas develops normally. In the adult, the pancreas has an increased enzymatic protein load and decreased expression of p-eIF4E targets such as c-Myc and Ccnd1.
4. Mnk1 is involved in normal secretory response of pancreatic acinar cells and plays a protective role in caerulein-induced acute pancreatitis as it is involved in down-regulation of enzymatic protein content.
5. Pancreata from *Mnk1*$^{-/-}$ mice upon multiple episodes of pancreatitis show reduced Ptf1a expression, however form less tubular complexes.

Conclusions for objective #3

6. Mnk1 knock-down in acinar cancer cells results in down-regulation of acinar gene expression what is potentiated upon stress induction.
7. During PDAC development Mnk1 is strongly down-regulated in cells that undergo acinar-to-ductal metaplasia and is seldom detected in precursor lesions. Nevertheless it is expressed in some tumor cells in vivo and in all PDAC cell lines.
8. Mnk1 plays a suppressory role in early steps of PDAC development possibly through its impact on acinar-to-ductal metaplasia.

Conclusiones

Conclusiones para el objetivo #1

1. Mnk1, pero no Mnk2, está expresado a niveles excepcionalmente altos en el páncreas donde es un nuevo marcador de células acinares y está regulado directamente por el complejo PTF1 (pancreatic transcription factor 1).

2. En células acinares de páncreas, la ruta Mnk1-eIF4E se activa mientras que la expresión de Mnk1 disminuye tras la inducción de pancreatitis aguda con ceruleína y estrés *in vitro.*

Conclusiones para el objetivo #2

3. En ausencia de Mnk1 en ratón, el páncreas se desarrolla de manera normal. En el adulto, se detecta un aumento en el contenido enzimático del páncreas y una disminución en la expresión de las dianas de p-eIF4E, como son c-Myc y Ccnd1.

4. Mnk1 está involucrado en el mantenimiento de la respuesta secretora en células acinares pancreáticas y tiene un papel protector en pancreatitis aguda inducida por ceruleína relacionado con la disminución del contenido proteico enzimático.

5. El páncreas de ratones *Mnk1*$^{-/-}$ muestran niveles bajos de expresión de Ptf1a tras múltiples episodios de pancreatitis, a pesar de ello se detectan un número menor de complejos tubulares.

Conclusiones para objetivo #3

6. El silenciamiento de Mnk1 en células acinares tumorales provoca una disminución en la expresión de genes acinares que se potencia tras inducción de estrés.

7. Durante el desarrollo de PDAC, los niveles de Mnk1 disminuyen drasticamente en las células en las que se observa metaplasia acino-ductal y es difícilmente detectable en las lesiones precursoras. Sin embargo, se expresa en algunas células tumorales *in vivo* y en todas las líneas tumorales derivadas de PDAC analizadas.

8. Mnk1 tiene un papel supresor en las fases iniciales de desarrollo de PDAC probablemente por su impacto en la metaplasia acino-ductal.

References

Adell T, Gomez-Cuadrado A, Skoudy A, Pettengill OS, Longnecker DS and Real FX. (2000). Role of the basic helix-loop-helix transcription factor p48 in the differentiation phenotype of exocrine pancreas cancer cells. Cell Growth Differ 11: 137-147.

Adler G, Kern HF and Scheele GA. (1986). Experimental models and concepts in acute pancreatitis. The exocrine pancreas: biology, pathobiology, diseases. G. V. e. al. New York, Raven.

Aghdassi AA, Mayerle J, Christochowitz S, Weiss FU, Sendler M and Lerch MM. (2011). Animal models for investigating chronic pancreatitis. Fibrogenesis Tissue Repair 4: 26-42.

Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, Redston M and DePinho RA. (2003). ctivated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev 17: 3112-3126.

Al-Adsani A, Burke ZD, Eberhard D, Lawrence KL, Shen CN, Rustgi AK, Sakaue H, Farrant JM and Tosh D. (2010). Dexamethasone treatment induces the reprogramming of pancreatic acinar cells to hepatocytes and ductal cells. PLoS One 5: e13650.

Amundadottir L, Kraft P, Stolzenberg-Solomon RZ, Fuchs CS, Petersen GM, Arslan AA, Bueno-de-Mesquita HB, Gross M, Helzlsouer K, Jacobs EJ, LaCroix A, Zheng W, Albanes D, Bamlet W, Berg CD, Berrino F, Bingham S, Buring JE, Bracci PM, Canzian F, Clavel-Chapelon F, Clipp S, Cotterchio M, de Andrade M, Duell EJ, Fox JW, Jr., Gallinger S, Gaziano JM, Giovannucci EL, Goggins M, Gonzalez CA, Hallmans G, Hankinson SE, Hassan M, Holly EA, Hunter DJ, Hutchinson A, Jackson R, Jacobs KB, Jenab M, Kaaks R, Klein AP, Kooperberg C, Kurtz RC, Li D, Lynch SM, Mandelson M, McWilliams RR, Mendelsohn JB, Michaud DS, Olson SH, Overvad K, Patel AV, Peeters PH, Rajkovic A, Riboli E, Risch HA, Shu XO, Thomas G, Tobias GS, Trichopoulos D, Van Den Eeden SK, Virtamo J, Wactawski-Wende J, Wolpin BM, Yu H, Yu K, Zeleniuch-Jacquotte A, Chanock SJ, Hartge P and Hoover RN. (2009). Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. Nat Genet 41: 986-990.

Amundadottir LT, Thorvaldsson S, Gudbjartsson DF, Sulem P, Kristjansson K, Arnason S, Gulcher JR, Bjornsson J, Kong A, Thorsteinsdottir U and Stefansson K. (2004). Cancer as a complex phenotype: pattern of cancer distribution within and beyond the nuclear family. PLoS Med 1: e65.

Andersson K and Sundler R. (2006). Posttranscriptional regulation of TNFalpha expression via eukaryotic initiation factor 4E (eIF4E) phosphorylation in mouse macrophages. Cytokine 33: 52-57.

Apte MV and Wilson JS. (2003). Stellate cell activation in alcoholic pancreatitis. Pancreas 27: 316–320.

Archer H, Jura N, Keller J, Jacobson M and Bar-Sagi D. (2006). A mouse model of hereditary pancreatitis generated by transgenic expression of R122H trypsinogen. Gastroenterology 131: 1844–1855.

Arquier N, Bourouis M, Colombani J and Leopold P. (2005). Drosophila Lk6 kinase controls phosphorylation of eukaryotic translation initiation factor 4E and promotes normal growth and development. Curr Biol 15: 19-23.

Baeyens L, Bonne S, Bos T, Rooman I, Peleman C, Lahoutte T, German M, Heimberg H and Bouwens L. (2009). Notch signaling as gatekeeper of rat acinar-to-beta-cell conversion in vitro. Gastroenterology 136: 1750-1760 e13.

Bardeesy N, Aguirre AJ, Chu GC, Cheng KH, Lopez LV, Hezel AF, Feng B, Brennan C, Weissleder R, Mahmood U, Hanahan D, Redston MS, Chin L and Depinho RA.

(2006). Both p16(Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. Proc Natl Acad Sci U S A 103: 5947-5952.
Bassi C, Sarr MG, Lillemoe KD and Reber HA. (2008). Natural history of intraductal papillary mucinous neoplasms (IPMN): current evidence and implications for management. J Gastrointest Surg 12: 645-650.
Bayne LJ, Beatty GL, Jhala N, Clark CE, Rhim AD, Stanger BZ and Vonderheide RH. (2012). Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. Cancer Cell 21: 822-835.
Benitez CM, Goodyer WR and Kim SK. (2012). Deconstructing pancreas developmental biology. Cold Spring Harb Perspect Biol 4: a012401.
Beres TM, Masui T, Swift GH, Shi L, Henke RM and MacDonald RJ. (2006). PTF1 is an organ-specific and Notch-independent basic helix-loop-helix complex containing the mammalian Suppressor of Hairless (RBP-J) or its paralogue, RBP-L. Mol Cell Biol 26: 117-130.
Bjornsti MA and Houghton PJ. (2004). The TOR pathway: a target for cancer therapy. Nat Rev Cancer 4: 335-348.
Bonal C, Thorel F, Ait-Lounis A, Reith W, Trumpp A and Herrera PL. (2009). Pancreatic inactivation of c-Myc decreases acinar mass and transdifferentiates acinar cells into adipocytes in mice. Gastroenterology 136: 309-319 e9.
Bragado MJ, Tashiro M and Williams JA. (2000). Regulation of the initiation of pancreatic digestive enzyme protein synthesis by cholecystokinin in rat pancreas in vivo. Gastroenterology 119: 1731-1739.
Brembeck FH, Schreiber FS, Deramaudt TB, L. C, Rhoades B, Swain G, Grippo P, Stoffers DA, Silberg DG and Rustgi AK. (2003). The mutant K-ras oncogene causes pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia in transgenic mice. Cancer Res 63: 2005-2009.
Buchler M, Fiess H, Uhl W and Malfertheiner P. (2002). Chronic pancreatitits: novel concepts in biology and therapy. Oxford, Blackwell Science.
Buxade M, Morrice N, Krebs DL and Proud CG. (2008). The PSF.p54nrb complex is a novel Mnk substrate that binds the mRNA for tumor necrosis factor alpha. J Biol Chem 283: 57-65.
Buxade M, Parra JL, Rousseau S, Shpiro N, Marquez R, Morrice N, Bain J, Espel E and Proud CG. (2005). The Mnks are novel components in the control of TNF alpha biosynthesis and phosphorylate and regulate HnRNP A1. Immunity 23: 177-189.
Buxade M, Parra-Palau JL and Proud CG. (2008). The Mnks: MAP kinase-interacting kinases (MAP kinase signal-integrating kinases). Front Biosci 13: 5359-5373.
Carreira S, Fueri C, Chaix JC and Puigserver A. (1996). Dietary modulation of the mRNA stability of trypsin isozymes and the two forms of secretory trypsin inhibitor in the rat pancreas. Eur J Biochem 239: 117-123.
Case RM. (1978). Synthesis, intracellular transport and discharge of exportable proteins in the pancreatic acinar cell and other cells. Biol Rev Camb Philos Soc 53: 211-354.
Cherla RP, Lee SY, Mees PL and Tesh VL. (2006). Shiga toxin 1-induced cytokine production is mediated by MAP kinase pathways and translation initiation factor eIF4E in the macrophage-like THP-1 cell line. J Leukoc Biol 79: 397-407.
Chrestensen CA, Eschenroeder A, Ross WG, Ueda T, Watanabe-Fukunaga R, Fukunaga R and Sturgill TW. (2007). Loss of MNK function sensitizes fibroblasts to serum-withdrawal induced apoptosis. Genes Cells 12: 1133-1140.

Chrestensen CA, Shuman JK, Eschenroeder A, Worthington M, Gram H and Sturgill TW. (2007). MNK1 and MNK2 regulation in HER2-overexpressing breast cancer lines. J Biol Chem 282: 4243-4252.

Cohn JA, Neoptolemos JP, Feng J, Yan J, Jiang Z, Greenhalf W, McFaul C, Mountford R and Sommer SS. (2005). Increased risk of idiopathic chronic pancreatitis in cystic fibrosis carriers. Hum Mutat 26: 303-307.

Collisson EA, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, Cooc J, Weinkle J, Kim GE, Jakkula L, Feiler HS, Ko AH, Olshen AB, Danenberg KL, Tempero MA, Spellman PT, Hanahan D and Gray JW. (2011). Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med 17: 500-503.

Crozier SJ, Sans MD, Guo L, D'Alecy LG and Williams JA. (2006). Activation of the mTOR signalling pathway is required for pancreatic growth in protease-inhibitor-fed mice. J Physiol 573: 775-786.

Czako L, Takacs T, Varga IS, Tiszlavicz L, Hai DQ, Hegyi P, Matkovics B and Lonovics J. (1998). Involvement of oxygen-derived free radicals in L-arginine-induced acute pancreatitis. Dig Dis Sci 43: 1770-1777.

Dabrowski A, Grady T, Logsdon CD and Williams JA. (1996). Jun kinases are rapidly activated by cholecystokinin in rat pancreas both in vitro and in vivo. J Biol Chem 271: 5686-5690.

DaSilva J, Xu L, Kim HJ, Miller WT and Bar-Sagi D. (2006). Regulation of sprouty stability by Mnk1-dependent phosphorylation. Mol Cell Biol 26: 1898-1907.

De Benedetti A and Harris AL. (1999). eIF4E expression in tumors: its possible role in progression of malignancies. Int J Biochem Cell Biol 31: 59-72.

Dembinski A, Warzecha Z, Konturek PC, Ceranowicz P, Stachura J, Tomaszewska R and Konturek SJ. (2000). Epidermal growth factor accelerates pancreatic recovery after caerulein-induced pancreatitis. Eur J Pharmacol 398: 159-168.

Dobrikov M, Dobrikova E, Shveygert M and Gromeier M. (2011). Phosphorylation of eukaryotic translation initiation factor 4G1 (eIF4G1) by protein kinase C{alpha} regulates eIF4G1 binding to Mnk1. Mol Cell Biol 31: 2947-2959.

Dolniak B, Katsoulidis E, Carayol N, Altman JK, Redig AJ, Tallman MS, Ueda T, Watanabe-Fukunaga R, Fukunaga R and Platanias LC. (2008). Regulation of arsenic trioxide-induced cellular responses by Mnk1 and Mnk2. J Biol Chem 283: 12034-12042.

Dreiling DA and Koller M. (1985). The natural history of alcoholic pancreatitis: update 1985. Mt Sinai J Med 52: 340-342.

Dreissigacker U, Mueller MS, Unger M, Siegert P, Genze F, Gierschik P and Giehl K. (2006). Oncogenic K-Ras down-regulates Rac1 and RhoA activity and enhances migration and invasion of pancreatic carcinoma cells through activation of p38. Cell Signal 18: 1156-1168.

Duncan R, Milburn SC and Hershey JW. (1987). Regulated phosphorylation and low abundance of HeLa cell initiation factor eIF-4F suggest a role in translational control. Heat shock effects on eIF-4F. J Biol Chem 262: 380-388.

Durbec JP and Sarles H. (1978). Multicenter survey of the etiology of pancreatic diseases. Relationship between the relative risk of developing chronic pancreaitis and alcohol, protein and lipid consumption. Digestion 18: 337-350.

Eilers M and Eisenman RN. (2008). Myc's broad reach. Genes Dev 22: 2755-2766.

Esni F, Ghosh B, Biankin AV, Lin JW, Albert MA, Yu X, MacDonald RJ, Civin CI, Real FX, Pack MA, Ball DW and Leach SD. (2004). Notch inhibits Ptf1 function and acinar cell

differentiation in developing mouse and zebrafish pancreas. Development 131: 4213-4224.
Farquhar MG and Palade GE. (1998). The Golgi apparatus: 100 years of progress and controversy. Trends Cell Biol 8: 2-10.
Fayard E, Schoonjans K, Annicotte JS and Auwerx J. (2003). Liver receptor homolog 1 controls the expression of carboxyl ester lipase. J Biol Chem 278: 35725-35731.
Ferlay J S-FE, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, Forman D, Bray F. (2013). Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer, in press.
Foitzik T, Lewandrowski KB, Fernandez-del Castillo C, Rattner DW, Klar E and Warshaw AL. (1994). Exocrine hyperstimulation but not pancreatic duct obstruction increases the susceptibility to alcohol-related pancreatic injury. Arch Surg 129: 1081-1085.
Fukuda A, Wang SC, Morris JPt, Folias AE, Liou A, Kim GE, Akira S, Boucher KM, Firpo MA, Mulvihill SJ and Hebrok M. (2011). Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. Cancer Cell 19: 441-455.
Fukunaga R and Hunter T. (1997). MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. Embo J 16: 1921-1933.
Furic L, Rong L, Larsson O, Koumakpayi IH, Yoshida K, Brueschke A, Petroulakis E, Robichaud N, Pollak M, Gaboury LA, Pandolfi PP, Saad F and Sonenberg N. (2010). eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. Proc Natl Acad Sci U S A 107: 14134-14139.
Gaiser S, Daniluk J, Liu Y, Tsou L, Chu J, Lee W, Longnecker DS, Logsdon CD and Ji B. (2011). Intracellular activation of trypsinogen in transgenic mice induces acute but not chronic pancreatitis. Gut 60: 1379-1388.
Gittes GK. (2009). Developmental biology of the pancreas: a comprehensive review. Dev Biol 326: 4-35.
Gorentla BK, Krishna S, Shin J, Inoue M, Shinohara ML, Grayson JM, Fukunaga R and Zhong XP. (2013). Mnk1 and 2 are dispensable for T cell development and activation but important for the pathogenesis of experimental autoimmune encephalomyelitis. J Immunol 190: 1026-1037.
Goto S, Yao Z and Proud CG. (2009). The C-terminal domain of Mnk1a plays a dual role in tightly regulating its activity. Biochem J 423: 279-290.
Grady T, Dabrowski A, Williams JA and Logsdon CD. (1996). Stress-activated protein kinase activation is the earliest direct correlate to the induction of secretagogue-induced pancreatitis in rats. Biochem Biophys Res Commun 227: 1-7.
Grady T, Liang P, Ernst SA and Logsdon CD. (1997). Chemokine gene expression in rat pancreatic acinar cells is an early event associated with acute pancreatitis. Gastroenterology 113: 1966-1975.
Grady T, Mah'Moud M, Otani T, Rhee S, Lerch MM and Gorelick FS. (1998). Zymogen proteolysis within the pancreatic acinar cell is associated with cellular injury. Am J Physiol 275: G1010-1017.
Grady T, Saluja A, Kaiser A and Steer M. (1996). Edema and intrapancreatic trypsinogen activation precede glutathione depletion during caerulein pancreatitis. Am J Physiol 271: G20-26.
Grasso D, Ropolo A, Lo Re A, Boggio V, Molejon MI, Iovanna JL, Gonzalez CD, Urrutia R and Vaccaro MI. (2011). Zymophagy, a novel selective autophagy pathway

mediated by VMP1-USP9x-p62, prevents pancreatic cell death. J Biol Chem 286: 8308-8324.

Grippo PJ and Sandgren EP. (2012). Acinar-to-ductal metaplasia accompanies c-myc-induced exocrine pancreatic cancer progression in transgenic rodents. Int J Cancer 131: 1243-1248.

Grund EM, Spyropoulos DD, Watson DK and Muise-Helmericks RC. (2005). Interleukins 2 and 15 regulate Ets1 expression via ERK1/2 and MNK1 in human natural killer cells. J Biol Chem 280: 4772-4778.

Grzmil M, Morin P, Jr., Lino MM, Merlo A, Frank S, Wang Y, Moncayo G and Hemmings BA. (2011). MAP kinase-interacting kinase 1 regulates SMAD2-dependent TGF-beta signaling pathway in human glioblastoma. Cancer Res 71: 2392-2402.

Gu G, Dubauskaite J and Melton DA. (2002). Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. Development 129: 2447-2457.

Guerra C, Collado M, Navas C, Schuhmacher AJ, Hernandez-Porras I, Canamero M, Rodriguez-Justo M, Serrano M and Barbacid M. (2011). Pancreatitis-induced inflammation contributes to pancreatic cancer by inhibiting oncogene-induced senescence. Cancer Cell 19: 728-739.

Guerra C, Schuhmacher AJ, Canamero M, Grippo PJ, Verdaguer L, Perez-Gallego L, Dubus P, Sandgren EP and Barbacid M. (2007). Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell 11: 291-302.

Guil S, Long JC and Caceres JF. (2006). HnRNP A1 relocalization to the stress granules reflects a role in the stress response. Mol Cell Biol 26: 5744-5758.

Gukovskaya AS and Gukovsky I. Which way to die: the regulation of acinar cell death in pancreatitis by mitochondria, calcium, and reactive oxygen species. Gastroenterology 140: 1876-1880.

Gukovskaya AS, Gukovsky I, Zaninovic V, Song M, Sandoval D, Gukovsky S and Pandol SJ. (1997). Pancreatic acinar cells produce, release, and respond to tumor necrosis factor-alpha. Role in regulating cell death and pancreatitis. J Clin Invest 100: 1853-1862.

Gukovsky I, Gukovskaya AS, Blinman TA, Zaninovic V and Pandol SJ. (1998). Early NF-kappaB activation is associated with hormone-induced pancreatitis. Am J Physiol 275: G1402-1414.

Guz Y, Montminy MR, Stein R, Leonard J, Gamer LW, Wright CV and Teitelman G. (1995). Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. Development 121: 11-18.

Halangk W, Lerch MM, Brandt-Nedelev B, Roth W, Ruthenbuerger M, Reinheckel T, Domschke W, Lippert H, Peters C and Deussing J. (2000). Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. J Clin Invest 106: 773-781.

Hale MA, Kagami H, Shi L, Holland AM, Elsasser HP, Hammer RE and MacDonald RJ. (2005). The homeodomain protein PDX1 is required at mid-pancreatic development for the formation of the exocrine pancreas. Dev Biol 286: 225-237.

Han B and Logsdon CD. (1999). Cholecystokinin induction of mob-1 chemokine expression in pancreatic acinar cells requires NF-kappaB activation. Am J Physiol 277: C74-82.

Hashi M, Yoshizawa F, Onozuka E, Ogata M and Hara H. (2005). Adaptive changes in translation initiation activities for rat pancreatic protein synthesis with feeding of a high-protein diet. J Nutr Biochem 16: 507-512.

Hashimoto N and Hara H. (2003). Dietary amino acids promote pancreatic protease synthesis at the translation stage in rats. J Nutr 133: 3052-7.

Hegyi P, Rakonczay Z, Jr., Sari R, Gog C, Lonovics J, Takacs T and Czako L. (2004). L-arginine-induced experimental pancreatitis. World J Gastroenterol 10: 2003-2009.

Hernandez-Munoz I, Skoudy A, Real FX and Navarro P. (2008). Pancreatic ductal adenocarcinoma: cellular origin, signaling pathways and stroma contribution. Pancreatology 8: 462-469.

Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N and Depinho RA. (2006). Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev 20: 1218-49.

Hidalgo M. (2010). Pancreatic cancer. N Engl J Med 362: 1605-1617.

Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, Ross S, Conrads TP, Veenstra TD, Hitt BA, Kawaguchi Y, Johann D, Liotta LA, Crawford HC, Putt ME, Jacks T, Wright CV, Hruban RH, Lowy AM and Tuveson DA. (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 4: 437-450.

Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH, Rustgi AK, Chang S and Tuveson DA. (2005). Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. Cancer Cell 7: 469-483.

Hiremath LS, Webb NR and Rhoads RE. (1985). Immunological detection of the messenger RNA cap-binding protein. J Biol Chem 260: 7843-7849.

Holmstrom SR, Deering T, Swift GH, Poelwijk FJ, Mangelsdorf DJ, Kliewer SA and MacDonald RJ. (2011). LRH-1 and PTF1-L coregulate an exocrine pancreas-specific transcriptional network for digestive function. Genes Dev 25: 1674-1679.

Hong SM, Heaphy CM, Shi C, Eo SH, Cho H, Meeker AK, Eshleman JR, Hruban RH and Goggins M. (2011). Telomeres are shortened in acinar-to-ductal metaplasia lesions associated with pancreatic intraepithelial neoplasia but not in isolated acinar-to-ductal metaplasias. Mod Pathol 24: 256-266.

Hou J, Lam F, Proud C and Wang S. (2012). Targeting Mnks for cancer therapy. Oncotarget 3: 118-131.

Houbracken I, de Waele E, Lardon J, Ling Z, Heimberg H, Rooman I and Bouwens L. (2011). Lineage tracing evidence for transdifferentiation of acinar to duct cells and plasticity of human pancreas. Gastroenterology 141: 731-41, 741 e1-4.

Hruban RH KD, Pitman MB. (2006). Tumors of the Pancreas. Washington, DC, Armed Forces Inst. Pathol.

Hu SI, Katz M, Chin S, Qi X, Cruz J, Ibebunjo C, Zhao S, Chen A and Glass DJ. (2012). MNK2 inhibits eIF4G activation through a pathway involving serine-arginine-rich protein kinase in skeletal muscle. Sci Signal 5: ra14.

Huang AM and Rubin GM. (2000). A misexpression screen identifies genes that can modulate RAS1 pathway signaling in Drosophila melanogaster. Genetics 156: 1219-1230.

Iacobuzio-Donahue CA, Ashfaq R, Maitra A, Adsay NV, Shen-Ong GL, Berg K, Hollingsworth MA, Cameron JL, Yeo CJ, Kern SE, Goggins M and Hruban RH. (2003). Highly expressed genes in pancreatic ductal adenocarcinomas: a comprehensive

characterization and comparison of the transcription profiles obtained from three major technologies. Cancer Res 63: 8614-8622.

Ijichi H, Chytil A, Gorska AE, Aakre ME, Bierie B, Tada M, Mohri D, Miyabayashi K, Asaoka Y, Maeda S, Ikenoue T, Tateishi K, Wright CV, Koike K, Omata M and Moses HL. (2011). Inhibiting Cxcr2 disrupts tumor-stromal interactions and improves survival in a mouse model of pancreatic ductal adenocarcinoma. J Clin Invest 121: 4106-4117.

Ijichi H, Chytil A, Gorska AE, Aakre ME, Fujitani Y, Fujitani S, Wright CV and Moses HL. (2006). Aggressive pancreatic ductal adenocarcinoma in mice caused by pancreas-specific blockade of transforming growth factor-beta signaling in cooperation with active Kras expression. Genes Dev 20: 3147-3160.

Imamura T, Asada M, Vogt SK, Rudnick DA, Lowe ME and Muglia LJ. (2002). Protection from pancreatitis by the zymogen granule membrane protein integral membrane-associated protein-1. J Biol Chem 277: 50725-50733.

Iovanna JL, Lechene de la Porte P and Dagorn JC. (1992). Expression of genes associated with dedifferentiation and cell proliferation during pancreatic regeneration following acute pancreatitis. Pancreas 7: 712-718.

Jauch R, Cho MK, Jakel S, Netter C, Schreiter K, Aicher B, Zweckstetter M, Jackle H and Wahl MC. (2006). Mitogen-activated protein kinases interacting kinases are autoinhibited by a reprogrammed activation segment. Embo J 25: 4020-4032.

Jauch R, Jakel S, Netter C, Schreiter K, Aicher B, Jackle H and Wahl MC. (2005). Crystal structures of the Mnk2 kinase domain reveal an inhibitory conformation and a zinc binding site. Structure 13: 1559-1568.

Jensen J, Pedersen EE, Galante P, Hald J, Heller RS, Ishibashi M, Kageyama R, Guillemot F, Serup P and Madsen OD. (2000). Control of endodermal endocrine development by Hes-1. Nat Genet 24: 36-44.

Jensen JN, Cameron E, Garay MV, Starkey TW, Gianani R and Jensen J. (2005). Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. Gastroenterology 128: 728-741.

Jessop NW and Hay RJ. (1980). Characteristics of two rat pancreatic exocrine cell lines derived from transplantable tumors. In Vitro 16: 212.

Joshi S, Kaur S, Redig AJ, Goldsborough K, David K, Ueda T, Watanabe-Fukunaga R, Baker DP, Fish EN, Fukunaga R and Platanias LC. (2009). Type I interferon (IFN)-dependent activation of Mnk1 and its role in the generation of growth inhibitory responses. Proc Natl Acad Sci U S A 106: 12097-12102.

Joshi S, Sharma B, Kaur S, Majchrzak B, Ueda T, Fukunaga R, Verma AK, Fish EN and Platanias LC. (2011). Essential role for Mnk kinases in type II interferon (IFNgamma) signaling and its suppressive effects on normal hematopoiesis. J Biol Chem 286: 6017-6026.

Kawaguchi Y, Cooper B, Gannon M, Ray M, MacDonald RJ and Wright CV. (2002). The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat Genet 32: 128-134.

Keller SA, Rosenberg MP, Johnson TM, Howard G and Meisler MH. (1990). Regulation of amylase gene expression in diabetic mice is mediated by a cis-acting upstream element close to the pancreas-specific enhancer. Genes Dev 4: 1316-1321.

Kesavan G, Sand FW, Greiner TU, Johansson JK, Kobberup S, Wu X, Brakebusch C and Semb H. (2009). Cdc42-mediated tubulogenesis controls cell specification. Cell 139: 791-801.

Kidd D and Raff JW. (1997). LK6, a short lived protein kinase in Drosophila that can associate with microtubules and centrosomes. J Cell Sci 110 (Pt 2): 209-219.

Kim HJ and Bar-Sagi D. (2004). Modulation of signalling by Sprouty: a developing story. Nat Rev Mol Cell Biol 5: 441-450.

Kiraly O, Boulling A, Witt H, Le Marechal C, Chen JM, Rosendahl J, Battaggia C, Wartmann T, Sahin-Toth M and Ferec C. (2007). Signal peptide variants that impair secretion of pancreatic secretory trypsin inhibitor (SPINK1) cause autosomal dominant hereditary pancreatitis. Hum Mutat 28: 469-476.

Kishino Y and Kawamura S. (1984). Pancreatic damage induced by injecting a large dose of arginine. Virchows Arch B Cell Pathol Incl Mol Pathol 47: 147-155.

Kjellerup RB, Kragballe K, Iversen L and Johansen C. (2008). Pro-inflammatory cytokine release in keratinocytes is mediated through the MAPK signal-integrating kinases. Exp Dermatol 17: 498-504.

Klein AP, Brune KA, Petersen GM, Goggins M, Tersmette AC, Offerhaus GJ, Griffin C, Cameron JL, Yeo CJ, Kern S and Hruban RH. (2004). Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. Cancer Res 64: 2634-2638.

Klimstra DS. (2007). Nonductal neoplasms of the pancreas. Mod Pathol 20 Suppl 1: S94-112.

Knauf U, Tschopp C and Gram H. (2001). Negative regulation of protein translation by mitogen-activated protein kinase-interacting kinases 1 and 2. Mol Cell Biol 21: 5500-5511.

Kopinke D and Murtaugh LC. (2010). Exocrine-to-endocrine differentiation is detectable only prior to birth in the uninjured mouse pancreas. BMC Dev Biol 10: 38.

Kopp JL, von Figura G, Mayes E, Liu FF, Dubois CL, Morris JPt, Pan FC, Akiyama H, Wright CV, Jensen K, Hebrok M and Sander M. (2012). Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. Cancer Cell 22: 737-750.

Korneeva NL, Soung YH, Kim HI, Giordano A, Rhoads RE, Gram H and Chung J. (2010). Mnk mediates integrin alpha6beta4-dependent eIF4E phosphorylation and translation of VEGF mRNA. Mol Cancer Res 8: 1571-1578.

Kotani J, Usami M, Nomura H, Iso A, Kasahara H, Kuroda Y, Oyanagi H and Saitoh Y. (1999). Enteral nutrition prevents bacterial translocation but does not improve survival during acute pancreatitis. Arch Surg 134: 287-292.

Krapp A, Knofler M, Frutiger S, Hughes GJ, Hagenbuchle O and Wellauer PK. (1996). The p48 DNA-binding subunit of transcription factor PTF1 is a new exocrine pancreas-specific basic helix-loop-helix protein. Embo J 15: 4317-4329.

Krapp A, Knofler M, Ledermann B, Burki K, Berney C, Zoerkler N, Hagenbuchle O and Wellauer PK. (1998). The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. Genes Dev 12: 3752-3763.

Kruse F, Komro CT, Michnoff CH and MacDonald RJ. (1988). The cell-specific elastase I enhancer comprises two domains. Mol Cell Biol 8: 893-902.

Kubisch CH, Sans MD, Arumugam T, Ernst SA, Williams JA and Logsdon CD. (2006). Early activation of endoplasmic reticulum stress is associated with arginine-induced acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 291: G238-245.

Kume K, Masamune A, Kikuta K and Shimosegawa T. (2006). [-215G>A; IVS3+2T>C] mutation in the SPINK1 gene causes exon 3 skipping and loss of the trypsin binding site. Gut 55: 1214.

Lachance PE, Miron M, Raught B, Sonenberg N and Lasko P. (2002). Phosphorylation of eukaryotic translation initiation factor 4E is critical for growth. Mol Cell Biol 22: 1656-1663.

Lardon J, De Breuck S, Rooman I, Van Lommel L, Kruhoffer M, Orntoft T, Schuit F and Bouwens L. (2004). Plasticity in the adult rat pancreas: transdifferentiation of exocrine to hepatocyte-like cells in primary culture. Hepatology 39: 1499-1507.

Lazaris-Karatzas A, Montine KS and Sonenberg N. (1990). Malignant transformation by a eukaryotic initiation factor subunit that binds to mRNA 5' cap. Nature 345: 544-7.

Lazaris-Karatzas A and Sonenberg N. (1992). The mRNA 5' cap-binding protein, eIF-4E, cooperates with v-myc or E1A in the transformation of primary rodent fibroblasts. Mol Cell Biol 12: 1234-1238.

Le Petit-Thevenin J, Verine A, Nganga A, Nobili O, Lombardo D and Bruneau N. (2001). Impairment of bile salt-dependent lipase secretion in AR4-2J rat pancreatic cells induces its degradation by the proteasome. Biochim Biophys Acta 1530: 184-198.

Lechene de la Porte P, Iovanna J, Odaira C, Choux R, Sarles H and Berger Z. (1991). Involvement of tubular complexes in pancreatic regeneration after acute necrohemorrhagic pancreatitis. Pancreas 6: 298-306.

Lerch MM, Saluja AK, Runzi M, Dawra R, Saluja M and Steer ML. (1993). Pancreatic duct obstruction triggers acute necrotizing pancreatitis in the opossum. Gastroenterology 104: 853-861.

Lesina M, Kurkowski MU, Ludes K, Rose-John S, Treiber M, Kloppel G, Yoshimura A, Reindl W, Sipos B, Akira S, Schmid RM and Algul H. (2011). Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. Cancer Cell 19: 456-469.

Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andren-Sandberg A and Domellof L. (1993). Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. N Engl J Med 328: 1433-1437.

Lowery MA, Klimstra DS, Shia J, Yu KH, Allen PJ, Brennan MF and O'Reilly EM. (2011). Acinar cell carcinoma of the pancreas: new genetic and treatment insights into a rare malignancy. Oncologist 16: 1714-1720.

Lu Z, Karne S, Kolodecik T and Gorelick FS. (2002). Alcohols enhance caerulein-induced zymogen activation in pancreatic acinar cells. Am J Physiol Gastrointest Liver Physiol 282: G501-507.

Ludwig CU, Menke A, Adler G and Lutz MP. (1999). Fibroblasts stimulate acinar cell proliferation through IGF-I during regeneration from acute pancreatitis. Am J Physiol 276: G193-198.

Luo X, Shin DM, Wang X, Konieczny SF and Muallem S. (2005). Aberrant localization of intracellular organelles, Ca2+ signaling, and exocytosis in Mist1 null mice. J Biol Chem 280: 12668-12675.

Luthen RE, Niederau C and Grendell JH. (1994). Glutathione and ATP levels, subcellular distribution of enzymes, and permeability of duct system in rabbit pancreas following intravenous administration of alcohol and cerulein. Dig Dis Sci 39: 871-879.

MacDonald RJ, Swift GH and Real FX. (2010). Transcriptional control of acinar development and homeostasis. Prog Mol Biol Transl Sci 97: 1-40.

Magenheim J, Ilovich O, Lazarus A, Klochendler A, Ziv O, Werman R, Hija A, Cleaver O, Mishani E, Keshet E and Dor Y. (2011). Blood vessels restrain pancreas branching, differentiation and growth. Development 138: 4743-4752.

Maitra A and Hruban RH. (2008). Pancreatic cancer. Annu Rev Pathol 3: 157-188.

Malka D, Hammel P, Maire F, Rufat P, Madeira I, Pessione F, Levy P and Ruszniewski P. (2002). Risk of pancreatic adenocarcinoma in chronic pancreatitis. Gut 51: 849-852.

Marcotrigiano J, Gingras AC, Sonenberg N and Burley SK. (1997). Cocrystal structure of the messenger RNA 5' cap-binding protein (eIF4E) bound to 7-methyl-GDP. Cell 89: 951-961.

Martin RC, Klimstra DS, Brennan MF and Conlon KC. (2002). Solid-pseudopapillary tumor of the pancreas: a surgical enigma? Ann Surg Oncol 9: 35-40.

Martinelli P, Canamero M, Del Pozo N, Madriles F, Zapata A and Real FX. (2012). Gata6 is required for complete acinar differentiation and maintenance of the exocrine pancreas in adult mice. Gut.

Masui T, Long Q, Beres TM, Magnuson MA and MacDonald RJ. (2007). Early pancreatic development requires the vertebrate Suppressor of Hairless (RBPJ) in the PTF1 bHLH complex. Genes Dev 21: 2629-2643.

Masui T, Swift GH, Deering T, Shen C, Coats WS, Long Q, Elsasser HP, Magnuson MA and MacDonald RJ. (2010). Replacement of Rbpj with Rbpjl in the PTF1 complex controls the final maturation of pancreatic acinar cells. Gastroenterology 139: 270-280.

Masui T, Swift GH, Hale MA, Meredith DM, Johnson JE and Macdonald RJ. (2008). Transcriptional autoregulation controls pancreatic Ptf1a expression during development and adulthood. Mol Cell Biol 28: 5458-5468.

Mayerle J, Schnekenburger J, Kruger B, Kellermann J, Ruthenburger M, Weiss FU, Nalli A, Domschke W and Lerch MM. (2005). Extracellular cleavage of E-cadherin by leukocyte elastase during acute experimental pancreatitis in rats. Gastroenterology 129: 1251-1267.

Mazzon E, Impellizzeri D, Di Paola R, Paterniti I, Esposito E, Cappellani A, Bramanti P and Cuzzocrea S. (2012). Effects of mitogen-activated protein kinase signaling pathway inhibition on the development of cerulein-induced acute pancreatitis in mice. Pancreas 41: 560-570.

Means AL, Meszoely IM, Suzuki K, Miyamoto Y, Rustgi AK, Coffey RJ, Jr., Wright CV, Stoffers DA and Leach SD. (2005). Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. Development 132: 3767-3776.

Meister T, Niehues R, Hahn D, Domschke W, Sendler M, Lerch MM and Schnekenburger J. (2010). Missorting of cathepsin B into the secretory compartment of CI-MPR/IGFII-deficient mice does not induce spontaneous trypsinogen activation but leads to enhanced trypsin activity during experimental pancreatitis--without affecting disease severity. J Physiol Pharmacol 61: 565-575.

Minich WB, Balasta ML, Goss DJ and Rhoads RE. (1994). Chromatographic resolution of in vivo phosphorylated and nonphosphorylated eukaryotic translation initiation factor eIF-4E: increased cap affinity of the phosphorylated form. Proc Natl Acad Sci U S A 91: 7668-7672.

Mizuno N, Naruse S, Kitagawa M, Ishiguro H and Hayakawa T. (2000). Effects of phospholipase A2 inhibitors on Ca2+ oscillations in pancreatic acinar cells. Pancreas 20: 77-83.

Mizunuma T, Kawamura S and Kishino Y. (1984). Effects of injecting excess arginine on rat pancreas. J Nutr 114: 467-471.

Molero X, Adell T, Skoudy A, Padilla MA, Gomez JA, Chalaux E, Malagelada JR and Real FX. (2007). Pancreas transcription factor 1alpha expression is regulated in pancreatitis. Eur J Clin Invest 37: 791-801.

Molero X, Vaquero EC, Flandez M, Gonzalez AM, Ortiz MA, Cibrian-Uhalte E, Servitja JM, Merlos A, Juanpere N, Massumi M, Skoudy A, Macdonald R, Ferrer J and Real FX. (2012). Gene expression dynamics after murine pancreatitis unveils novel roles for Hnf1alpha in acinar cell homeostasis. Gut 61: 1187-1196.

Mulkeen AL, Yoo PS and Cha C. (2006). Less common neoplasms of the pancreas. World J Gastroenterol 12: 3180-3185.

Nakhai H, Siveke JT, Mendoza-Torres L and Schmid RM. (2008). Conditional inactivation of Myc impairs development of the exocrine pancreas. Development 135: 3191-3196.

Nemoda Z and Sahin-Toth M. (2006). Chymotrypsin C (caldecrin) stimulates autoactivation of human cationic trypsinogen. J Biol Chem 281: 11879-11886.

Neuzillet C, Hammel P, Tijeras-Raballand A, Couvelard A and Raymond E. (2012). Targeting the Ras-ERK pathway in pancreatic adenocarcinoma. Cancer Metastasis Rev.

Niedzwiecka A, Marcotrigiano J, Stepinski J, Jankowska-Anyszka M, Wyslouch-Cieszynska A, Dadlez M, Gingras AC, Mak P, Darzynkiewicz E, Sonenberg N, Burley SK and Stolarski R. (2002). Biophysical studies of eIF4E cap-binding protein: recognition of mRNA 5' cap structure and synthetic fragments of eIF4G and 4E-BP1 proteins. J Mol Biol 319: 615-635.

Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL and Wright CV. (1996). PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 122: 983-995.

Ohlsson H, Karlsson K and Edlund T. (1993). IPF1, a homeodomain-containing transactivator of the insulin gene. Embo J 12: 4251-429.

O'Loghlen A, Gonzalez VM, Jurado T, Salinas M and Martin ME. (2007). Characterization of the activity of human MAP kinase-interacting kinase Mnk1b. Biochim Biophys Acta 1773: 1416-1427.

O'Loghlen A, Gonzalez VM, Pineiro D, Perez-Morgado MI, Salinas M and Martin ME. (2004). Identification and molecular characterization of Mnk1b, a splice variant of human MAP kinase-interacting kinase Mnk1. Exp Cell Res 299: 343-355.

Origanti S and Shantz LM. (2007). Ras transformation of RIE-1 cells activates cap-independent translation of ornithine decarboxylase: regulation by the Raf/MEK/ERK and phosphatidylinositol 3-kinase pathways. Cancer Res 67: 4834-4842.

Ornitz DM, Palmiter RD, Messing A, Hammer RE, Pinkert CA and Brinster RL. (1985). Elastase I promoter directs expression of human growth hormone and SV40 T antigen genes to pancreatic acinar cells in transgenic mice. Cold Spring Harb Symp Quant Biol 50: 399-409.

Pandol SJ. (2010). The exocrine pancreas. San Rafael (CA), Morgan & Claypool Life Sciences.

Pandol SJ, Periskic S, Gukovsky I, Zaninovic V, Jung Y, Zong Y, Solomon TE, Gukovskaya AS and Tsukamoto H. (1999). Ethanol diet increases the sensitivity of rats to pancreatitis induced by cholecystokinin octapeptide. Gastroenterology 117: 706-716.

Parra JL, Buxade M and Proud CG. (2005). Features of the catalytic domains and C termini of the MAPK signal-integrating kinases Mnk1 and Mnk2 determine their differing activities and regulatory properties. J Biol Chem 280: 37623-37633.

Parra-Palau JL, Scheper GC, Harper DE and Proud CG. (2005). The Drosophila protein kinase LK6 is regulated by ERK and phosphorylates the eukaryotic initiation factor eIF4E in vivo. Biochem J 385: 695-702.

Parra-Palau JL, Scheper GC, Wilson ML and Proud CG. (2003). Features in the N and C termini of the MAPK-interacting kinase Mnk1 mediate its nucleocytoplasmic shuttling. J Biol Chem 278: 44197-44204.

Pemberton L, Taylor-Papadimitriou J and Gendler SJ. (1992). Antibodies to the cytoplasmic domain of the MUC1 mucin show conservation throughout mammals. Biochem Biophys Res Commun 185: 167-175.

Petersen GM, Amundadottir L, Fuchs CS, Kraft P, Stolzenberg-Solomon RZ, Jacobs KB, Arslan AA, Bueno-de-Mesquita HB, Gallinger S, Gross M, Helzlsouer K, Holly EA, Jacobs EJ, Klein AP, LaCroix A, Li D, Mandelson MT, Olson SH, Risch HA, Zheng W, Albanes D, Bamlet WR, Berg CD, Boutron-Ruault MC, Buring JE, Bracci PM, Canzian F, Clipp S, Cotterchio M, de Andrade M, Duell EJ, Gaziano JM, Giovannucci EL, Goggins M, Hallmans G, Hankinson SE, Hassan M, Howard B, Hunter DJ, Hutchinson A, Jenab M, Kaaks R, Kooperberg C, Krogh V, Kurtz RC, Lynch SM, McWilliams RR, Mendelsohn JB, Michaud DS, Parikh H, Patel AV, Peeters PH, Rajkovic A, Riboli E, Rodriguez L, Seminara D, Shu XO, Thomas G, Tjonneland A, Tobias GS, Trichopoulos D, Van Den Eeden SK, Virtamo J, Wactawski-Wende J, Wang Z, Wolpin BM, Yu H, Yu K, Zeleniuch-Jacquotte A, Fraumeni JF, Jr., Hoover RN, Hartge P and Chanock SJ. (2010). A genome-wide association study identifies pancreatic cancer susceptibility loci on chromosomes 13q22.1, 1q32.1 and 5p15.33. Nat Genet 42: 224-228.

Petersen OH and Tepikin AV. (2008). Polarized calcium signaling in exocrine gland cells. Annu Rev Physiol 70: 273-299.

Petrucco S, Wellauer PK and Hagenbuchle O. (1990). The DNA-binding activity of transcription factor PTF1 parallels the synthesis of pancreas-specific mRNAs during mouse development. Mol Cell Biol 10: 254-264.

Phillips A and Blaydes JP. (2008). MNK1 and EIF4E are downstream effectors of MEKs in the regulation of the nuclear export of HDM2 mRNA. Oncogene 27: 1645-1649.

Phillips PA, McCarroll JA, Park S, Wu MJ, Pirola R, Korsten M, Wilson JS and Apte MV. (2003). Rat pancreatic stellate cells secrete matrix metalloproteinases: implications for extracellular matrix turnover. Gut 52: 275-282.

Pinho AV, Rooman I, Reichert M, De Medts N, Bouwens L, Rustgi AK and Real FX. (2011). Adult pancreatic acinar cells dedifferentiate to an embryonic progenitor phenotype with concomitant activation of a senescence programme that is present in chronic pancreatitis. Gut 60: 958-966.

Ponnappa BC, Hoek JB, Waring AJ and Rubin E. (1987). Effect of ethanol on amylase secretion and cellular calcium homeostasis in pancreatic acini from normal and ethanol-fed rats. Biochem Pharmacol 36: 69-79.

Pontoglio M, Barra J, Hadchouel M, Doyen A, Kress C, Bach JP, Babinet C and Yaniv M. (1996). Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. Cell 84: 575-585.

Powers RE, Grady T, Orchard JL and Gilrane TB. (1993). Different effects of hyperstimulation by similar classes of secretagogues on the exocrine pancreas. Pancreas 8: 58-63.

Puri S and Hebrok M. (2010). Cellular plasticity within the pancreas--lessons learned from development. Dev Cell 18: 342-356.

Pylayeva-Gupta Y, Lee KE, Hajdu CH, Miller G and Bar-Sagi D. (2012). Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. Cancer Cell 21: 836-847.

Pyronnet S, Dostie J and Sonenberg N. (2001). Suppression of cap-dependent translation in mitosis. Genes Dev 15: 2083-2093.

Pyronnet S, Imataka H, Gingras AC, Fukunaga R, Hunter T and Sonenberg N. (1999). Human eukaryotic translation initiation factor 4G (eIF4G) recruits mnk1 to phosphorylate eIF4E. Embo J 18: 270-279.

Raimondi S, Maisonneuve P and Lowenfels AB. (2009). Epidemiology of pancreatic cancer: an overview. Nat Rev Gastroenterol Hepatol 6: 699-708.

Ramnath RD, Sun J and Bhatia M. (2010). PKC delta mediates pro-inflammatory responses in a mouse model of caerulein-induced acute pancreatitis. J Mol Med (Berl) 88: 1055-1063.

Rannou Y, Salaun P, Benaud C, Khan J, Dutertre S, Giet R and Prigent C. (2012). MNK1 kinase activity is required for abscission. J Cell Sci 125: 2844-2852.

Rao MS, Dwivedi RS, Subbarao V, Usman MI, Scarpelli DG, Nemali MR, Yeldandi A, Thangada S, Kumar S and Reddy JK. (1988). Almost total conversion of pancreas to liver in the adult rat: a reliable model to study transdifferentiation. Biochem Biophys Res Commun 156: 131-136.

Ray KC, Bell KM, Yan J, Gu G, Chung CH, Washington MK and Means AL. (2011). Epithelial tissues have varying degrees of susceptibility to Kras(G12D)-initiated tumorigenesis in a mouse model. PLoS One 6: e16786.

Rebours V, Boutron-Ruault MC, Schnee M, Ferec C, Maire F, Hammel P, Ruszniewski P and Levy P. (2008). Risk of pancreatic adenocarcinoma in patients with hereditary pancreatitis: a national exhaustive series. Am J Gastroenterol 103: 111-119.

Reiling JH, Doepfner KT, Hafen E and Stocker H. (2005). Diet-dependent effects of the Drosophila Mnk1/Mnk2 homolog Lk6 on growth via eIF4E. Curr Biol 15: 24-30.

Roberts NJ, Jiao Y, Yu J, Kopelovich L, Petersen GM, Bondy ML, Gallinger S, Schwartz AG, Syngal S, Cote ML, Axilbund J, Schulick R, Ali SZ, Eshleman JR, Velculescu VE, Goggins M, Vogelstein B, Papadopoulos N, Hruban RH, Kinzler KW and Klein AP. (2012). ATM mutations in patients with hereditary pancreatic cancer. Cancer Discov 2: 41-46.

Rooman I and Real FX. (2012). Pancreatic ductal adenocarcinoma and acinar cells: a matter of differentiation and development? Gut 61: 449-458.

Rosendahl J, Witt H, Szmola R, Bhatia E, Ozsvari B, Landt O, Schulz HU, Gress TM, Pfutzer R, Lohr M, Kovacs P, Bluher M, Stumvoll M, Choudhuri G, Hegyi P, te Morsche RH, Drenth JP, Truninger K, Macek M, Jr., Puhl G, Witt U, Schmidt H, Buning C, Ockenga J, Kage A, Groneberg DA, Nickel R, Berg T, Wiedenmann B, Bodeker H, Keim V, Mossner J, Teich N and Sahin-Toth M. (2008). Chymotrypsin C (CTRC) variants that

diminish activity or secretion are associated with chronic pancreatitis. Nat Genet 40: 78-82.

Rovira M, Scott SG, Liss AS, Jensen J, Thayer SP and Leach SD. (2010). Isolation and characterization of centroacinar/terminal ductal progenitor cells in adult mouse pancreas. Proc Natl Acad Sci U S A 107: 75-80.

Rowlett RM, Chrestensen CA, Nyce M, Harp MG, Pelo JW, Cominelli F, Ernst PB, Pizarro TT, Sturgill TW and Worthington MT. (2008). MNK kinases regulate multiple TLR pathways and innate proinflammatory cytokines in macrophages. Am J Physiol Gastrointest Liver Physiol 294: G452-459.

Ruggero D, Montanaro L, Ma L, Xu W, Londei P, Cordon-Cardo C and Pandolfi PP. (2004). The translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in lymphomagenesis. Nat Med 10: 484-486.

Saghir AN, Tuxworth WJ, Jr., Hagedorn CH and McDermott PJ. (2001). Modifications of eukaryotic initiation factor 4F (eIF4F) in adult cardiocytes by adenoviral gene transfer: differential effects on eIF4F activity and total protein synthesis rates. Biochem J 356: 557-566.

Sahin-Toth M and Toth M. (2000). Gain-of-function mutations associated with hereditary pancreatitis enhance autoactivation of human cationic trypsinogen. Biochem Biophys Res Commun 278: 286-289.

Saluja AK, Lerch MM, Phillips PA and Dudeja V. (2007). Why does pancreatic overstimulation cause pancreatitis? Annu Rev Physiol 69: 249-269.

Sans MD, DiMagno MJ, D'Alecy LG and Williams JA. (2003). Caerulein-induced acute pancreatitis inhibits protein synthesis through effects on eIF2B and eIF4F. Am J Physiol Gastrointest Liver Physiol 285: G517-528.

Sans MD, Lee SH, D'Alecy LG and Williams JA. (2004). Feeding activates protein synthesis in mouse pancreas at the translational level without increase in mRNA. Am J Physiol Gastrointest Liver Physiol 287: G667-675.

Satoh A, Gukovskaya AS, Nieto JM, Cheng JH, Gukovsky I, Reeve JR, Jr., Shimosegawa T and Pandol SJ. (2004). PKC-delta and -epsilon regulate NF-kappaB activation induced by cholecystokinin and TNF-alpha in pancreatic acinar cells. Am J Physiol Gastrointest Liver Physiol 287: G582-591.

Schafer C, Ross SE, Bragado MJ, Groblewski GE, Ernst SA and Williams JA. (1998). A role for the p38 mitogen-activated protein kinase/Hsp 27 pathway in cholecystokinin-induced changes in the actin cytoskeleton in rat pancreatic acini. J Biol Chem 273: 24173-24180.

Scheper GC, Morrice NA, Kleijn M and Proud CG. (2001). The mitogen-activated protein kinase signal-integrating kinase Mnk2 is a eukaryotic initiation factor 4E kinase with high levels of basal activity in mammalian cells. Mol Cell Biol 21: 743-754.

Scheper GC, Parra JL, Wilson M, Van Kollenburg B, Vertegaal AC, Han ZG and Proud CG. (2003). The N and C termini of the splice variants of the human mitogen-activated protein kinase-interacting kinase Mnk2 determine activity and localization. Mol Cell Biol 23: 5692-5705.

Scheper GC, van Kollenburg B, Hu J, Luo Y, Goss DJ and Proud CG. (2002). Phosphorylation of eukaryotic initiation factor 4E markedly reduces its affinity for capped mRNA. J Biol Chem 277: 3303-3309.

Schnekenburger J, Weber IA, Hahn D, Buchwalow I, Kruger B, Albrecht E, Domschke W and Lerch MM. (2009). The role of kinesin, dynein and microtubules in pancreatic secretion. Cell Mol Life Sci 66: 2525-2237.

Sendler M, Dummer A, Weiss FU, Kruger B, Wartmann T, Scharffetter-Kochanek K, van Rooijen N, Malla SR, Aghdassi A, Halangk W, Lerch MM and Mayerle J. (2013). Tumour necrosis factor alpha secretion induces protease activation and acinar cell necrosis in acute experimental pancreatitis in mice. Gut 62: 430-439.

Seymour PA, Freude KK, Tran MN, Mayes EE, Jensen J, Kist R, Scherer G and Sander M. (2007). SOX9 is required for maintenance of the pancreatic progenitor cell pool. Proc Natl Acad Sci U S A 104: 1865-1870.

Sharer N, Schwarz M, Malone G, Howarth A, Painter J, Super M and Braganza J. (1998). Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. N Engl J Med 339: 645-652.

Shenberger JS, Zhang L, Hughlock MK, Ueda T, Watanabe-Fukunaga R and Fukunaga R. (2007). Roles of mitogen-activated protein kinase signal-integrating kinases 1 and 2 in oxidant-mediated eIF4E phosphorylation. Int J Biochem Cell Biol 39: 1828-1842.

Shi Y, Frost P, Hoang B, Yang Y, Fukunaga R, Gera J and Lichtenstein A. (2012). MNK kinases facilitate c-myc IRES activity in rapamycin-treated multiple myeloma cells. Oncogene 32: 190-197.

Shibata S, Morino S, Tomoo K, In Y and Ishida T. (1998). Effect of mRNA cap structure on eIF-4E phosphorylation and cap binding analyses using Ser209-mutated eIF-4Es. Biochem Biophys Res Commun 247: 213-216.

Shveygert M, Kaiser C, Bradrick SS and Gromeier M. (2010). Regulation of eukaryotic initiation factor 4E (eIF4E) phosphorylation by mitogen-activated protein kinase occurs through modulation of Mnk1-eIF4G interaction. Mol Cell Biol 30: 5160-5167.

Siegel R, Naishadham D and Jemal A. (2013). Cancer statistics, 2013. CA Cancer J Clin 63: 11-30.

Siegmund E, Lohr JM and Schuff-Werner P. (2004). [The diagnostic validity of non-invasive pancreatic function tests--a meta-analysis]. Z Gastroenterol 42: 1117-1128.

Singh VP, Saluja AK, Bhagat L, van Acker GJ, Song AM, Soltoff SP, Cantley LC and Steer ML. (2001). Phosphatidylinositol 3-kinase-dependent activation of trypsinogen modulates the severity of acute pancreatitis. J Clin Invest 108: 1387-1395.

Sipos B, Frank S, Gress T, Hahn S and Kloppel G. (2009). Pancreatic intraepithelial neoplasia revisited and updated. Pancreatology 9: 45-54.

Siveke JT, Lubeseder-Martellato C, Lee M, Mazur PK, Nakhai H, Radtke F and Schmid RM. (2008). Notch signaling is required for exocrine regeneration after acute pancreatitis. Gastroenterology 134: 544-555.

Slack JM. (1995). Developmental biology of the pancreas. Development 121: 1569-80.

Slentz-Kesler K, Moore JT, Lombard M, Zhang J, Hollingsworth R and Weiner MP. (2000). Identification of the human Mnk2 gene (MKNK2) through protein interaction with estrogen receptor beta. Genomics 69: 63-71.

Solar M, Cardalda C, Houbracken I, Martin M, Maestro MA, De Medts N, Xu X, Grau V, Heimberg H, Bouwens L and Ferrer J. (2009). Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. Dev Cell 17: 849-860.

Sonenberg N. (2008). eIF4E, the mRNA cap-binding protein: From basic discovery to translational research. Biochem Cell Biol 86: 178–183.

Stathis A and Moore MJ. (2010). Advanced pancreatic carcinoma: current treatment and future challenges. Nat Rev Clin Oncol 7: 163-172.

Steinle AU, Weidenbach H, Wagner M, Adler G and Schmid RM. (1999). NF-kappaB/Rel activation in cerulein pancreatitis. Gastroenterology 116: 420-430.

Steward MC, Ishiguro H and Case RM. (2005). Mechanisms of bicarbonate secretion in the pancreatic duct. Annu Rev Physiol 67: 377-409.

Strobel O, Dor Y, Alsina J, Stirman A, Lauwers G, Trainor A, Castillo CF, Warshaw AL and Thayer SP. (2007). In vivo lineage tracing defines the role of acinar-to-ductal transdifferentiation in inflammatory ductal metaplasia. Gastroenterology 133: 1999-2009.

Su KH, Cuthbertson C and Christophi C. (2006). Review of experimental animal models of acute pancreatitis. HPB (Oxford) 8: 264-286.

Svitkin YV, Herdy B, Costa-Mattioli M, Gingras AC, Raught B and Sonenberg N. (2005). Eukaryotic translation initiation factor 4E availability controls the switch between cap-dependent and internal ribosomal entry site-mediated translation. Mol Cell Biol 25: 10556-10565.

Szmola R and Sahin-Toth M. (2007). Chymotrypsin C (caldecrin) promotes degradation of human cationic trypsin: identity with Rinderknecht's enzyme Y. Proc Natl Acad Sci U S A 104: 11227-11232.

Teich N, Le Marechal C, Kukor Z, Caca K, Witzigmann H, Chen JM, Toth M, Mossner J, Keim V, Ferec C and Sahin-Toth M. (2004). Interaction between trypsinogen isoforms in genetically determined pancreatitis: mutation E79K in cationic trypsin (PRSS1) causes increased transactivation of anionic trypsinogen (PRSS2). Hum Mutat 23: 22-31.

Tomoo K, Shen X, Okabe K, Nozoe Y, Fukuhara S, Morino S, Ishida T, Taniguchi T, Hasegawa H, Terashima A, Sasaki M, Katsuya Y, Kitamura K, Miyoshi H, Ishikawa M and Miura K. (2002). Crystal structures of 7-methylguanosine 5'-triphosphate (m(7)GTP)- and P(1)-7-methylguanosine-P(3)-adenosine-5',5'-triphosphate (m(7)GpppA)-bound human full-length eukaryotic initiation factor 4E: biological importance of the C-terminal flexible region. Biochem J 362: 539-544.

Tsunoda Y and Owyang C. (1995). High-affinity CCK receptors are coupled to phospholipase A2 pathways to mediate pancreatic amylase secretion. Am J Physiol 269: G435-44.

Tu XH, Song JX, Xue XJ, Guo XW, Ma YX, Chen ZY, Zou ZD and Wang L. (2012). Role of bone marrow-derived mesenchymal stem cells in a rat model of severe acute pancreatitis. World J Gastroenterol 18: 2270-229.

Ueda T, Sasaki M, Elia AJ, Chio, II, Hamada K, Fukunaga R and Mak TW. (2010). Combined deficiency for MAP kinase-interacting kinase 1 and 2 (Mnk1 and Mnk2) delays tumor development. Proc Natl Acad Sci U S A 107: 13984-13990.

Ueda T, Watanabe-Fukunaga R, Fukuyama H, Nagata S and Fukunaga R. (2004). Mnk2 and Mnk1 are essential for constitutive and inducible phosphorylation of eukaryotic initiation factor 4E but not for cell growth or development. Mol Cell Biol 24: 6539-6549.

Vaccaro MI. (2012). Zymophagy: selective autophagy of secretory granules. Int J Cell Biol 2012: 396705.

Vasseur S, Folch-Puy E, Hlouschek V, Garcia S, Fiedler F, Lerch MM, Dagorn JC, Closa D and Iovanna JL. (2004). p8 improves pancreatic response to acute pancreatitis by

enhancing the expression of the anti-inflammatory protein pancreatitis-associated protein I. J Biol Chem 279: 7199-7207.
Vila MR, Lloreta J and Real FX. (1994). Normal human pancreas cultures display functional ductal characteristics. Lab Invest 71: 423-31.
Villasenor A, Chong DC, Henkemeyer M and Cleaver O. (2010). Epithelial dynamics of pancreatic branching morphogenesis. Development 137: 4295-4305.
Vonlaufen A, Wilson JS and Apte MV. (2008). Molecular mechanisms of pancreatitis: current opinion. J Gastroenterol Hepatol 23: 1339-1348.
Wagner AC, Metzler W, Hofken T, Weber H and Goke B. (1999). p38 map kinase is expressed in the pancreas and is immediately activated following cerulein hyperstimulation. Digestion 60: 41-47.
Wagner M, Luhrs H, Kloppel G, Adler G and Schmid RM. (1998). Malignant transformation of duct-like cells originating from acini in transforming growth factor transgenic mice. Gastroenterology 115: 1254-1262.
Wartmann T, Mayerle J, Kahne T, Sahin-Toth M, Ruthenburger M, Matthias R, Kruse A, Reinheckel T, Peters C, Weiss FU, Sendler M, Lippert H, Schulz HU, Aghdassi A, Dummer A, Teller S, Halangk W and Lerch MM. (2010). Cathepsin L inactivates human trypsinogen, whereas cathepsin L-deletion reduces the severity of pancreatitis in mice. Gastroenterology 138: 726-737.
Waskiewicz AJ, Flynn A, Proud CG and Cooper JA. (1997). Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. Embo J 16: 1909-1920.
Waskiewicz AJ, Johnson JC, Penn B, Mahalingam M, Kimball SR and Cooper JA. (1999). Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 in vivo. Mol Cell Biol 19: 1871-1880.
Weaver C, Bishop AE and Polak JM. (1994). Pancreatic changes elicited by chronic administration of excess L-arginine. Exp Mol Pathol 60: 71-87.
Wendel HG, De Stanchina E, Fridman JS, Malina A, Ray S, Kogan S, Cordon-Cardo C, Pelletier J and Lowe SW. (2004). Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. Nature 428: 332-337.
Wendel HG, Silva RL, Malina A, Mills JR, Zhu H, Ueda T, Watanabe-Fukunaga R, Fukunaga R, Teruya-Feldstein J, Pelletier J and Lowe SW. (2007). Dissecting eIF4E action in tumorigenesis. Genes Dev 21: 3232-3237.
Werner J, Saghir M, Warshaw AL, Lewandrowski KB, Laposata M, Iozzo RV, Carter EA, Schatz RJ and Fernandez-Del Castillo C. (2002). Alcoholic pancreatitis in rats: injury from nonoxidative metabolites of ethanol. Am J Physiol Gastrointest Liver Physiol 283: G65-73.
Wheater MJ, Johnson PW and Blaydes JP. (2010). The role of MNK proteins and eIF4E phosphorylation in breast cancer cell proliferation and survival. Cancer Biol Ther 10: 728-735.
Whitcomb DC. (2012). Genetics of alcoholic and nonalcoholic pancreatitis. Curr Opin Gastroenterol 28: 501-506.
Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK, Jr., Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC and Ehrlich GD. (1996). Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 14: 141-145.
Whitcomb DC and Lowe ME. (2007). Human pancreatic digestive enzymes. Dig Dis Sci 52: 1-17.

Willemer S and Adler G. (1989). Histochemical and ultrastructural characteristics of tubular complexes in human acute pancreatitis. Dig Dis Sci 34: 46-55.

Willemer S, Kloppel G, Kern HF and Adler G. (1989). Immunocytochemical and morphometric analysis of acinar zymogen granules in human acute pancreatitis. Virchows Arch A Pathol Anat Histopathol 415: 115-123.

Williams JA. (2010a). Regulation of acinar cell function in the pancreas. Curr Opin Gastroenterol 26: 4784-83.

Williams JA. (2010b). Isolation of rodent pancreatic acinar cells and acini by collagenase digestion. The Pancreapedia: Exocrine Pancreas Knowledge Base.

Williams JA, Korc M and Dormer RL. (1978). Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini. Am J Physiol 235: 517-524.

Wilson JS, Korsten MA, Apte MV, Thomas MC, Haber PS and Pirola RC. (1990). Both ethanol consumption and protein deficiency increase the fragility of pancreatic lysosomes. J Lab Clin Med 115: 749–755.

Wu C, Kraft P, Stolzenberg-Solomon R, Steplowski E, Brotzman M, Xu M, Mudgal P, Amundadottir L, Arslan AA, Bueno-de-Mesquita HB, Gross M, Helzlsouer K, Jacobs EJ, Kooperberg C, Petersen GM, Zheng W, Albanes D, Boutron-Ruault MC, Buring JE, Canzian F, Cao G, Duell EJ, Elena JW, Gaziano JM, Giovannucci EL, Hallmans G, Hutchinson A, Hunter DJ, Jenab M, Jiang G, Khaw KT, Lacroix A, Li Z, Mendelsohn JB, Panico S, Patel AV, Qian ZR, Riboli E, Sesso H, Shen H, Shu XO, Tjonneland A, Tobias GS, Trichopoulos D, Virtamo J, Visvanathan K, Wactawski-Wende J, Wang C, Yu K, Zeleniuch-Jacquotte A, Chanock S, Hoover R, Hartge P, Fuchs CS, Lin D and Wolpin BM. (2012). Genome-wide association study of survival in patients with pancreatic adenocarcinoma. Gut.

Xu H, Zhu J, Smith S, Foldi J, Zhao B, Chung AY, Outtz H, Kitajewski J, Shi C, Weber S, Saftig P, Li Y, Ozato K, Blobel CP, Ivashkiv LB and Hu X. (2012). Notch-RBP-J signaling regulates the transcription factor IRF8 to promote inflammatory macrophage polarization. Nat Immunol 13: 642-650.

Zaninovic V, Gukovskaya AS, Gukovsky I, Mouria M and Pandol SJ. (2000). Cerulein upregulates ICAM-1 in pancreatic acinar cells, which mediates neutrophil adhesion to these cells. Am J Physiol Gastrointest Liver Physiol 279: G666-676.

Zhou Q, Brown J, Kanarek A, Rajagopal J and Melton DA. (2008). In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 455: 627-632.

Zhou Q, Law AC, Rajagopal J, Anderson WJ, Gray PA and Melton DA. (2007). A multipotent progenitor domain guides pancreatic organogenesis. Dev Cell 13: 103-114.

Ziaei S, Shimada N, Kucharavy H and Hubbard K. (2011). MNK1 expression increases during cellular senescence and modulates the subcellular localization of HnRNP A1. Exp Cell Res 318: 500-508.