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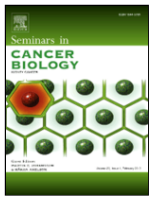

Review

G protein-coupled receptor kinases (GRKs) in tumorigenesis and cancer progression: GPCR regulators and signaling hubs

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ABSTRACT

Increasing evidences point to G protein-coupled receptor kinases (GRKs), a subfamily of protein kinase A/G/C-like kinases, as relevant players in cancer progression, in a cell-type and tumor-specific way. Alterations in the expression and/or activity of particular GRKs have been identified in several types of tumors, and demonstrated to modulate the proliferation, survival or invasive properties of tumor cells by acting as integrating signaling nodes. GRKs are able to regulate the functionality of both G protein-coupled receptors (GPCR) and growth factor receptors and to directly control cytosolic, cytoskeletal or nuclear signaling components of pathways relevant for these processes. Furthermore, many chemokines as well as angiogenic and inflammatory factors present in the tumor microenvironment act through GPCR and other GRK-modulated signaling modules. Changes in the dosage of certain GRKs in the tumor stroma can alter tumor angiogenesis and the homing of immune cells, thus putting forward these kinases as potentially relevant modulators of the carcinoma-fibroblast-endothelial-immune cell network fostering tumor development and dissemination. A better understanding of the alterations in different GRK isoforms taking place during cancer development and metastasis in specific tumors and cell types and of its impact in signaling pathways would help to design novel therapeutic strategies.

1. Introduction

Cancer progression is a highly complex process that implicates multiple and sequential changes in tumor cells as well as in their interactions with different types of cells (fibroblasts, vascular or immune cells) present in the "tumor microenvironment" [1]. The alterations of signaling pathways governing proliferation, survival, angiogenesis, invasive migration, metastasis, metabolism or the immune response are key events in cancer initiation and progression. Such alterations in signal transduction homeostasis can be caused by variations in the normal levels of chemical messengers or in the responsiveness of cells to such signals, because of oncogenic mutations and/or altered expression patterns or functionality in their receptors or in the downstream components (kinases/phosphatases, G protein switches, transcription factors) of these signaling cascades. In this scenario, oncogenes often cooperate with non-genetically altered signaling nodes (onco-modulators) to strength tumoral hallmarks and lead to cancer development.

G-protein-coupled receptors (GPCR) constitute the largest family of membrane receptors and are involved in a wide variety of physiological functions. Increasing evidence is putting forward the role of GPCR and their ligands in different aspects of tumor biology [2,3]. In this context, G-protein-coupled receptor kinases (GRKs), a subfamily of AGC (protein kinase A/G/C-like) kinases originally identified as inhibitors of GPCR signaling, are emerging as potentially relevant onco-modulators. GRKs are able to modulate the response to many GPCR involved in tumoral signaling as well as to act as hubs regulating several cellular processes related to cancer progression via its interaction with other components of transduction cascades [4,5]. In this review, we provide an outline of the potential impact of altered GRK functionality on cancer-related signaling processes and discuss the emerging data indicating the implication of given GRK isoforms in tumor progression, in a cell-type and tumor-specific way.

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2. The GRK subfamily of AGC kinases

Agonist-activated GPCRs couple to heterotrimeric G proteins, thus facilitating exchange of GDP by GTP in the Gα subunits, which subsequently dissociate from the βγ dimers. Free Gα and βγ subunits transiently interact with effectors (such as adenylyl cyclases, phospholipases, or small GTPases, among others) to trigger canonical transduction cascades. Ligand-bound GPCR also become specifically phosphorylated by GRKs in the third cytoplasmic loop and/or the C-terminal tail. This event promotes the recruitment to the phosphorylated receptor of the cytosolic proteins β-arrestins, leading to uncoupling from G proteins, a process termed GPCR desensitization [6]. Besides this initial inhibitory role, β-arrestins were reported to act as scaffold proteins for several endocytosis adaptors and signaling mediators, thus triggering receptor internalization and recycling and the modulation of additional signaling cascades by GPCR [7]. Therefore, GRKs would also control the balance between the G protein-dependent and β-arrestin-dependent branches of GPCR signaling [4].

Seven members (GRK1 to GRK7) of the GRK family have been identified in vertebrates and grouped in three subfamilies: visual GRKs, present in cones and rods, include GRK1 (originally termed rhodopsin kinase) and GRK7; GRK2, comprising GRK2 and GRK3; and GRK4, to which GRK4, GRK5 and GRK6 belong [6,8,9] (Fig. 1). Although a detailed comparative analysis of the tissue and cell-type specific expression pattern of GRKs is not available, non-visual kinases are ubiquitous, with the only exception of GRK4, which appears predominantly expressed in testes, kidney, brain and uterus [6].

All GRKs are multidomain proteins, with a central catalytic serine/threonine kinase region of circa 330 residues that belongs to the AGC kinase family. However, different from most AGC kinases, GRKs are not activated by phosphorylation of the activation segment or hydrophobic motif, but require and induced re-arrangement to become active. GRKs have thus developed specific features required for their interaction and conformational activation by their ligand-bound GPCR substrates at the plasma membrane [6,10–12]. GRKs have an N-terminal RH (RGS – regulator of G protein signaling homology) domain proposed to help to stabilize the active configuration of the small lobe of the kinase domain. On the other hand, a circa 20 residue helical segment at the N-terminus appears to be essential for GPCR phosphorylation [11,13]. In addition, the C-terminal domain of GRKs contain determinants for its targeting to the membrane, including prenylation motifs in GRK1 and GRK7, palmitoylation sites in GRK4 and 6, positive lipid-binding regions in GRK4, 5 and 6, and a plecsktrin homology (PH) domain in GRK2 and 3 able to interact with free Gβγ subunits (Fig. 1). This structural organization would allow coordinated GRK membrane recruitment and activation. Moreover, the non-catalytic domains of GRKs also present a variety of sites for phosphorylation-dependent modulation and for interaction with other cellular partners, such as the specific association of GRK2/3 members with Gαq alpha subunits via their RH domain (see below).

Since there are hundreds of GPCR and only five non-visual GRKs, each GRK must be able to phosphorylate many different receptors, thus emerging as important signaling hubs. Moreover, accumulating evidence is showing that GRKs, and in particular the most studied GRK2 and GRK5 isoforms, interact with and/or phosphorylate other non-GPCR proteins, including receptor-tyrosine kinases (RTKs) and a variety of cytosolic or nuclear signaling components of pathways relevant for cancer progression [4–6,14–16] (see also below). A better understanding of GRK specificity toward particular GPCR and how GPCR-related and non-canonical functions of given GRKs are integrated in specific cell types and pathological conditions will be key to unravel the role of these kinases in tumor biology.

3. Potential impact of changes in GRK functionality in cancer-related signaling pathways

3.1. Integrated regulation of GPCR cascades and other signaling modules in tumor cells

The role of GPCR cascades in cancer initiation and progression is being increasingly noted and has being recently reviewed [2,3,17,18].

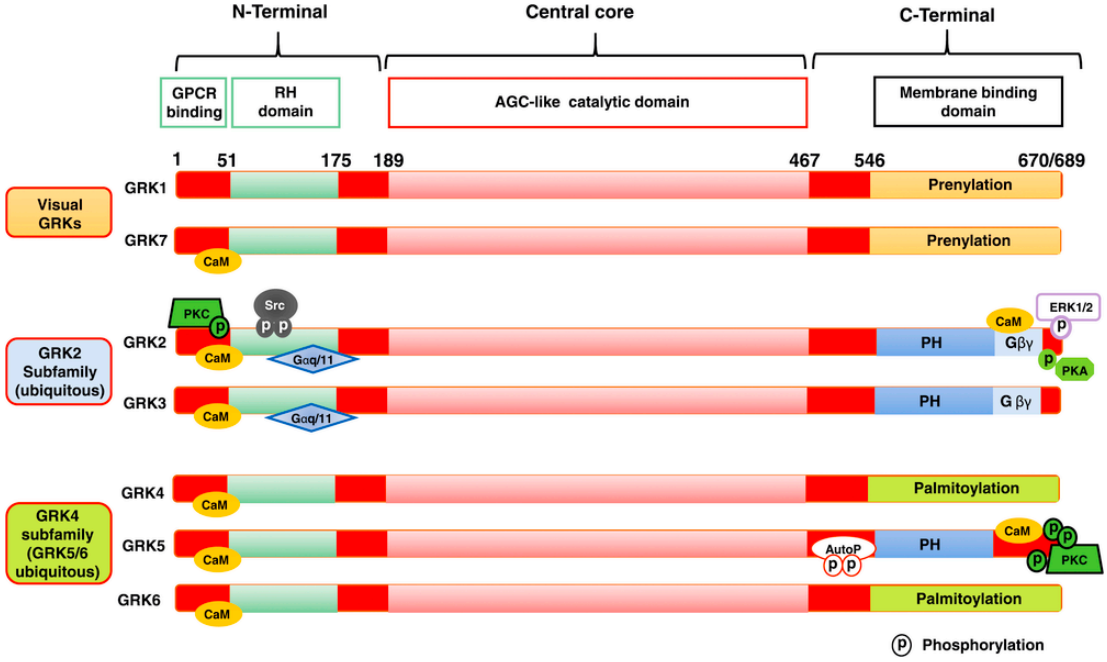

Fig. 1. The GRK subfamily. GRKs share a similar central catalytic domain that belongs to the AGC kinase family, an N-terminal RH (RGS – regulator of G protein signaling homology) domain and a C-terminal region that includes determinants for its targeting to the membrane, such as prenylation or palmitoylation motifs, plecsktrin homology (PH) domains or Gβγ subunits binding regions. The identified sites of interaction with G protein subunits, with modulators as calmodulin (CaM) or phosphorylation by different kinases are indicated.

Altered GPCR signaling can occur by a variety of mechanisms and may involve both tumor cells and other cell types in the tumor microenvironment. In transformed cells, overexpression of specific GPCR (for instance, receptors for lysophosphatidic acid (LPA), sphingosine-1-phosphate (S1P), angiotensin, endothelin, prostaglandins or the plasma membrane estrogen receptor GPR30) has been reported. Enhanced function of these GPCR would foster cell proliferation and survival by modulating core cascades related to these processes, including small GTPases, MAPK and PI3K/AKT/mTOR pathways, as well as Wnt/beta-catenin or YAP/TAZ-dependent transcription programs [2,3,18,19]. The presence of activating or inactivating GPCR mutations (estimated to take place in circa 20% of human cancers) [20], or activating mutations in Gα protein subunits may also lead to altered GPCR signaling in specific tumor types and patients. It should be noted that, in addition to modifying heterotrimeric G protein signaling, changes in GPCR expression or functionality may trigger tumor progression via β-arrestin-dependent cascades (reviewed in [21,22]) and/or transactivation of growth factor receptors belonging to the receptor tyrosine kinase (RTK) family [2,23]. Such crosstalk can take place via the paracrine release of growth factors or the β-arrestin/Src-mediated activation of RTKs. Conversely, RTK activation can also modulate GPCR signal transduction at different levels [24–26]. In such complex scenario, changes in GRK expression or activity taking place in tumoral cells may alter GPCR signaling through a variety of mechanisms. According to their canonical role, enhanced levels of a given GRK isoform would decrease G protein-dependent signaling triggered by their preferential GPCR substrates. Conversely, down-regulation of GRKs would foster GPCR cascades. In addition, changes in GRKs could also alter the balance between the G protein-dependent and β-arrestin branches of GPCR signaling and the recruitment of β-arrestin interactors [4]. Interestingly, differential phosphorylation by particular GRKs of ligand-bound GPCRs (termed "phosphorylation barcoding") has been suggested to determine the recruitment of β-arrestins in specific conformations, leading to the association of different sets of partners, for instance, endocytosis adaptors vs MAPKs (reviewed in [4]). Therefore, it is tempting to speculate that alterations in the relative dosage of endogenous GRKs in a certain tumor cell type may also change the GPCR barcodes and trigger alterations in β-arrestin-dependent signaling.

Similar mechanisms of modulation by GRKs may affect GPCR closely related to cell migration, invasion and metastasis expressed either endogenously or aberrantly in tumor cells, such as CXCR4, CCR7 or CCR10 chemokine receptors, receptors for LPA, prostaglandins or thrombin or adhesion GPCRs [2,3].

Moreover, the integrated effect of GRKs in all these tumoral hallmarks may also involve non-GPCR targets (Fig. 2). Accumulating evidence indicate that GRKs, and in particular GRK2 and GRK5, the more studied members of this family, can phosphorylate (or interact with) other signaling proteins relevant to cancer progression in certain contexts and cell types (reviewed in [5,6,27,28]). These include RTKs for PDGF (GRK2 and 5) or EGF (GRK2); downstream components such as IRS1, MEK, p38MAPK, RhoA, GIT-1, AKT, EPAC-1, HDAC6 (GRK2) or β-arrestin-1 (GRK5); transcriptional modulators as Smad2/3 (GRK2), HDAC5 (GRK5) or NFκB (GRK5); key players in stress response and survival as p53 (GRK5), nucleophosmin (GRK5) or Mdm2 (GRK2); or cytoskeletal proteins as tubulin (GRK2 and 5), moesin (GRK5) or ezrin (GRK2).

Consistent with such complexity, the effect of changes in GRK expression on cell proliferation/survival and migration/invasion is not straightforward and appears to depend on both the cell type and the stimuli involved (see below in the sections devoted to the role of given GRKs in different tumors). As examples of such variety of mechanisms, enhanced GRK2 levels have been shown to foster MAPK signaling upon S1P1 or integrin receptor activation in epithelial cells via interaction with GIT1, leading to cell migration [29], which can also be fostered in response to EGF via GRK2 phosphorylation of HDAC6 [30]. In other contexts, increased GRK2 dosage potentiates β-arrestin-MAPK activation by the chemokine receptor CXCR7 in astrocytes [31] or up-regulate EGFR-triggered pathways in vascular smooth muscle cells [32], epithelial cells [33,34] and breast cancer cells, in the latter case by modulating the HDAC6/Pin1 axis ([34], see below). Conversely, increased GRK2 dosage counteracts PDGF-dependent proliferation of thyroid can-

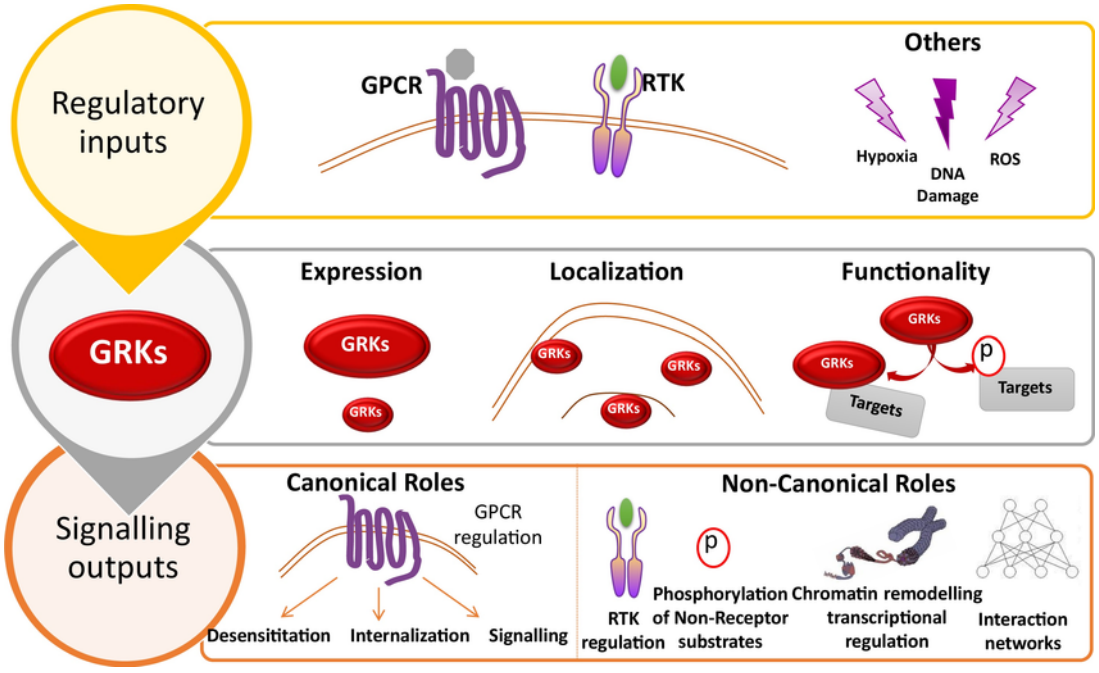

Fig. 2. GRKs as signaling nodes allowing the coordinated modulation of GPCR and non-GPCR pathways. The expression levels, localization and activity of GRKs are modulated by a variety of mechanisms upon stimulation of GPCR or tyrosine kinase receptors or other regulatory inputs. In turn, GRKs can promote the coordinated control of many cellular processes via both canonical and non-canonical mechanisms. See main text for details.

cer cells [35] and smooth muscle [36], or IGF1-dependent growth in human hepatocellular carcinoma (HCC) cells [37], probably as a result of inhibitory phosphorylation of these receptors. Similarly, both tumor-promoting and inhibitory effects have been reported for GRK5 [28]. Down-modulation of this kinase enhances TSH receptor signaling to cAMP in thyroid cells, resulting in increased proliferation. On the contrary, high GRK5 levels appear to contribute to enhanced survival in osteosarcoma cells via p53 phosphorylation and degradation [38] or to increased invasion in prostate cancer cells by phosphorylating moesin [39].

Understanding the specific contributions of such potential mechanisms (both GPCR-dependent and independent) and their integration in specific tumors, cell types, and the changing pathophysiological contexts taking place during tumor development and metastasis remains a main challenge for future research in this area.

3.2. Modulation of signaling in other cell types present in the tumor microenvironment

The reciprocal interactions among transformed cells, the tumor-associated vasculature, fibroblasts and infiltrated immune cells are key for cancer progression. This interplay is orchestrated by an array of signaling mediators, chemokines, angiogenic and inflammatory factors present in the tumor microenvironment (Fig. 3). Many of these ligands act through GRK-modulated GPCR or via other signaling pathways also functionally connected to members of this kinase family. Since cancer can be understood as a global loss of the cellular quiescence required for tissue homeostasis, the impact of GRKs on tumor progression may also involve the alteration of the quiescent states of different types of stromal cells during this process.

The potential role of GRK dosage in fibroblast function in tumor contexts has not been specifically investigated to date. Quiescent fibroblasts provide a tumor suppressor environment, which is disrupted when these cells proliferate and secrete soluble factors and extracellular matrix components that foster the growth of surrounding epithelial and vascular cells. Cancer-associated fibroblasts (CAFS) are particularly relevant for the development of breast, prostate, pancreatic, lung or colorectal cancer, among other types [40]. Notably, diverse GPCR (such as adenosine A2b receptor, the OPRK1 opioid receptor or GPR37) and other GRK-modulated pathways (TGF and PDGF receptors, Gq, RalGDS, among others) are known to modulate fibroblast quiescence initiation and maintenance programs [41], thus encouraging future research on the role of GRK dosage in CAFs.

Chemokines locally released in the tumor microenvironment are important modulators of tumor cell survival, growth, motility and metastatic homing acting through CXCR4, CCR7, or CCR10 receptors, among others [2,42,43]. In general, GRK2 and other GRKs have been reported to control the intensity and duration of chemokine-triggered signaling in lymphocytes and neutrophils during inflammation due to its ability to trigger desensitization of particular chemokine receptors [44,45]. However, the role of changes in the dosage of specific GRKs taking place during cancer progression in the modulation of signaling/migration/invasion by chemokines in tumor cells has not been investigated in detail, and requires to be addressed in the future [46].

Importantly, chemokines can also act on endothelial cells to modulate angiogenesis and recruit macrophages and leukocytes to the tumor milieu. In turn, these immune cells will locally release VEGF and other angiogenic factors as well as prostaglandins and other inflammatory cytokines, further fueling the expression of chemokines and angiogenic ligands by both tumoral and stromal cells and fostering tumor progression (reviewed in [2,47,48]). Tumor-associated macrophages and the

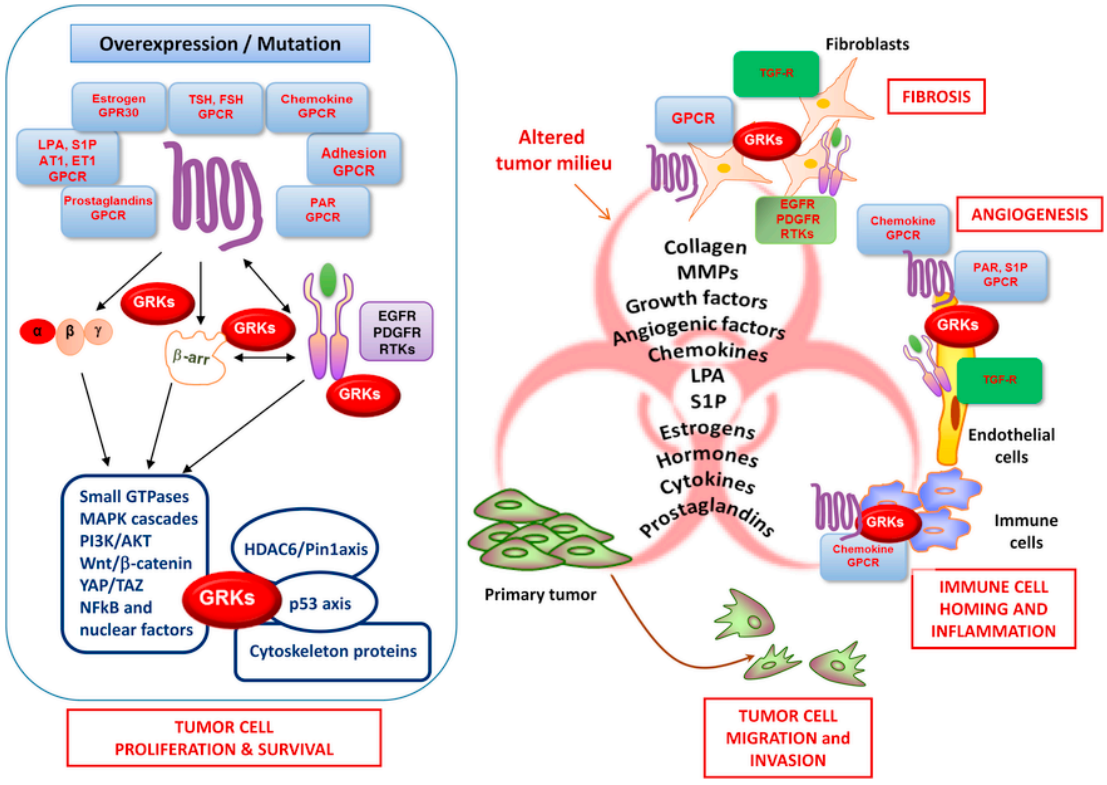

Fig. 3. Potential impact of changes in GRK functionality in cancer-related signaling pathways in both tumor cells and in other cell types present in the tumor microenvironment. Changes in the expression or activity of given GRKs can take place in either the primary tumor or in other relevant cells in the tumor microenvironment (fibroblasts, endothelial, macrophages and other recruited immune cells). Such changes in GRKs may alter at the indicated steps the pathways triggered by the variety of signals present in the tumor milieu governing proliferation, survival or invasion of transformed cells, as well as angiogenesis, fibrosis, inflammation or the homing and activation of immune cells. See main text for details.

local chemokine environment have also been linked to immunosuppression and immune cell evasion, critical for cancer progression. The fact that several GRKs can modulate a variety of chemokine receptors as well as other GPCRs for angiogenic and inflammatory factors (S1P, thrombin, prostaglandins) [5,6,44,47] put forward these kinases as potentially relevant modulators of the carcinoma-endothelial-immune cell network.

Regarding the role of GRKs in the tumor vasculature, down-regulation of GRK2 in endothelial cells (EC) increases the response to angiogenic stimuli (VEGF, S1P) and impairs endothelial TGF-β signaling and EC interaction with pericytes. These effects lead to the formation of immature and leaky vessels, known features of the tumor microvasculature [49]. Interestingly, GRK2 levels decrease in human breast cancer vessels, whereas endothelium-specific GRK2 ablation fosters tumor growth in mice, along with enhanced intra-tumoral hypoxia and macrophage infiltration [49]. In this line, reduced GRK2 levels also alters the endothelial production of pro-inflammatory and pro-angiogenic factors and of chemokines known to specifically recruit myeloid cells to tumors [47,49]. It is tempting to suggest that downmodulation of GRK2 at EC may be a common feature among different types of cancer, leading to pleiotropic effects on angiogenesis by regulating both ALK1/ALK5 TGF-β receptors and diverse angiogenic GPCR receptors. Interestingly, decreased GRK2 levels have been reported in models of Kaposi's sarcoma, a highly disseminated angiogenic tumor linked to infection by Kaposi's sarcoma-associated herpesvirus (KSHV) [50]. KSHV encodes the miR-K3 micro-RNA, which inhibits EC GRK2 expression, in turn leading to the stimulation of the chemokine CXCR2/AKT signaling axis, negatively regulated by GRK2. Other potentially interesting target in this context is the calcitonin receptor-like receptor, a family B GPCR. This protein forms the adrenomedullin (ADM) AM1 and AM2 receptors in association with RAMP2 or RAMP3, and that is negatively modulated by GRK2 in model systems [51]. In addition to its cardiovascular role as a potent vasodilator, ADM levels are upregulated in a variety of tumor types, including breast and melanoma, and appear to play an important role in promoting tumor growth by enhancing angiogenesis, proliferation or inflammation. Interestingly, melanoma-derived tumors grown in Tie2Cre-GRK2 f/f mice showed a marked increase of ADM, which correlated with macrophage infiltration [47,49]. These results suggest that GRK2 downmodulation in this murine model might favor ADM secretion by myeloid cells and the activity of ADM receptors in both macrophages and endothelial cells, leading to exacerbated angiogenesis.

Other studies appear to support a role for other GRK isoforms in tumor angiogenesis, although whether this is a consequence of its specific alteration in the vascular cells has not been investigated (reviewed in [47]). GRK3 down-modulation has been correlated with increased growth of glioblastoma cells by mechanisms including a paracrine, endothelial-mediated effect [52], whereas over-expression of GRK3 in prostate tumor cells promotes tumor growth and metastasis through the induction of angiogenesis [53]. GRK5 expression in endothelial cells attenuates the secretion of several pro-angiogenic cytokines by inhibiting NFκB activity [54], leading to impaired in vivo angiogenesis in models of wound healing and chronic ischemia. However, whether vascular GRK5 levels might influence tumor angiogenesis was not investigated. On the other hand, altered chemokine receptor signaling and enhanced angiogenesis has been implicated in the increased sensitivity of GRK6 knockout mice to tumor progression and metastasis in the Lewis Lung carcinoma heterotopic murine model. GRK6 depletion in host animals favored higher levels of stromal MMP-9 and MMP-2 metalloproteases and angiogenesis, along with increased infiltration of polymorphonuclear leukocytes and enhanced CXCR2 signaling [55], suggesting that an improved chemotactic motility, tumor homing and pro-tumoral neutrophil activity was taking place.

In summary, GPCRs and other GRK-modulated signaling modules appear to play a central role in tumoral endothelium functionality and in the homing of immune cells to the tumor microenvironment. Thus, it is tempting to suggest that concurrent changes in the dosage of different GRKs in vascular endothelial cells and in circulating monocytes and other immune cell types might cooperate in fueling tumor progression. This constitutes an interesting issue to address in future studies.

4. Multiple mechanisms may trigger altered GRK expression and functionality in tumor contexts

The emerging role of GRKs as modulators of multiple processes related to cancer progression discussed in previous sections underscores the need for a comprehensive study of its functional status and expression levels in specific tumors and in related stromal cells. A better knowledge of the endogenous patterns of expression of GRK isoforms and their relative dosage in normal tissues will also help to better understand the effects triggered by changes taking place during tumor development.

Inspection of different "omics" sources of information indicates that, at the genomic level, GRK amplification (mostly observed for GRK2 or GRK6) or mutations/deletions (most frequent than amplification for GRK3 or GRK5) take place (usually at frequencies from 5% to 25%) in cohorts of different tumor types (http://cbioportal.org) [56,57]. Changes in GRK gene expression have also been reported in mRNA arrays for a variety of cancer cohorts, although little is known about the regulation of GRK transcription in tumoral contexts, and the absence of significant mRNA changes does not rule out the occurrence of altered protein levels or activity. The information available in the Human Protein Atlas [58] also suggests the occurrence of up or down-modulation in the normal expression of given GRK isoforms in subsets of cancer samples. A more detailed analysis using different validated antibodies and higher sample sizes are needed to confirm these data (see sections devoted to the role of GRK isoforms in specific tumors below).

Moreover, accumulating evidence (mostly related to GRK2 and GRK5) indicates that GRK functionality and expression are regulated at multiple post-transcriptional levels, leading to changes in protein activity, localization or stability [4,59]. Therefore, the occurrence of such potential modulatory mechanisms should be carefully analyzed in cancer contexts.

Although binding to active GPCRs constitutes the best-established mechanism for triggering GRK activity, their functional status can be modulated by a variety of post-translational modifications ([59] and interactions with other proteins). GRK2/3 kinases are stimulated by Gβγ subunits released upon GPCR activation and by phospahtidylinositol-4,5-bisphosphate (PIP2) via binding to the PH domain [60,61]. Members of the GRK4/5/6 subfamily are stimulated by PIP2 via binding to the N-terminal domain [61] or inhibited by association of calcium-calmodulin in the same region [62]. Caveolin 1, a protein suggested to play a multifaceted role in cancer progression [63] has been reported to bind and inhibit several GRK isoforms [64]. On the other hand, the Raf kinase inhibitor protein (RKIP), frequently downregulated in cancers and reported to participate in tumorigenesis [65], acts as a physiological inhibitor of GRK2 [66]. It is tempting to suggest that alterations in these modulatory mechanisms might contribute to altered GRK function in tumor settings.

Interestingly, stimulation of growth factor receptors and of kinases known to be altered in cancer contexts have also been reported to modulate GRK function via phosphorylation. Both GRK2 and GRK5 are tyrosine-phosphorylated and activated by the PDGFR, ensuring feedback PDGFR desensitization [67,68]. EGFR stimulation also leads to stimulatory GRK2 tyrosine phosphorylation [69], as does c-Src, which enhances GRK2 catalytic activity toward both soluble and membrane

substrates [70]. On the other hand, GRK2 phosphorylation at S670 by ERK1/2 disrupts its interaction with GPCR or GIT1 [29,71], while enabling the phosphorylation of HDAC6 [30]. These data suggest that, in the context of high MAPK activation, the repertoire of GRK2 substrates and partners will be modified [45]. In other situations, stimulation of protein kinase C (PKC) pathways may alter the pattern of GPCR modulation by GRKs, since PKC-mediated phosphorylation of GRK5 has an inhibitory effect, whereas it enhances GRK2 by increasing its recruitment to the membrane (reviewed in [6]).

Regarding subcellular localization-related modulation, the presence of a putative nuclear localization signal (NLS) in the members of the GRK4 subfamily suggest that some GRKs may play signaling roles in the nucleus, potentially linking GPCR to transcriptional regulation [72,73]. In cardiomyocytes, nuclear GRK5 triggers the phosphorylation and subsequent nuclear exit of HDAC5 [74], thus allowing myocyte enhancer factor-2 (MEF2)-dependent transcription. Interestingly, GRK5 has been reported to phosphorylate the nuclear protein nucleophosmin 1, involved in the modulation of apoptosis, proper nuclei assembly, centrosome duplication and cytokinesis, at the same residue modified by the Polo-like kinase 1 [75]. The extent of phosphorylation of nucleophosmin 1 by GRK5 is related to the sensitivity of cells to undergo apoptosis. GRK5 also displays a functional nuclear export sequence (NES). It has been reported that stimulation of Gq-coupled GPCRs promotes calcium-calmodulin binding to GRK5, in turn leading to NES sequence unmasking and nuclear exit of the kinase [72,73]. It is tempting to suggest that deregulation of GPCR activity and/or calcium signaling in some tumor contexts might alter the nuclear shuttling of GRK5 and contribute to cellular transformation.

The presence of GRKs in other cellular structures may be also relevant for tumor progression. It has been reported that both GRK2 and GRK5 localize in centrosomes. At such location, GRK5 promotes G2/M transition by phosphorylating p53 [76] whilst GRK2 promotes early mitogen-dependent centrosome separation [77]. Therefore, altered levels of GRK5 and GRK2 may affect cell cycle progression and spindle organization, respectively. A better characterization of the signaling pathways controlling the centrosomal localization of GRKs in normal and cancer settings is needed. GRK2 has been also found in the mitochondria. Mitochondrial GRK2 may promote a harmful effect by increasing superoxide levels and altering ATP production in cardiomyocytes [78–80]. However, other authors have reported that increased GRK2 levels foster ATP accumulation and tolerance to ischemia in skeletal muscle [81]. Interestingly, phosphorylation of GRK2 at residue Ser670 by extracellular signal-regulated kinases (ERKs) results in enhanced GRK2 binding to heat shock protein Hsp90 and mitochondrial targeting [78]. Since over-activation of ERKs and over-expression of Hsp90 are frequent in many tumors, it will be interesting to elucidate whether GRK2 dosage is increased in the mitochondria of transformed cells and its potential impact on mitochondrial metabolism reprogramming in tumor progression.

GRK2 is also regulated at the level of protein stability by the proteasome pathway [82]. Upon activation of certain GPCRs, GRK2 is degraded in a complex process involving kinase phosphorylation by both c-Src and MAPK kinases and β-arrestins as scaffolds for recruitment of the Mdm2 E3-ubiquitin ligase [83–85]. On the contrary, activation of the PI3K/Akt pathway by certain RTKs triggers the nuclear localization of Mdm2, thus impeding Mdm2-mediated GRK2 degradation and enhancing GRK2 protein levels [86]. Given the relevant role for Mdm2 in oncogenesis, it would be of interest to investigate the relationship between Mdm2 and GRK2 in tumoral contexts.

Future research will need to address whether similar mechanisms of modulation of protein stability occur for other GRKs. In addition, the potential regulation of GRKs by microRNAs frequently altered in cancer progression is to our knowledge an unexplored field that would open new ways to understand alterations in their levels in pathological contexts.

5. Roles of specific GRK isoforms in different tumor types

5.1. Tumor-type specific roles of GRK2 and GRK3 in cancer progression

Emerging evidence indicates that GRK2 would act as an onco-modulator, contributing to cancer progression in a tumor and cell-type-specific way (Table 1).

Recent reports indicate that changes in GRK2 dosage in transformed mammary cells [34] and in the tumor endothelium [49] plays a concurrent role in breast cancer development. In breast epithelial cells, signaling cascades downstream estrogen, EGF or HER-2 receptors, frequently hyper-activated in luminal and in certain non-luminal types of breast cancer [87], lead to enhanced GRK2 expression via enhanced stimulation of the PI3K/AKT pathway [34]. As a consequence, GRK2 protein levels are increased in breast cancer cell lines and mice experimental models, and in a significant proportion of two independent cohorts of patients diagnosed with invasive ductal carcinoma. Interestingly, the ADRBK1 gene (coding for GRK2) is located at the 11q13.2 band and is thus part of the 11q13 chromosomal region. This region is believed to contain amplification units which might be amplified independently in circa 13% of breast cancer patients [88]. The core 1 region bearing the ADRBK1 gene was amplified in 33% of CylinD1-positive breast tumors, showing a strong association between copy number status and gene expression level. The 11q13 amplicon correlates with local relapses of breast cancer, associates with the estrogen receptor-positive status and predicts poor prognosis. Consistently, amplification frequencies of circa 5% are found for ADRBK1 in diverse patient cohorts of invasive carcinoma and ductal invasive carcinoma (http://cbioportal.org).

Increasing GRK2 levels promotes the acquisition of oncogenic features (potentiation of EGF and heregulin mitogenic and survival signaling pathways, growth under low-serum or normal conditions, resistance to different chemotherapeutic agents, anchorage-independent growth) by both luminal MCF7 and basal MDA-MB-231 basal cancer cells. Moreover, enhanced GRK2 expression increases their ability to trigger tumor growth in vivo in both xenograft and orthotopic mice models [34]. Consistent with a key role in breast cancer progression, decreasing GRK2 levels in either luminal or basal cancer cells prevents tumor growth in vivo [89] and sensitizes cultured cells to the effects of chemotherapeutic agents.

The molecular mechanisms underlying the positive effects of GRK2 on breast cancer progression appear to involve the coordinated modulation of key cancer-driving nodes such as the histone deacetylase 6 (HDAC6) and the prolyl-isomerase Pin1, also known to be over-expressed in these tumor contexts [90–95]. We have uncovered that in settings of enhanced levels of these proteins, GRK2 phosphorylates and activates HDAC6. This is favored by increased phosphorylation of GRK2 on S670 by ERK1/2 (a pathway often hyper-activated in both luminal and basal breast cancer contexts). Enhanced HDAC6 activity in turn leads to de-acetylation of Pin1, thus enhancing its stability and the interaction with key downstream cell growth and survival regulators [34]. It is likely, however, that the functionality of other pathways relevant to breast tumorigenesis would be altered by enhanced GRK2 expression, including other partners modulated by HDAC6 as well as GPCR and other GRK2 interactors related to cancer hallmarks [34]. A phospho-proteome profiling have recently identified a GPCR cluster as a signature of a subset of breast cancers [96]. Exploring the potential role of GRK2 in the modulation of cross talk among growth factor receptors and GPCR such as chemokine receptors in breast cancer cells is an attractive area for future research.

Table 1
Roles of specific GRK isoforms in different tumor types.

Cancer type	GRK isoform			Biological model	Ref.
	mRNA/ protein	Molecular mechanisms	Cellular process		
Breast	GRK2			Invasive ductal carcinoma patients, cell lines, orthotopic and xenograft mouse models	[34]
	Up/Up	HDAC6/Pin1 axis AKT/ERK cascades	Increased proliferation and survival		
	GRK3			Breast cancer patients, cell lines, orthotopic mouse models	[46]
	Down/ND	CXCR4 desensitization and signaling	Increased migration and metastasis		
	GRK4			Ductal carcinoma patients and cell lines	[123]
	Up/Up	β-arrestin1-mediated ERK and JNK signaling	Increased proliferation		
Prostate	GRK2			Adenocarcinoma patients	[103]
	Down/Down	ND	Differentiation		
	GRK2			Neuroendocrine prostate and metastatic castration-resistant prostate cancer patients	[106]
	Up/Up	ND	ND		
	GRK3			Metastatic castration-resistant prostate cancer patients, cell lines and orthotopic mouse models	[53]
	Up/Up	Downmodulation of angiogenesis inhibitors	Increased angiogenesis, growth and metastasis		
	GRK3			Cell lines	[112]
	Up/Up	ND	Increased differentiation		
	GRK5			Cell lines and xenograft mouse tumors	[28,39]
	ND/Up	G2/M progression Moesin phosphorylation	Increased proliferation and migration		
Pancreas	GRK2			Pancreatic carcinoma patients	[125]
	Up/Up	ND	Higher stage and invasion		
	GRK2			Ductal adenocarcinoma patients and cell lines	[101]
	Up/Up	ND	Increased proliferation		
Ovary	GRK2 and GRK4			Granulosa cell cancer patients	[102]
	ND/Up	ND	ND		
Thyroid	GRK2			Differentiated thyroid carcinoma patients and cell lines	[35,113]
	No change/Up	ND	Decreased proliferation		
	GRK5			Differentiated thyroid carcinoma patients	[113]
	Down/Down	TSHR desensitization and signaling	Increased proliferation		
Glioblastoma	GRK2			Mesenchymal glioblastoma patients	[52]
	Up/Up	ND	ND		
	GRK3			Classical glioblastoma patients	[52]
	Down/Down	CXCR4 desensitization and signaling	Increased proliferation		
	GRK5			Glioblastoma multiforme patients and cell lines	[114]
	Up/Up	ND	Increased proliferation		
Osteosarcoma	GRK5			Cell lines	[38]
	ND/ND	p53 phosphorylation and degradation	Decreased apoptosis and radiosensitivity		
Colon	GRK5			Cell lines	[116]
	ND/Down	PGE2 receptor desensitization and signaling	Increased proliferation		
Lung	GRK6			Adenocarcinoma patients	[119]
	Down/Down	ND	Decreased survival		
Medullo-blastoma	GRK6			Medulloblastoma patients and cell lines	[118]
	Down/Down	CXCR4 desensitization and signaling	Increased migration		
Myeloma	GRK6			Primary multiple myeloma patients and cell lines	[122]
	Up/Up	STAT3 phosphorylation	Increased survival		
Kaposi's sarcoma	GRK2			Patients and cell lines	[50]
	Down/Down	CXCR2 desensitization and AKT signaling	Increased invasion		
	GRK5			Cell lines	[117]

Table 1 (*Continued*)

Cancer type	GRK isoform		Biological model	Ref.
	mRNA/ protein	Molecular mechanisms	Cellular process	
	ND/ND	KSHV-GPCR desensitization and signaling	Increased proliferation	

Summary of the main reported alterations and effects of specific GRKs in different tumor types. See text for more details. ND, not determined.

Regardless of such possible alternative mechanisms, the switch-on of the GRK2-HDAC6-Pin1 axis emerges as a relevant molecular signature in breast cancer. It is tempting to suggest that GRK2 expression levels in breast tumor cells may foster the resistance to therapeutic agents targeting growth factors or estrogen receptors, HDAC6 or cytotoxic compounds. Partial responses to pan-HDAC inhibitors have been shown [97] in GRK2-overexpressing cells, while extra GRK2 dosage decreases the cytotoxic effectiveness of HDAC6 inhibitors [34]. In addition, an inverse GRK2-p53 correlation is observed in xenograft tumor models and in samples of patients with breast cancer, suggesting that higher GRK2 expression would favor down-modulation of wild-type p53 protein in parallel to the activation of the pro-survival AKT cascade [34], which would counteract the effects of cytotoxic compounds. Previous data have also shown that in the presence of DNA damaging agents such as doxorubicin, GRK2 accumulates and compensates the activation of p53 triggered by G2/M checkpoints, thus limiting apoptosis of arrested cells [98]. Therefore, it could be speculated that treatment of certain types of breast cancer may benefit from the combined use of already used HDAC6 inhibitors and of those being developed for Pin1 [99] or GRK2 [100].

However, as discussed above, a more complex picture arises when considering other cells in the tumor microenvironment. Breast tumor cells secrete unknown factors able to down-regulate GRK2 levels in endothelial cells, and endothelial GRK2 expression is decreased in human breast cancer vessels [49]. Down-regulation of GRK2 in endothelial cells is relevant in triggering the tumor angiogenic switch, by altering the response to TGF-β and other angiogenic stimuli. In this way, decreased endothelial GRK2 levels promote leaky and immature vessels and lead to enhanced hypoxia and macrophage infiltration, thus fostering tumor growth in mice [49]. These data open new questions regarding the integrated impact of potential GRK2 inhibitors in breast cancer development.

An enhanced GRK2 expression and a positive role in tumor progression have also been reported in pancreatic cancer. RNAi-mediated silencing of GRK2 significantly inhibited growth of Panc-1 cancer cells by inducing cell cycle arrest by undefined mechanisms. GRK2 protein was present in both epithelial as well as in subsets of infiltrating immune cells in circa 50% of samples from patients of pancreatic ductal adenocarcinoma was versus non-neoplastic tissues [101]. The use of GRK2 overexpression as a potential indicator of unfavorable prognosis in pancreatic cancer has also been suggested [5].

Changes in GRK2 functionality have been reported in some endocrine cell tumors. Higher GRK2 protein levels are present in granulosa cell ovarian tumors compared to nonmalignant cells [102], although potential pathogenic mechanisms were not explored. Enhanced GRK2 levels were reported in a limited cohort of non-medullary differentiated thyroid carcinoma, while GRK2 overexpression reduced proliferation in two poorly differentiated thyroid cell lines, suggesting that GRK2 roles may change with the differentiation status [35]. In differentiated carcinomas, the activity of the TSHR/Gs/AMPc signaling pathway drives tumor growth, and consistently desensitization of this GPCR is markedly reduced in this tumor type due to the down-modulation of GRKs distinct to GRK2 (mainly GRK5). This fact suggested that the increased activity of GRK2 in this context would modulate other proliferation or survival pathways independent of TSH.

Conflicting results have been reported in prostate cancer. GRK2 down-modulation was observed in a subset of high-grade prostate adenocarcinomas [103], whereas the C-terminal domain of GRK2 inhibited prostate cancer cell proliferation in vitro and in vivo via undetermined mechanisms [104]. Notably, the ADRBK1 gene is amplified at a frequency of 25% in neuroendocrine prostate cancer (http://cbioportal.org). These aggressive tumors often arises in later stages of castration-resistant prostate cancer and are characterized by loss of androgen receptor expression and activation of the PI3K pathway, among other molecular features [105]. Since androgen-independent growth of prostate cancer cells upon therapeutic androgen withdrawal can be promoted by overexpression of the EGF receptor (EGFR) family member HER2, it is tempting to suggest that GRK2 upregulation would favor tumor progression in this context. Consistently, a kinase-substrate enrichment phosphor-proteomic analysis has recently implicated GRK2/3 activation in metastatic castration-resistant prostate cancer, stressing the interest in investigating the role of these proteins in prostate cancer progression [106].

Studies in cultured cell models suggest an inhibitory role for GRK2 in IGF-1-dependent proliferation and migration pathways in hepatocellular carcinoma cells (HCC) [107,108]. However, its overall effect may be not straightforward and depend on the integration of its impact on different growth factor and cell differentiation signaling modules altered in HCC. Although detailed information about GRK2 expression in HCC patients is lacking, data from the Atlas Human Protein and the cBioportal suggest that either downregulation or overexpression of GRK2 may take place in this cancer type, in which chromosomal amplification of genes in the 11q13.2 region has also been reported [109].

Emerging data suggest that decreasing GRK2 might favor tumor progression in some other contexts. As discussed in a previous section, down-modulation of GRK2 has been observed in Kaposi's sarcoma leading to enhanced endothelial cell migration [50]. On the other hand, preliminary data from our laboratory indicate a putative inhibitory role of GRK2 in the progression of skin, cervix and head and neck squamous cell carcinomas (SCC), with very low levels of GRK2 present in undifferentiated and high-grade tumors (Palacios et al., in preparation).

Such dual role depending on the tumor type also applies for GRK3. Analysis of public mRNA microarray datasets indicated that GRK3 is expressed at significant lower levels in breast tumors, particularly in the basal, triple negative subtype, although this was not confirmed at the protein level [46]. An inverse correlation between GRK3 and CXCR4 expression was found in these cancer subtypes, and an enhanced ratio of CXCR4/GRK3 transcript copy was noted to correlate with the invasiveness potential of breast cancer cell lines. Consistent with the notion that decreased GRK3 levels would foster CXCR4-mediated invasion and metastasis, silencing of GRK3 enhanced CXCL12-triggered MDA-MB-231 basal cancer cell migration, whereas kinase overexpression had the opposite effect [46], likely by modulating CXCR4 receptor internalization and β-arrestin recruitment. GRK3 dosage did not appear to influence cell proliferation or detachment-induced death. Furthermore, GRK3 downmodulation enhanced mammary tumor formation

and lung and liver metastasis in an in vivo syngeneic mouse model [46].

Overall these data suggest that decreased GRK3 in basal breast cancer would mostly affect CXCR4 signaling related to cell migration and metastasis. This kinase has been shown to play a pivotal role in regulating CXCL12-mediated migration and CXCR4 signaling in the context of immune deficiency and inflammation in both human and mouse [110,111]. On the contrary, GRK2 functionality is upregulated in both luminal and basal breast cancer cells and favors tumor progression by fostering growth factor signaling and the HDAC6/Pin1 axis. Therefore, it would be of interest to investigate how such concurrent and opposite alterations in GRK2 and GRK3 may cooperate in promoting aberrant GPCR and growth factor signaling in basal breast cells through their respective preferential targets in such contexts.

Interestingly, reduced GRK3 mRNA levels have also been noted in the classical subtype of glioblastoma. GRK3 expression is down-regulated by EGFR activation, and in glioblastoma models decreased GRK3 led to sustained CXCR4 signaling and enhanced tumor growth [52], although additional effects of GRK3 dosage on the crosstalk between tumor and endothelial cells was also apparent. Conversely, higher GRK2 mRNA levels were apparent in the mesenchymal glioma subtype, what may facilitate tumor growth and invasiveness by the CXCL12/CXCR7 axis, since CXCR7 is also overexpressed in these cells and GRK2 fosters the CXCL12/CXCR7 signaling in astrocytes [31].

A different role for GRK3 has been put forward in prostate cancer. GRK3 protein labeling was higher in a human prostate carcinoma microarray compared with benign prostatic hyperplasias, especially in non-skeletal tumor metastases [53]. GRK3 was found to be essential for the survival and proliferation of prostate human metastatic cell lines in culture. Overexpression of this kinase was sufficient to potentiate both tumor growth and metastasis in poorly metastatic DU145 and LNCaP cells in mouse xenograft models. As for the mechanisms involved, no differences in growth rate, migration, or invasion were detected between control and GRK3-expressing PC3 prostate cancer cells. However, the latter cells strongly promoted endothelial cell migration and an increase in proliferating micro-vessels in both the primary tumor and in the metastases [53]. Enhanced GRK3 dosage appears to down-regulate the expression of thrombospondin-1 (TSP-1) and plasminogen activator inhibitor 2 (PAI-2). These factors have previously been reported to act as angiogenesis inhibitors in human tumors by decreasing endothelial proliferation and migration or inhibiting the urokinase plasminogen activator, respectively (see references in [53]). The mechanisms by which GRK3 blocks TSP-1 and PAI-2 expression thus leading to enhanced angiogenesis are unknown.

GRK3 levels are also high in neuroendocrine prostate cancer [112], an aggressive subtype of prostate tumors arising after radiation therapy or androgen deprivation therapies. In these treatment contexts, the enhanced activation of the cAMP response element binding protein (CREB) pathway would directly target and induce the GRK3 gene, consistent with the positive correlation between GRK3 and CREB expression in human prostate cancers. GRK3 overexpression in PAC cells increased the expression of neuroendocrine markers, whereas silencing blocked CREB-induced neuroendocrine differentiation and inhibited proliferation.

Overall, available data indicate that, despite their high degree of homology, the role of GRK2 and GRK3 is non-redundant and that these kinases play independent roles in the progression of specific types of cancer.

5.2. Roles of GRK4/5/6 family members in progression of specific tumor types

As discussed in prior sections, GRK5 is the member of this GRK subfamiliy more thoroughly investigated in terms of regulation and functional interaction with different signaling pathways. As other GRKs, GRK5 seems to be able to play a dual role in cancer development, either favoring or inhibiting oncogenic cascades depending on the context (reviewed in [28]). However, there is limited information regarding changes in GRK5 expression or functionality in human tumors.

GRK5 down-modulation at the protein level takes place in differentiated thyroid carcinoma, leading to enhanced TSH-mediated cAMP signaling and increased proliferation [113]. On the other hand, GRK5 expression increases with tumor grade in glioblastoma multiforme samples, being particularly upregulated in stem cells compared to differentiated glioblastoma cells freshly isolated from these specimens. Glioblastoma stem cells are highly invasive and more resistant to chemotherapy and radiation. Interestingly, silencing GRK5 decreased the proliferation of glioblastoma stem cells [114].

Other reports in experimental models suggest a varied role for GRK5 in tumor development. Regarding prostate cancer, GRK5 silencing inhibited proliferation of the PC3 cell line by G2/M arrest during cell cycle (reviewed in [114]). Moreover, GRK5 plays a relevant role fostering the migration and invasion capabilities of different prostate cell lines by phosphorylating moesin at the T66 residue [39], and silencing GRK5 reduced tumor development and metastasis by prostate cancer cells in xenograft mouse models. Notably, both GRK5 and GRK6 are enriched in exosomes from prostate cancer cells [115], along with c-Src, IGF-Receptor and focal adhesion kinase. In human osteosarcoma cell lines, high GRK5 levels appear to contribute to enhanced survival by phosphorylating p53 at the T55 residue, promoting its degradation and inhibiting p53-mediated apoptosis [38]. Consistently, GRK5 knockout mice display altered p53 levels and higher susceptibility to radiation. On the contrary, in model colon cancer cell lines, GRK5 appear to act as a negative modulator of tumor growth. In the HCT116 line, tazarotene-induced gene 1 (TIG1), a retinoid-inducible tumor suppressor gene, induces GRK5 expression, which in turn inhibits prostaglandin E2-mediated cell proliferation [116]. GRK5 also inhibits Kaposi's sarcoma cell proliferation and transformation by counteracting the effects of a constitutively active GPCR encoded by the human herpesvirus 8 [117]. These potentially relevant results will have to be complemented with studies aimed at determining changes in GRK5 levels, localization or functionality during progression of these different tumor types.

Regarding other members of the subfamily, reduced GRK6 expression has been noted in a limited cohort of medulloblastoma, patients, particularly in the sonic-hedgehog responsive subgroup, leading to increased tumor progression through enhanced CXCR4 signaling, whereas GRK6 overexpression impaired CXCL12-dependent migration [118]. As noted in a previous section, altered chemokine receptor signaling has also been implicated in the enhanced sensitivity of GRK6 knockout mice to tumor progression and metastasis in the Lewis Lung carcinoma heterotopic murine model [55]. Interestingly, GRK6 expression has been recently reported to be downregulated at both the mRNA and the protein level in a cohort of lung adenocarcinoma patients and correlated with worse overall survival [119]. The Human Protein Atlas data suggest that global immunoreactivities of GRK6, GRK5 and GRK3 decrease in NSCLC tumors, while expression of GRK2 is up-regulated in some cases. Since nicotine promotes the translocation of β-arrestin1 to the nucleus in this cell type, where it complexes with the transcription factor E2F1 to regulate proliferative genes [120], it might be speculated that altered dosage of GRKs could affect β-arrestin1 availability for this pathway related to smoking abuse. Similarly, emerging data point to aberrant methylation of the GRK6 promoter leading to its down-regulation in hypopharyngeal squamous cell carcinoma [121]. Therefore, most of the reports to date point to a down-regulation of GRK6 in cancer contexts leading to enhanced GPCR signaling. However, GRK6 inhibition has been shown to be lethal for different human multiple myeloma cell lines [122], suggesting that this kinase may play

a positive role in the progression of this tumor type. In myeloma cells, binding to the heat shock protein HSP90 regulates GRK6 protein expression, and GRK6 silencing reduces activation of the Signal transducer and activator of transcription 3 (STAT3) and promotes cytotoxicity [122], indicating that other mechanisms may contribute to the role of GRK6 in tumor development.

Finally, the expression of certain isoforms of GRK4 has been found to be elevated in a limited cohort of ovarian granulosa cell tumors [102]. This kinase has also been suggested to play a positive role in breast tumorigenesis [123]. The presence of different GRK4 splicing variants was detected in a proportion of invasive lobular and ductal breast carcinomas, whereas no protein labeling was apparent in normal breast tissue. GRK4 overexpression in MCF7 breast cancer cells enhanced ERK1/2 and JNK signaling and proliferation, in a kinase-activity-dependent way, whereas GRK4 silencing promoted the opposite effect. Notably, silencing of β-arrestin 1 and 2 blocked the effects of GRK4 overexpression, suggesting that this kinase plays a positive role in breast tumor proliferation by favoring β-arrestin pathways [123].

6. Concluding remarks

The data summarized in this review strongly suggest that altered functionality of particular GRKs can have an important impact on several signaling pathways and cellular processes related to the hallmarks of cancer, involving both cells of the primary tumor and of the tumor microenvironment, thus contributing to the progression of specific cancer types. A number of key issues should be addressed in future research to gain further insight on the role of GRKs and help in the design of therapeutic strategies.

First, a detailed study of the expression levels, localization and functional status of particular GRKs in specific tumors at different stages of progression is needed. This would require adequate experimental tools, such as isoform-specific antibodies, as well as phosphor-antibodies able to detect changes in the phosphorylation status of GRKs known to modify its activity and interactome and/or sites of GRK phosphorylation in cellular substrates [4,6,14,34]. Since changes in the dosage of different GRKs might occur in parallel in endothelial cells or in circulating monocytes and other immune cell types and contribute to foster tumor progression and metastasis, these investigations should also include when possible other cell types present in the tumor microenvironment.

In addition to experiments with cultured cell lines, xenograft and orthotopic tumor mouse models using tissue and cell-type GRK knockout or transgenic animals (as in [49]) will help to investigate the integrated impact on tumor development of up-regulating or down-regulating GRK expression in given tumors or stromal cell types. Such information could also be key to assess the feasibility of therapeutic strategies using GRK inhibitors or agents able to foster GRK expression.

We also need to gain further insight on the effect of oncogenic drivers and/or stimuli present in the tumor milieu in promoting changes in GRK expression, activity or localization in a tumor and cell type-specific way and on the molecular mechanisms (at the genomic, transcriptional, miRNA or protein stability levels) involved.

Future studies should also investigate how GPCR-related and non-canonical functions of given GRKs are integrated in specific cell types and pathological conditions, cooperating with oncogene-governed pathways or allowing compensatory signaling cascade networks to strengthen cancer progression and metastasis. The role of GRK dosage might be particularly relevant in the context of acquired resistance to therapeutic drugs targeting components of signaling networks, conditions that often lead to the emergence of compensatory or alternative pathways [124]. It is tempting to suggest that changes in GRK functionality might in some contexts modulate the cross-talk among GPCR, RTK and other oncogenic networks, so determination of alterations of the expression of GRKs in tumor contexts could be useful as a biomarker predictive of resistance or in designing combination therapies.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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