EBioMedicine 40 (2019) 7–8

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

EBioMedicine
Published by THE LANCET

Commentary

The dark side of radiotherapy-induced cell death in cancer

Sonia Alcalá [a,b,c], Bruno Sainz Jr. [a,b,c,*]

[a] Department of Biochemistry, Universidad Autónoma de Madrid (UAM), Madrid, Spain
[b] Department of Cancer Biology, Instituto de Investigaciones Biomédicas "Alberto Sols" (IIBM), CSIC-UAM, Madrid, Spain
[c] Chronic Diseases and Cancer Area 3, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain

Pancreatic ductal adenocarcinoma (PDAC), currently the fourth leading cause of cancer-related death worldwide, is projected to become the second leading cause by 2030 [1]. In addition to its high metastatic capacity, this extremely aggressive malignancy is characterized by its inherent resistance to chemotherapy and radiotherapy [3]. These attributes are believed to be due to the existence of a subpopulation of stem-like cells within the tumor known as cancer stem cells (CSCs). CSCs constitute a biologically unique subset of rare cells within the bulk tumor cell population that possess, by definition, inherent self-renewal properties, multipotency and an exclusive ability to initiate and recapitulate the parental tumor upon serial passage in immunodeficient mice [2]. In addition, CSCs participate in the multistep process of oncogenesis, giving rise to the clonogenic core of the tumor, and these cells are also necessary for long-term tumor maintenance, are key drivers of metastasis, and represent the underlying cause of chemo- and radio-resistance [3].

Reversing CSC-mediated chemo- and radio-resistance would greatly improve overall patient survival; however, to date, we are still far from understanding the complex process of therapy resistance. In *EBioMedicine*, Chen et al. build upon their previous findings [4], and show in this new study that radiotherapy-induced dying tumor cells not only secrete the high-mobility group box 1 (HMGB1) protein, an extracellular damage-associated molecular pattern molecule, but secreted HMGB1 can bind to Toll-like receptor 2 (TLR2) on pancreatic CD133$^+$ CSCs to promote their self-renewal and tumorigenic capacity [5]. Thus, the authors propose HGMB1 as a previously unrecognized driver of pancreatic CSC proliferation in the presence of radiotherapy. Interestingly, while HMGB1 has been extensively studied in PDAC, its role as a tumor suppressor or tumor promoter has been debated [8]. For example, Kang et al. recently showed that intracellular HMGB1 can act as a tumor suppressor in early-stage PDAC development, by sustaining chromosome stability and limiting pro-inflammatory nucleosome release and activity in a K-ras-driven mouse model of PDAC [6]. On the flipside, extracellular HGMB1 can act as a pro-tumor protein by enhancing, for example, receptor for advanced glycation end products (RAGE)-dependent inflammatory responses [7], and HGMB1 has also been associated with pancreatic cancer invasion and metastasis, chemotherapy resistance, autophagy and immunogenic cell death (reviewed in [8]).

While HMGB1 may likely have a dual role in tumor development, and its pro- or anti-tumor properties may be tumor type specific, in this study Cheng et al. highlight a previously undefined and context-specific role for this protein in PDAC and specifically in pancreatic CSCs[5]. Following anti-cancer therapy, dying (necrotic or apoptotic) cells are believed to secrete factors that can promote chemoresistance or stimulate the regrowth of neighboring resident tumor cells. Cheng and colleagues wisely associate radiotherapy-induced tumor cell death with HMGB1 secretion and enrichment of the CD133$^+$ pancreatic CSC population. They provide, for the first time, a mechanism by which the "necrosis niche", the intratumor microenvironment containing dying cells, debris and their released soluble factors, can enrich for CSCs following radiotherapy *via* secreted HMGB1. They show that radiotherapy enriches for CD133$^+$ CSCs from PDAC cell lines *in vitro* and *in vivo*, dying cells secrete HMGB1, and secreted HMGB1 or recombinant HMGB1 can positively regulate CD133$^+$ CSC *in vitro* stemness and *in vivo* tumorigenicity *via* binding to TLR2, which is expressed to high levels of CD133$^+$ cells. HMGB1 can bind and activate TLR2, TLR4, and TLR9, as well as RAGE, CD24 and certain integrins. Interestingly, the authors observed that HMGB1 enrichment of CSCs was TLR2- and not TLR4-specific as overexpression of TLR4, silencing of TLR4 or pharmacological inhibition of TLR4 revealed an unexpected antagonistic effect of TLR4 on the HMGB1/TLR2 axis-induced stemness effect in CD133$^+$ cells. It remains to be determined if TLR9, RAGE, CD24 or integrins are also involved in this HMGB1-mediated pro-CSC signaling process. Nonetheless, the authors were also able to show that the HMGB1/TLR2 axis maintains and enhances the stemness of CD133$^+$ CSCs by activating Wnt/β-catenin signaling, highlighting several putative therapeutic targets.

Certainly, some pre-clinical and clinical aspects still remain unresolved and should be further investigated in follow-up studies. For example, while the *in vivo* studies in which CD133$^+$ SW1990 cancer cells were stimulated with conditioned media from irradiated HMGB1+ cells, conditioned media from HMGB1-silenced cells or with rhHMGB1 showed that HMGB1 can promote increased tumor growth over 27 days, the use of K-ras-driven mouse models of PDAC with temporal and conditional knockout of HMGB1 prior to irradiation may provide more conclusive insights into the role HMGB1 in PDAC radio-

DOI of original article: https://doi.org/10.1016/j.ebiom.2018.12.016.
* Corresponding author at: Department of Biochemistry, Universidad Autónoma de Madrid, Calle del Arzobispo Morcillo 4, Madrid 28029, Spain.
E-mail address: bruno.sainz@uam.es (B. Sainz).

https://doi.org/10.1016/j.ebiom.2019.01.047

resistance. Likewise, several strategies have been proposed to inhibit HMGB1 expression, activity and release in a direct or indirect manner for the treatment of cancer, as reviewed in [9]. For example, the triterpenoid saponin glucoside of glycyrrhizic acid, Glycyrrhizin (GR), can inhibit the chemotactic and mitogenic activities of HMGB1 [10], and anti-HMGB1 antibodies could neutralize secreted HMGB1 *in vivo*. The efficacy of these and other HMGB1 inhibitors, alone or in combination with Wnt/β-catenin signaling inhibitors, should be tested in the context of radiation therapy *in vivo* to conclusively determine the clinical relevance of HMGB1 in pancreatic cancer radio-resistance. Nevertheless, this study offers a strong rational to continue investigating the role of HMGB1 in PDAC, and provides important evidence to continue elucidating how the "therapy-induced dying cell niche" may be more harmful than helpful with respect to enriching or activating the CSC compartment in PDAC.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The work of Dr. Sainz is supported by a Rámon y Cajal Merit Award from the Ministerio de Economía y Competitividad, Spain, a Coordinated grant from the Fundación Asociación Española Contra el Cáncer (AECC), and a Fondo de Investigaciones Sanitarias (FIS) grant PI15/01507 (co-financed through Fondo Europeo de Desarrollo Regional (FEDER) "Una manera de hacer Europa") from the Instituto de Salud Carlos III (ISCIII), Spain.

References

[1] Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014;74(11):2913–21.
[2] Nguyen LV, Vanner R, Dirks P, Eaves CJ. Cancer stem cells: an evolving concept. Nat Rev Cancer 2012;12(2):133–43.
[3] Hermann PC, Sainz Jr B. Pancreatic cancer stem cells: a state or an entity? Semin Cancer Biol 2018;53:223–31.
[4] Chen X, Zhang L, Jiang Y, et al. Radiotherapy-induced cell death activates paracrine HMGB1-TLR2 signaling and accelerates pancreatic carcinoma metastasis. J Exp Clin Cancer Res 2018;37(1):77.
[5] Chen X, Cheng F, Liu Y, et al. Toll-like receptor 2 and Toll-like receptor 4 exhibit distinct regulation of cancer cell stemness mediated by cell death-induced high-mobility group box 1. EBioMedicine 2019. https://doi.org/10.1016/j.ebiom.2018.12.016.
[6] Kang R, Xie Y, Zhang Q, et al. Intracellular HMGB1 as a novel tumor suppressor of pancreatic cancer. Cell Res 2017;27(7):916–32.
[7] Kang R, Zhang Q, Hou W, et al. Intracellular Hmgb1 inhibits inflammatory nucleosome release and limits acute pancreatitis in mice. Gastroenterology 2014;146(4):1097–107.
[8] Cebrian MJ, Bauden M, Andersson R, Holdenrieder S, Ansari D. Paradoxical Role of HMGB1 in pancreatic cancer: tumor suppressor or tumor promoter? Anticancer Res 2016;36(9):4381–9.
[9] He SJ, Cheng J, Feng X, Yu Y, Tian L, Huang Q. The dual role and therapeutic potential of high-mobility group box 1 in cancer. Oncotarget 2017;8(38):64534–50.
[10] Mollica L, De Marchis F, Spitaleri A, et al. Glycyrrhizin binds to high-mobility group box 1 protein and inhibits its cytokine activities. Chem Biol 2007;14(4):431–41.