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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Acinar-to-Ductal Metaplasia Induced by Transforming Growth Factor Beta Facilitates KRAS^{G12D}-driven Pancreatic Tumorigenesis

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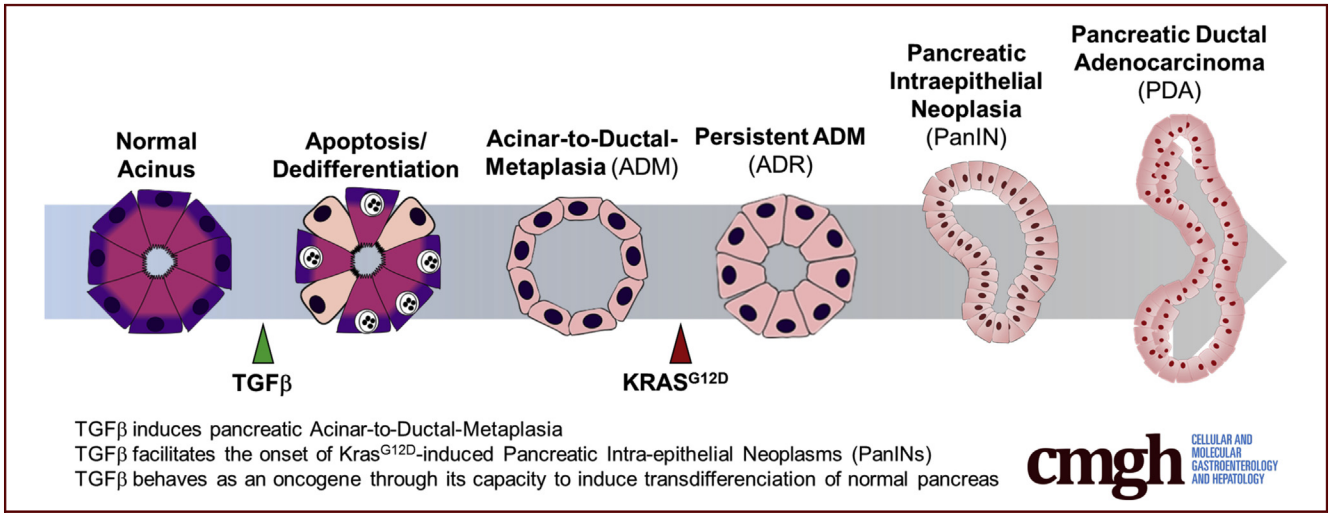

SUMMARY

Acinar-to-Ductal Metaplasia (ADM) is considered the main origin of pancreatic pre-neoplastic lesions that eventually develop into Pancreatic Ductal Adenocarcinoma (PDA). ADM could be a decisive step during tumorigenesis, selecting plastic cells for a more aggressive subsequent tumorigenesis. Our results indicate that TGFβ, a cytokine overexpressed during early and late pancreatic tumorigenesis, is an inducer of ADM and set up a favorable context for the emergence of oncogene-driven neoplastic lesions. This questions the usual dichotomist vision of TGFβ in PDA.

BACKGROUND & AIMS: Transforming growth factor beta (TGFβ) acts either as a tumor suppressor or as an oncogene, depending on the cellular context and time of activation. TGFβ activates the canonical SMAD pathway through its interaction with the serine/threonine kinase type I and II heterotetrameric receptors. Previous studies investigating TGFβ-mediated signaling in the pancreas relied either on loss-of-function approaches or on ligand overexpression, and its effects on acinar cells have so far remained elusive.

METHODS: We developed a transgenic mouse model allowing tamoxifen-inducible and Cre-mediated conditional activation of

a constitutively active type I TGFβ receptor (TβRI^{CA}) in the pancreatic acinar compartment.

RESULTS: We observed that *TβRI^{CA}* expression induced acinar-to-ductal metaplasia (ADM) reprogramming, eventually facilitating the onset of KRAS^{G12D}-induced pre-cancerous pancreatic intraepithelial neoplasia. This phenotype was characterized by the cellular activation of apoptosis and dedifferentiation, two hallmarks of ADM, whereas at the molecular level, we evidenced a modulation in the expression of transcription factors such as *Hnf1β*, *Sox9,* and *Hes1*.

CONCLUSIONS: We demonstrate that TGFβ pathway activation plays a crucial role in pancreatic tumor initiation through its capacity to induce ADM, providing a favorable environment for KRAS^{G12D}-dependent carcinogenesis. Such findings are highly relevant for the development of early detection markers and of potentially novel treatments for pancreatic cancer patients. *(Cell Mol Gastroenterol Hepatol 2017;4:263–282; http://dx.doi.org/10.1016/j.jcmgh.2017.05.005)*

Keywords: Pancreas; Cancer; TGFβ; Acinar-to-Ductal Metaplasia; KRAS^{G12D}.

In mammals, the pancreas is a bifunctional organ.[1] The exocrine pancreas, composed of acinar cells and ducts, accounts for more than 90% of the organ's mass. Acinar cells secrete digestive enzymes that are further collected in a network of ducts that discharge pancreatic juices into the duodenum. The endocrine tissue, represented by islets of Langerhans embedded in the exocrine pancreas, controls blood glucose levels by secreting hormones such as insulin and glucagon. Embryonically, all pancreatic lineages arise from a multipotent progenitor present at day E9 in the mouse endoderm.[2] The fate of the pancreas is dictated by the expression of progenitor markers such as SOX9 and CK19 for ductal cells, MIST1, PTF1A, CPA1, and ELA1 for acinar cells, and NGN3 for endocrine cells.[2–4] Note that PTF1A is required for the specification of the pancreatic multipotent progenitor cells and is later expressed only in the adult acinar compartment.[5]

The adult pancreas is highly plastic[6] to ensure organ integrity in response to internal (intracellular activation of digestive enzymes, obstruction of main ducts potentially as a result of gallstones) or external stresses (alcohol, trauma). Repair and regeneration of the injured organ are orchestrated by many cell types including acinar cells, centroacinar cells, ductal cells, immune cells, and stellate cells.[7,8] The existence of a resident stem cell population in the organ remains controversial.[9,10] Many studies[1,11,12] have focused on post-injury pancreatic regeneration and demonstrated the crucial role of acinar cells during this process. Indeed, under severe stress conditions such as pancreatitis, acinar cells undergo acinar-to-ductal metaplasia (ADM), a morphologic and transcriptional conversion into duct-like cells with embryonic progenitor cell properties.[5,7,13] These metaplastic cells are then capable of proliferating to replenish the damaged organ. In the case of a sustained stress signal or concomitant oncogenic activation such as KRAS activating mutations (eg, KRAS^{G12D}), the metaplastic cells cannot revert to a differentiated state, as generally observed in the case of an acute stress. This pancreatic erosion process constitutes a favorable setting for the onset of low-grade pancreatic intraepithelial neoplasia (PanIN).[14] Progression toward PanINs of higher grade and eventually pancreatic ductal adenocarcinoma (PDA) is associated with recurrent mutations or genetic/epigenetic alterations in tumor suppressor genes (*INK4/ARF*, *TP53*).[15,16] Importantly, *SMAD4/DPC4*, a core component of the transforming growth factor beta (TGFβ) signaling pathway, is deleted in 50% of PDAs.[16–18] Members of the TGFβ superfamily of transforming growth factors are involved in embryonic development, regulation of homeostasis, and the pathogenesis of a variety of diseases.[19,20] TGFβ signaling occurs through a heterotetrameric receptor complex composed of 2 subunits, the type I and type II TGFβ receptors (TβRI and TβRII, respectively). On binding to its receptors, TGFβ enables TβRII to transphosphorylate TβRI, which in turn activates the canonical SMAD pathway (phosphorylation of SMAD2 and SMAD3 that further interact with SMAD4 to accumulate inside the nucleus) and other signaling pathways (MAPK, RHOA, and PI3K/AKT).[21] In cancer, TGFβ behaves as either a tumor suppressor or a tumor promoter. Indeed, TGFβ is generally considered to be a tumor suppressor early in tumor development (tumor initiation) by restricting epithelial cell growth (through cytostasis and apoptosis).[19,22,23] However, in later stages of tumorigenesis (tumor progression), TGFβ has oncogenic properties through its capacity to regulate cellular plasticity by stimulating biological processes, including extracellular matrix deposition, immune evasion, epithelial-to-mesenchymal transition (EMT), and stemness.[22,24–27]

Unlike the majority of previously published studies in which the TGFβ signaling pathway was abrogated, our present work addresses the consequences of TGFβ cell-autonomous activation in the pancreatic epithelial lineage. To this end, we generated a unique mouse model enabling us to express a constitutively activated TβRI receptor (TβRI^{CA}) exclusively in pancreatic acinar cells, starting either in the embryo or after birth. Hence, we demonstrate that the specific activation of TGFβ signaling in pancreatic acinar cells induced ADM reprogramming and eventually facilitated the onset of KRAS^{G12D}-induced PanINs and PDA progression.

[a]Authors share co-first authorship.

Abbreviations used in this paper: **ADM, acinar-to-ductal metaplasia; AFI, acinar fatty infiltration; EMT, epithelial-to-mesenchymal transition; PanIN, pancreatic intraepithelial neoplasia; PBS, phosphate-buffered saline; PDA, pancreatic ductal adenocarcinoma; RT-qPCR, reverse transcription quantitative polymerase chain reaction; TGFβ, transforming growth factor beta; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.**

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