A novel role of hepatic epithelial transforming growth factor- β signaling in cholangiocarcinogenesis

Man Liu¹, Myth T. S. Mok^{1,3}, Alfred S. L. Cheng^{1,2,3}

¹School of Biomedical Sciences, ²State Key Laboratory of Digestive Disease and Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong SAR, China; ³Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen 518057, China

Correspondence to: Prof. Alfred S. L. Cheng, School of Biomedical Sciences and State Key Laboratory of Digestive Disease, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China. Email: alfredcheng@cuhk.edu.hk.

Submitted Feb 07, 2016. Accepted for publication Feb 18, 2016.

doi: 10.21037/atm.2016.03.01

View this article at: <http://dx.doi.org/10.21037/atm.2016.03.01>

Transforming growth factor- β (TGF- β) signaling regulates a broad range of cellular processes including cell proliferation, differentiation and apoptosis (1). Based on the current knowledge, TGF- β is the main pro-fibrogenic cytokine in the liver that induces fibrosis by activating the hepatic stellate cells (HSCs) (2). However, the role of TGF- β in hepatocarcinogenesis is not as clear as in hepatic fibrogenesis because of the dual functions of TGF- β as both a tumor suppressor and promoter (3). In tumor microenvironment, many cell types are responsive to TGF- β signaling leading to complex effects on cancer initiation and progression. It is now generally accepted that TGF- β acts as a tumor suppressor at early stage of cancer development by inhibiting cell cycle progression and inducing malignant cell apoptosis. However, in late stage, TGF- β acts as a tumor promoter by increasing tumor invasiveness and metastasis. The pro-tumorigenic effect of TGF- β is evident by the induction of a mesenchymal phenotype in epithelial tumor cells, also known as epithelial-to-mesenchymal transition (EMT) after prolonged exposure to TGF- β (4). Indeed, overexpressed TGF- β has been related to increased tumor progression and poor clinical outcomes in different types of cancers (5). Given the critical role of TGF- β in tumor progression, TGF- β has been regarded as a promising target for cancer therapy (6).

Liver is a multicellular organ composed of diverse cell types, including epithelial cells (e.g., hepatocytes, cholangiocytes, etc.) and mesenchymal cells (e.g., HSCs, liver macrophages (Kupffer cells), sinusoidal endothelial cells, etc.) (7). Among these cells, HSCs can be directly stimulated by TGF- β , in which the TGF- β signaling is propagated by TGF- β receptors,

Smad2/3/4 and miRNAs, leading to transcriptional changes for fibrogenic phenotype (8). The resulting fibrosis can further progress to cirrhosis, and eventually hepatocellular carcinoma (HCC) (8). Of note, Smad7 is an antagonist of this TGF- β -Smad pathway through a negative feedback mechanism (9). In addition to fibrosis, TGF- β has also been implicated in liver cancer development. TGF- β is an immune regulator that takes part in innate and adaptive immune response (10). Elevated TGF- β in tumor microenvironment is widely reported to impair cancer immune surveillance by induction of M2 macrophage polarization (11), inhibition of NK cell maturation (12), impairment of antigen presenting function of dendritic cells (13), and induction of regulatory T cell (Treg) and myeloid derived suppressive cell (MDSC) expansion (14), which all contribute to immune tolerance and promote tumor immune escape and progression. Despite the diverse effects of TGF- β , its exact roles in individual hepatocellular compartments have not been clearly distinguished. To evaluate the therapeutic values of hepatic TGF- β -targeted drugs, it is necessary to characterize the TGF- β functions in context- and cell-specific manners.

In a recent issue of *Gastroenterology*, Mu *et al.* reported a comprehensive *in vivo* study on the functions of TGF- β in the epithelial compartment of injured liver (15). They first confirmed the activation of TGF- β signaling in epithelial cells (hepatocytes and cholangiocytes) and mesenchymal cells (HSCs) in both human cirrhotic liver and murine injured livers [treated with carbon tetrachloride (CCl₄), bile duct ligation (BDL) or *Mdr2* knockout]. To dissect the cell-specific roles of TGF- β , the authors generated double

transgenic mice devoid of epithelial TGF- β receptor II (*TBR2*^{lko}) and compared them with controls for liver fibrosis development. Surprisingly, they found that epithelial *TBR2* affected neither liver injury nor fibrosis development in all three CCl₄, BDL and *Mdr2* knockout mouse models. Moreover, expression of epithelial *TBR2* is not related to the formation of diethylnitrosamine (DEN)-induced HCCs and the associated expression of *Afp*, *Cd133*, and *mKi67*. These results contradict with a previous finding that reported the positive regulation of TGF- β on liver fibrosis and HCC development (16), though in which a different knockout mouse model deficient in *Tak1* (a downstream mediator of TGF- β) was used and the results might not be as directly relevant to TGF- β as those from *TBR2*^{lko} mouse model.

To further investigate the functional role of epithelial TGF- β signaling in liver carcinogenesis, Mu *et al.* generated more knockout models including *PTEN*^{lko} and *TBR2 PTEN*^{lko}. Both single and double knockout mice were born normally. Intriguingly, all the *TBR2 PTEN*^{lko} mice developed cholangiocarcinomas and died around age 5-7 months, whereas *PTEN*^{lko} mice displayed no tumors or mortality at the same ages. Consistent with the phenotype, cholangiocyte- and cholangiocarcinoma-related markers such as *Ehf*, *Reg1* and *Dmbt1* were also up-regulated in *TBR2 PTEN*^{lko} mice compared with *PTEN*^{lko} controls. In addition, considerable expansion of cholangiocytes was found in *TBR2 PTEN*^{lko} mice. These findings suggest that epithelial TGF- β signaling has a protective role against cholangiocarcinoma formation, which contrasts with the previous results from Shuang group that TGF- β can promote EMT in human cholangiocarcinoma cell line TFK-1, resulting in the acquisition of cancer stem cell traits, and increased invasiveness and metastasis of cholangiocarcinoma (17).

To determine whether the TGF- β signaling in cholangiocytes and/or hepatocytes contributes to the cholangiocarcinogenesis in *TBR2 PTEN*^{lko} mice, Mu *et al.* generated more mouse models for cholangiocyte-specific knockout [*TBR2 PTEN* ^{Δ Cbol(Prom1)} and *TBR2 PTEN* ^{Δ Cbol(K19)}] and hepatocyte-specific knockout (*TBR2 PTEN* ^{Δ Hep}). After treatment with DDC diet, rapid development of cholangiocarcinoma (<20 weeks) was evident in *TBR2 PTEN* ^{Δ Cbol(Prom1)} and *TBR2 PTEN* ^{Δ Cbol(K19)} mice, wherein cholangiocytes expanded in the absence of *TBR2* and *PTEN*, and were regarded as the major cell type responsible for cholangiocarcinogenesis. Similar to the cholangiocyte-specific knockout models, *TBR2 PTEN* ^{Δ Hep}

mice also developed cholangiocarcinoma, but in a significantly lower rate (>52 weeks), of which tumors exhibited comparable gene expression patterns to those of human cholangiocarcinoma. Based on these results, the authors concluded that *TBR2* ablation in hepatocyte-derived cholangiocytes, rather than hepatocytes, promotes cholangiocarcinoma development.

TGF- β -dependent pathways are among the most complex molecular signaling cascades that can exert pleiotropic effects in a broad range of cell types in multiple organs. Numerous studies have reported the functional roles of TGF- β signaling in liver pathogenesis, particularly fibrogenesis and carcinogenesis. Nevertheless, the consensus is mainly confined to the pro-fibrogenic role of TGF- β in HSCs. The recent study by Mu *et al.* comprehensively proved that epithelial TGF- β signaling has insignificant effects on both liver fibrogenesis and carcinogenesis, but it can suppress cholangiocarcinoma formation by inhibiting the proliferation of hepatocyte-derived cholangiocytes. These results clearly demonstrate the cell-specific and opposite actions of TGF- β in the liver. However, it should be noted that all cholangiocarcinoma data in the Mu study were derived from mouse models that are devoid of not only *TBR2*, but also *PTEN*. It is unclear why the *TBR2*^{lko} group was omitted in all *in vivo* cholangiocarcinoma experiments, therefore it is hard to interpret whether the observed phenotypic changes primarily resulted from the loss of *TBR2*, or both *TBR2* and *PTEN*. Another shortcoming of this study is the lack of mechanistic characterizations and validations in relevant cell models, particularly those related to *PTEN* pathways, which would otherwise help address the relationship of *PTEN* and *TBR2* in cholangiocarcinoma development, and resolve the discrepancies among different studies. In addition to the liver-residential cells, infiltrating immunoregulatory cells are also susceptible to TGF- β actions and can potentially react in different manners. Moreover, the TGF- β -Smad pathway can be epigenetically regulated in the gastrointestinal system (18). Continuous studies of the regulation of TGF- β pathway and its effects on distinct cell types in the liver will provide more specific insights on the therapeutic potential and delivery approach of TGF- β -targeted inhibitors in treating liver diseases.

Acknowledgements

Funding: This work was supported by the Collaborative Research Fund (C4017-14G) of the Hong Kong Research Grants Council and the National Natural Science Foundation

of China (373492, 81302167).

Footnote

Provenance: This is a Guest Commentary commissioned by Guest Editor Haitao Zhao, MD, PhD (Department of Liver Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China).

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

- Ikushima H, Miyazono K. TGFbeta signalling: a complex web in cancer progression. *Nat Rev Cancer* 2010;10:415-24.
- Gressner AM, Weiskirchen R, Breitkopf K, et al. Roles of TGF-beta in hepatic fibrosis. *Front Biosci* 2002;7:d793-807.
- Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. *Nat Genet* 2001;29:117-29.
- Miettinen PJ, Ebner R, Lopez AR, et al. TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. *J Cell Biol* 1994;127:2021-36.
- Pickup M, Novitskiy S, Moses HL. The roles of TGFβ in the tumour microenvironment. *Nat Rev Cancer* 2013;13:788-99.
- Yingling JM, Blanchard KL, Sawyer JS. Development of TGF-beta signalling inhibitors for cancer therapy. *Nat Rev Drug Discov* 2004;3:1011-22.
- Ramadori G, Saile B. Mesenchymal cells in the liver--one cell type or two? *Liver* 2002;22:283-94.
- Fabregat I, Moreno-Càceres J, Sánchez A, et al. TGF-β signaling and liver disease. *FEBS J* 2016. [Epub ahead of print].
- Achyut BR, Yang L.. Transforming growth factor-β in the gastrointestinal and hepatic tumor microenvironment. *Gastroenterology* 2011;141:1167-78.
- Yang L, Pang Y, Moses HL. TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. *Trends Immunol* 2010;31:220-7.
- Gong D, Shi W, Yi SJ, Chen H. TGFβ signaling plays a critical role in promoting alternative macrophage activation. *BMC Immunol* 2012;13:31.
- Marcoe JP, Lim JR, Schaubert KL, et al. TGF-β is responsible for NK cell immaturity during ontogeny and increased susceptibility to infection during mouse infancy. *Nat Immunol* 2012;13:843-50.
- Tanaka H, Shinto O, Yashiro M, et al. Transforming growth factor β signaling inhibitor, SB-431542, induces maturation of dendritic cells and enhances anti-tumor activity. *Oncol Rep* 2010;24:1637-43.
- Ryzhov SV, Pickup MW, Chytil A, et al. Role of TGF-β signaling in generation of CD39+CD73+ myeloid cells in tumors. *J Immunol* 2014;193:3155-64.
- Mu X, Pradere JP, Affò S, et al. Epithelial transforming growth factor-β signaling does not contribute to liver fibrosis but protects mice from cholangiocarcinoma. *Gastroenterology* 2016;150:720-33.
- Yang L, Inokuchi S, Roh YS, et al. Transforming growth factor-β signaling in hepatocytes promotes hepatic fibrosis and carcinogenesis in mice with hepatocyte-specific deletion of TAK1. *Gastroenterology* 2013;144:1042-54.e4.
- Shuang ZY, Wu WC, Xu J, et al. Transforming growth factor-β1-induced epithelial-mesenchymal transition generates ALDH-positive cells with stem cell properties in cholangiocarcinoma. *Cancer Lett* 2014;354:320-8.
- Yang W, Mok MT, Li MS, et al. Epigenetic silencing of GDF1 disrupts SMAD signaling to reinforce gastric cancer development. *Oncogene* 2015. [Epub ahead of print].

Cite this article as: Liu M, Mok MT, Cheng AS. A novel role of hepatic epithelial transforming growth factor-β signaling in cholangiocarcinogenesis. *Ann Transl Med* 2016;4(5):101. doi: 10.21037/atm.2016.03.01