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Targeting Transforming Growth Factor Beta Signaling in Liver Cancer

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Hepatocellular carcinoma (HCC), accounting for 85–90% of all primary liver cancers, is among the most prevalent and lethal cancers in the world, with five-year survival for advanced cases <11%. Ineffective detection of tumors and drug resistance are some of the causal factors for therapy failure. Thus, there is an urgent need to understand the molecular mechanisms underlying drug resistance and HCC initiation. Development of HCC is associated with alterations in the transforming growth factor beta (TGF- β) signaling pathway, which plays a critical role in cell proliferation, apoptosis, differentiation, motility, lineage specificity, as well as normal stem cell homeostasis (1–4). Mediated by TGF- β ligands that activate TGF- β receptors (TGFBR1 and TGFBR2) to stimulate downstream molecules against decapentaplegic (SMAD) proteins (SMAD2/3/4), the TGF- β signaling pathway positively or negatively regulates transcription of various gene targets involved in diverse biological functions. In addition, many other proteins—including the SMAD3 adaptor spectrin beta, nonerythrocytic 1 (SPTBN1); the TGFBR-associated protein Smad anchor for receptor activation; and the SMAD corepressors SNO/SKI—function as positive or negative regulators of TGF- β signaling (1). In cancers like HCC, TGF- β -SMAD signaling can be either tumor suppressing or tumor promoting (1, 5, 6). The complexity of this pathway makes it extremely hard to target for cancer therapy.

Before targeting the TGF- β pathway in HCC, there are fundamental questions that need to be addressed: at “which stage of cancer,” under “what cellular context,” through “what mechanisms” does TGF- β signaling promote HCC development and progression? Gene expression analysis of The Cancer Genome Atlas data from more than 400 HCC samples reveals that more than >40% of HCC samples carry genetic alternations in at least one of the TGF- β signaling pathway members, proving TGF- β signaling pathway is a big player in HCC pathogenesis (3). However, the mechanisms by which those alterations contribute to HCC remain unclear. Mouse models provide a system for addressing mechanistic questions.

In this issue of *Hepatology*, a study by Dr. Zhang et al. used a novel mouse model to address the precise role of TGF- β signaling in HCC initiation (4). They isolated tumor-initiating cells (TICs) from the livers of Tgfbr2 conditional mice (Tgfbr2^{f/f}) challenged by the carcinogen diethylnitrosamine (DEN) and carrying a Tgfbr2 allele flanked with loxP sites (locus of X-over P1 site) that can be recognized by cyclization recombination (Cre)-recombinase. They then transplanted these TICs by splenic injection to recipient mice with

an intact immune system. In the recipient mice, Tgfr2 is inactivated only in the transplanted TICs after injection of Cre-expressing adenovirus. There are advantages of this system over other mouse models, such as xenograft and transgenic mouse models. This approach enables the study of tumor development in immune-competent animals, which is closer to physiological than xenograft studies with immune-deficient mice. In contrast to transgenic studies, in this system the role of Tgfr2 in the TICs can be addressed without the confounding complications of non-cell autonomous effects from targeting Tgfr2 in other cells in liver or in other organs. Furthermore, cancer stem cells, which TICs resemble, tend to be resistant to chemotherapy. Thus, understanding the role of TGF- β signaling in TICs has clinical implications for HCC management. The conclusion from this study is that Tgfr2 functions as a tumor suppressor in HCC TICs; loss of TGF- β signaling in these cancer stem-like cells promotes HCC progression. The translational prediction from this finding is that at early stages of HCC inhibition of TGF- β signaling would be detrimental.

Zhang et al. found that TICs deficient in Tgfr2 form HCC, whereas TICs with intact Tgfr2 have a reduced capability to form tumors in recipient mice, supporting a tumor suppressive role of Tgfr2 (4). This study provides a direct evidence of tumor-suppressive effects of TGF- β signaling within the cancer cells, rather than the tumor stroma or microenvironment. Other studies in mice with the loss one of allele of TGF- β pathway members—Tgfr1, Tgfr2, Smad3 adaptor Sptn1 and Smad3—also indicated a tumor-suppressive role for this pathway (1, 2, 4). However, those studies could not rule out effects on the tumor stroma or microenvironment for the increase in HCC development in the heterozygous mice.

Another interesting result is that these TICs only formed tumors in liver, suggesting that only the liver is a permissive environment for colonization, survival, and proliferation of these TICs and the consequent development of HCC. We speculate that liver specific-growth factors or cytokines and chemokines provide signals for TICs to survive and expand, whereas the TICs may exhaust their self-renewal capacity in other tissues and organs that lack these factors or through elimination by the immune response of the host mice. The authors defined TICs isolated from the DEN-injured liver as cells with abundant cluster of differentiation 44, a marker of cancer stem cells and high abundance of the liver progenitor cell markers epithelial cell adhesion molecule octamer 4, Sox2 (SRY [sex determining region Y]-box 2), and Kruppel-like factor 4. However, they did not experimentally prove that these cells had self-renewal capacity (4). Whether these cells are true TICs with some capacity for self-renewal or DEN-primed liver progenitors awaits further analysis. Exposure mice to the carbon tetrachloride (CCl₄) is used to induce TICs proliferation. However, systemic treatment of CCl₄ in the recipient mice induced DNA damage in both the TICs and host liver cells (4), suggesting that CCl₄ challenge may serve as a second “hit” in HCC formation. Further studies are needed to determine whether CCl₄ converts DEN-primed TICs into HCC-initiating cells or CCl₄-induced liver damage creates a permissive environment for DEN-primed TICs, enabling initiation of HCC.

Additionally, this study uncovers a mechanism underlying the TGF- β tumor-suppressive role in HCC. The authors showed that TGF- β signaling inhibited Sox2-mediated transcription of the long noncoding RNA H19. H19 promoted HCC development by inhibiting apoptosis involving p53 and p21, which differs from other studies showing that H19 promotes TGF- β -

induced endothelial-to-mesenchymal transition (EMT) in ovarian cancer (7). In HCC, TGF- β signaling is tumor suppressive: loss of TGF- β signaling increases H19, which reduces apoptosis. In ovarian cancer, TGF- β signaling is tumor promoting: TGF- β signaling increases H19 to promote EMT. This discrepancy illustrates the context-dependent functions of TGF- β signaling and of long noncoding RNAs. These context-dependent effects must be taken into consideration when evaluating therapeutic options (Fig. 1). It is not surprising that TGF- β pathway functions as a tumor suppressor in premalignant cells or early stage of cancers, as TICs represent, when cell cycle arrest and apoptosis pathways are intact. Once the tumor cells overcome cell cycle arrest and apoptosis mediated in the later stages of cancer, TGF- β signaling can promote tumor progression. Zhang et al. induced the TICs with the carcinogen DEN and deletion of Tgfbr2. Whether DEN induced other genetic alterations in the TICs is unknown. There are chances that TICs survived from DEN-induced DNA damage through p53 mutations. A recent study from Moon et al. reported that TGF- β signaling pathway inhibits HCC progression driven by oncogenic H-RasG12V while promotes HCC growth in the context of p53 deficiency through the regulation of Snail in transposon-based mouse models of HCC (6). Further analysis is needed regarding the p53 status of the TICs isolated from mice with DEN-induced liver damage.

Identifying key driver genes of HCC is critical to improving early detection and treatment strategies for this deadly form of cancer. This article in Hepatology, showing that TGF- β signaling functions as a tumor suppressor in TICs, provides clues to solving the “when and where to target TGF- β signaling” in HCC. From a translational perspective, one can predict that through the loss-of-function mutations or deletion in TGF- β pathway genes in early-stage or late-stage HCC, patients might be resistant to chemotherapy. In late-stage patients, this would be due to the presence of TICs with defective TGF- β signaling; these cells would grow after treatment causing a relapse. However, this prediction depends on the presence of other genetic alterations that can affect the outcome of TGF- β signaling. However, these results reinforce the importance of screening patients for such genetic alterations or reduced abundance of the core components to inform HCC management.

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Figure 1.

Context-dependent dichotomy of TGF-β signaling in HCC. TGF-β signaling cooperates with transcription factors and coactivators or corepressors to activate or repress target gene expression. Depending on the cellular context, TGF-β signaling has dual role in HCC. (Left) In early stages of tumorigenesis when p53 is intact, TGF-β signaling exerts its tumor-suppressive role mainly stimulating expression of genes involved in cell cycle arrest and apoptosis. (Right) In later stages of tumor progression or when p53 is mutated, TGF-β signaling becomes tumor-promoting through a reduction in the expression of the cell cycle arrest and apoptotic genes and an increase in the expression of genes associated with EMT. Whether TGF-β-mediated changes in the expression of genes that inhibit cell growth or promote cell senescence occurs in the absence of functional p53 remains to be determined. Only representative genes in each category are shown. Genes noted in red are stimulated by TGF-β signaling; genes in blue are repressed. Abbreviations: Bim, Bcl-2-interacting mediator of cell death; DAPK, death-associated protein kinase; IGF2, insulin-like growth factor 2; TERT, telomerase reverse transcriptase.