Targeting HCC Therapy: On or Off ToPiX?

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Primary liver cancer, the fifth most frequently diagnosed cancer worldwide, is the second most common cause of cancer mortality [1]. Among primary liver cancers, hepatocellular carcinoma (HCC) accounts for 70–85 % of the total liver cancer burden worldwide [2]. Epidemiological evidence suggests that several environmental factors are involved in the development of HCC, including infection with hepatitis B (HBV) and C (HCV) viruses. Globally, HBV is the most frequent underlying cause of HCC, with an estimated 300 million persons with chronic infection worldwide. Chronic HCV infection is also a major risk factor for the development of HCC. There is preliminary evidence indicating that host genetic factors may contribute to progression of HCV [3]. Assessment of host genetic aberrations revealed mutations of the well-known tumor suppressor gene *p53* and amplification of the human chromosome 20q region in up to 30 % of HCC cases [4].

Targeting protein for Xenopus kinesin-like protein 2 (TPX2) is a microtubule-associated protein encoded by a gene located on human chromosome 20q11. Several studies report that multiple tumor types overexpress TPX2; TPX2 expression in HCC tissues correlates with the tumor–node–metastasis stage (TNM), tumor number, and tumor differentiation. Liu et al. [5] described TPX2 as a novel prognostic marker for predicting overall 5-year and

disease-free survival of HCC patients. In vitro, TPX2 knockdown significantly inhibited cell proliferation and viability in Hep3B and HepG2 cells. Moreover, TPX2 knockdown noticeably slowed down tumor growth in a nude mouse xenograft model and prominently suppressed HCC cell invasion and migration [6]. TPX2 localizes to the nucleus during S-phase and G2 and at the mitotic spindle poles during mitosis [7] emphasizing its likely involvement in cell division. Yet, the full connection of TPX2 to HCC remains largely undetermined, as only few studies have investigated this protein.

In this issue of *Digestive Diseases and Sciences*, Lian et al. [8] investigated the effects of TPX2 on the cell cycle, on apoptosis, and on the epithelial-to-mesenchymal transition (EMT) fundamental to tumorigenesis. They reported that TPX2 mRNA expression levels were elevated in HCC tumors in 70 paired tumor versus adjacent non-tumor liver tissues, with expression of TPX2 localized to the nuclei. Using siRNA-mediated knockdown loss of TPX2, the authors reported G2/M cell cycle arrest, reduced cell proliferation, and the induction of apoptosis in three cell lines, with alteration of the key cell cycle regulators cyclin A1, B1, D1, and E1, and p21, a regulator of cyclin D1.

Genomic lesions in a cell are usually repaired before the onset of DNA replication and cell division. If the damage is not repairable, programmed cell death termed apoptosis occurs to limit proliferation of cells with defective DNA. The tumor suppressor protein *p53* induces cell cycle arrest or apoptosis, partly through Bax, a transcriptional target of *p53*. Bax expression was increased in the TPX2-siRNA knockdown HCC-derived cell lines, indicating that TPX2 drives damaged cells through the cell cycle, promoting tumorigenesis. The apoptosis-related enzymes cleaved caspase-3 and caspase-8 were upregulated following TPX2 loss, further supporting the anti-apoptosis function of TPX2 (Fig. 1).

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Mechanistically, the authors identified that the phosphatidylinositol 3-kinase (PI3-kinase) signaling pathway, key for the regulation of cellular survival and proliferation, is involved in the regulation of TPX2-mediated events; for example, in the absence of TPX2, phosphorylation of the important PI3-kinase-related signaling molecule Akt and, to a lesser extent, phosphorylation of extracellular signal-regulated kinase (ERK)1/2 were reduced. Functional consequences of TPX2 loss included reduced cell migration and invasion, which correlated with an increase in the cell adhesion molecule E-cadherin, a decrease in β -catenin, and a decrease in markers commonly associated with EMT such as N-cadherin, Slug, and matrix metalloproteinase.

Interestingly, β -catenin, a cytoplasmic binding partner of E-cadherin, tightly regulated during early liver development [9], which contributes to cell adhesion while also regulating the Wntless-related integration site (Wnt)/ β -catenin pathway, was downregulated after TPX2 suppression. Activation of the β -catenin pathway frequently occurs in mouse and human HCC involving somatic mutations [10], in addition to transcriptional repression of negative regulators, underscoring a potential central mechanism of TPX2 in the activation of Wnt/ β -catenin during hepatocellular carcinogenesis.

TPX2 expression is a major regulator of cell cycle progression: Aurora kinase (AURKA), a serine–threonine kinase essential for the regulation of mitotic events such as spindle assembly, which interacts with the Tpx2 pathway, is activated through an auto-phosphorylation process after binding with TPX2 [11]. Since AURKA also inhibits the pro-apoptotic function of p53, it is conceivable that, based on the findings in this paper, TPX2 and AURKA mediate pro-survival signaling in HCC.

As already stated, the major risk factors for HCC include liver injury complicating chronic infection with the HBV, HCV, and alcoholism, with the vast majority of HCC cases developing in the presence of underlying cirrhosis

[12]. The authors state that $\sim 85\%$ patients were HBV-infected, as compared to only three HCV-infected patients, with no reported alcohol use. Due to the lack of control cases, the effect of infection with either virus on the proliferation process could not be determined. Nevertheless, since the HBV-encoded HBx gene can induce multipolar spindles causing centrosome abnormalities, and given the involvement of TPX2 in spindle assembly, one might hypothesize that TPX2 helps mediate HBV-mediated tumorigenesis. Future studies will be necessary to delineate the contributions of TPX2 to virus-induced early events such as aneuploidy and the progression from a preneoplastic condition to HCC in chronically HBV-infected individuals.

The ultimate clinical “payoff” of this work is the discovery of novel, highly tumor-specific molecular targets enabling the development of potent and safe tumor therapies. To realize this goal, additional studies will be necessary to determine the feasibility of targeting this gene for therapy as it most likely also has important functions in normal proliferating cells that may not be specific for cancer cells. While haploinsufficient *tpx2* knockout mice have increased susceptibility to spontaneous tumor formation, homozygous knockout animals display severe developmental defects that culminate in early lethality between embryonic days 8.5 and 17.5 [13]. Together, these results not only highlight the evolutionarily conserved importance of *tpx2*, but also hint at the challenges faced using TPX2 as a therapeutic target. It is hoped that by discovering new tumor-related pathways such as those affected by TPX2, more promising molecular targets and improved therapies will be identified.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61:69–90.
2. Perz JF, Armstrong GF, Ferrington LA, et al. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol*. 2006;45:529–538.
3. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*. 2007;132:2557–2576.
4. Wong N, Lai P, Pang E, et al. Genomic aberrations in human hepatocellular carcinomas of differing etiologies. *Clin Cancer Res*. 2000;6:4000–4009.
5. Liu TUK, Zhang H, Zheng X, Xiao Y, Liu O. TPX2 as a novel prognostic biomarker for hepatocellular carcinoma. *Hepatol Res*. 2014. doi:10.1111/hepr.12428.
6. Huang Y, Guo W, Kan H. TPX2 is a prognostic marker and contributes to growth and metastasis of human hepatocellular carcinoma. *Int J Mol Sci*. 2014;15:181148–181161.
7. Pérez de Castro I, Malumbres M. Mitotic stress and chromosomal instability in cancer: the case for TPX2. *Genes Cancer*. 2012;3:721–730.

Fig. 1 Summary of TPX2 function on cell cycle, survival, motility, and signaling

8. Bo Liang B, Jia C, Huang Y, et al. TPX2 level correlates with hepatocellular carcinoma cell proliferation, apoptosis, and EMT. *Dig Dis Sci*. (Epub ahead of print). doi:[10.1007/s10620-015-3730-9](https://doi.org/10.1007/s10620-015-3730-9).
9. Micsenyi A, Tan X, Sneddon T, Luo JH, Michalopoulos GK, Monga SP. Beta-catenin is temporally regulated during normal liver development. *Gastroenterology*. 2004;126:1134–1146.
10. de La CA, Romagnolo B, Billuart P, et al. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc Natl Acad Sci USA*. 1998;95:8847–8851.
11. Tsai MY, Wiese C, Cao K, et al. A Ran signalling pathway mediated by the mitotic kinase Aurora A in spindle assembly. *Nat Cell Biol*. 2003;5:242–248.
12. Forner A, Llovet JM, Bruix. Hepatocellular Carcinoma. *Lancet*. 2012;379:1245–1255.
13. Aguirre-Portoles C, Bird AW, Hyman A, Canamero M, Perez de Castro I, Malumbres M. Tpx2 controls spindle integrity, genome stability, and tumour development. *Cancer Res*. 2012;72:1518–1528.