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Targeting hepatocellular carcinoma through TGF-β pathway E3 Ligases

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Although RING-finger E3 ligases are instrumental in the regulation of inflammatory, apoptosis and cancer, their role in hepatocellular cancer (HCC) was unknown. We therefore analyzed the TCGA database 29 TGF-β pathway associated E3 ligases in HCC and identified mRNA alteration in 55% of the tumors, most prominently for UCHL5, PJA, WWP2, SMURF1/2 and KEAP1. We recently uncovered increased PJA1 expression in TGF-β deficient (β2SP$^{+/-}$/Smad3$^{+/-}$) mice, which develop a human stem cell syndrome and HCC. Analyses of primary HCC datasets reveal increased PJA1 correlates with decreased levels of TGF-β/Smad3 and their regulated genes including Smad9 and TGFBR3. PJA1 interacts with the Smad3 MH2 domain and β2SP N- and C-terminal domains to promote ubiquitin-mediated Smad3 degradation in a TGF-β dependent manner. In addition, we found that PJA1 expression in HCC is negatively associated with the expression of c-FOS and SERPINE1. Overexpression of a RING-domain-deleted PJA1 reduced HCC cell proliferation and PJA1 knockdown significantly decreased HCC anchorage-independent growth and tumorigenicity. E3 ligase inhibitors, RTA402/405, originally identified as inhibitors for KEAP1, blocking KEAP1-dependent Nrf2 ubiquitination was shown to inhibit HCC cell proliferation and tumor growth in nude mice. RTA402/405 increased β2SP and TGF-β signaling target expression and luciferase activity and RTA402 was shown to bind PJA1 RING-finger domain by molecular docking experiment. Finally, our results demonstrate that PJA1 promotes liver cancer stem cell formation in Smad3$^{+/-}$ mice. Taken together, these studies demonstrate that loss of β2SP and Smad3 through PJA1 could play an important role in the development of HCC and reveals that PJA1 may be novel therapeutic targets for this lethal disease.