EPH receptor B2 augments cancer stemness and drug resistance involving a Wnt/β-catenin positive feedback signaling loop in hepatocellular carcinoma

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Sorafenib has been using for a decade as a first line treatment of advanced hepatocellular carcinoma (HCC) with a median of 3-month survival benefit. However, advanced HCC is still incurable, partly owing to drug resistance. Accumulating evidence revealed that tumor-initiating cells (T-ICs) confers intrinsically resistance to convention therapies, and targeting the genes in regulating the traits of T-ICs may represent promising therapeutic approach. Our previous study demonstrated that upregulation of several key kinases in sorafenib-resistant HCC clones, derived from PDTXs, with enhanced T-IC properties, among which EPHB2 was most significant. Here, we aims to characterize the functional role EPH receptor B2 (EPHB2) in regulating cancer stemness and drug resistance in HCC, and its underlying mechanisms.

Overexpression and knockdown and cell sorting approaches were performed to characterize the *in vitro* and *in vivo* functional role of EPHB2 in maintaining T-ICs properties. Using lentiviral-based overexpression and knockdown approaches, EPHB2 was found to regulate the traits of T-ICs, including tumorigenicity, self-renewal, the resistance to chemotherapy and targeted therapy, and the expression of liver T-IC markers. Similarly, $EPHB2^{High}$ HCC cells were endowed with these enhanced T-IC properties. In addition, using Tet-On inducible knockdown approach, the suppression of EPHB2 sensitized to sorafenib treatment. In clinical setting, a stepwise increase in the expression of EPHB2 was observed from normal to fibrotic liver to HCC. In large cohorts, EPHB2 was significantly upregulated in HCC clinical samples, compared to paired non-tumor counterparts; and EPHB2 overexpression was associated with shorter disease free survival.

Molecular pathways mediating the phenotypic alterations was identified through RNA sequencing analysis. Wnt/β-catenin pathway was found as the downstream effector of EPHB2 mediating liver T-IC function. Consistently, EphB2 knockdown was associated with decreased β-catenin expression, as detected immunoflorescence and western blotting. TOP/FOP luciferase signal and the gene expressions of CMYC and CCND1, downstream target of Wnt/beta-catenin were also significant attenuated. Similarly, EPHB2 overexpression and $EPHB2^{High}$ were associated with increased β-catenin expression, TOP/FOP luciferase signal, and CMYC and CCND1 expressions. Taken together with the observation that Ccnstitutively active β-catenin HCC cells increased EPHB2 expression, EPHB2 expression was regulated by the wnt/β-catenin positive feedback loop in HCC.

In conclusion, EPHB2 regulate cancer stemness and drug resistance via inducing Wnt/β-catenin positive feedback loop, and may specifically modulate sensitivity of liver T-ICs to the effects of

sorafenib treatment. Targeting EPHB2 in combination with sorafenib is possibly a novel therapeutic regimen to combat HCC.

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