UBE2T: a molecular regulator for cancer stemness and drug resistance in hepatocellular carcinoma

Nicole Pui Yu Ho[1], Terence Kin Wah Lee[1,2]

[1]Hong Kong Polytechnic University, Applied Biology and Chemical Technology, Hong Kong, Hong Kong, [2]Hong Kong Polytechnic University, State Key Laboratory of Chemical Biology and Drug Discovery, Hong Kong, Hong Kong

Hepatocellular carcinoma (HCC) is one of the common cancers worldwide. Increasing evidence showed the critical role of cancer stem cells (CSCs) on tumor relapse and therapeutic resistance. Since normal stem cells and CSCs share high similarity in genetic profile, we would like to identify the molecules/pathways crucial in liver CSCs by determining what molecules involved in normal liver stem cells during liver regeneration. Comparison of expression profiles between early regenerating liver and intact liver revealed the upregulation of DNA Damage Response pathways in the self-renewing liver, in which Ubiquitin Conjugating Enzyme E2 T (UBE2T) was the most significantly upregulated. This, together with the publicly available dataset (GSE5975) shows upregulation of UBE2T in EpCAM-enriched liver CSC populations, suggest the potential role of UBE2T on regulation of cancer stemness. By qPCR analysis, UBE2T was overexpressed in 91% of the clinical HCC specimens and associated with aggressive phenotype and poorer patients' survival. Significant overexpression of UBE2T in protein level was also confirmed by Western Blot and IHC analyses. By lentiviral based overexpression and knock-down approaches, we demonstrated the role of UBE2T in regulation of liver CSC properties, including self-renewal, tumorigenicity, drug resistance and expression of liver CSC markers. In orthotopic HCC model, UBE2T was found to play pivotal role in lung metastasis in vivo. In order to identify downstream target of UBE2T for regulation of cancer, tandem affinity purification coupled with mass spectrometry identified was employed. Upon analysis, Mule, the E3 ubiquitin ligase, was identified to be the novel protein binding partner of UBE2T. Being the E2 ubiquitination enzyme, UBE2T was found to physically bind and regulate the protein expression of Mule via ubiquitination. Since Mule was found to directly degrade β-catenin protein, UBE2T regulated liver T-IC functions through direct regulation of Mule-mediated β-catenin degradation. Such effect on Mule-mediated β-catenin regulation was abolished when E2 activity of UBE2T was impaired by replacing wildtype form of UBE2T with the E2-dead mutant (C86A). In conclusion, we have uncovered the novel UBE2T/Mule/β-catenin signaling cascade in regulation liver CSCs, which provides attractive therapeutic target for potential treatment of HCC.