

REGULATORY ROLE OF MIRNA-1246 AND WNT/B-CATENIN PATHWAY INTERACTION IN CD133+ LIVER CANCER STEM CELLS-DRIVEN HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC), the main type of liver cancer in human, is one of the most prevalent and deadly malignancies in the world. Despite advances in therapy, prognosis remains dismal, largely attributed to our limited understanding on information related to the progressive development of the disease, particularly in their cancer-initiating and stem cell-like properties. There is increasing evidence in recent years to support the role of miRNAs in the regulation of cancer stem cell (CSC) maintenance. However, miRNAs in hepatic CSCs remain understudied. Our group has previously identified a functional subset of liver CSCs marked by the CD133 phenotype (1-2). Utilizing a comprehensive transcriptome sequencing approach, we recently compared the differential miRNA profiles of CD133+ liver CSCs and CD133- differentiated cells isolated from HCC cells Huh7 and PLC8024, and identified a significant up-regulation of miR-1246 in CD133+ liver CSC subset. This observation was further validated in additional CD133 sorted HCC cells (Huh7, PLC8024, HepG2 and SNU182). Expression of miR-1246 and CD133 was also found to positively correlate across a panel of liver cell lines. Functional studies were subsequently performed using lentiviral-based miR-1246 knockdown in Huh7 and HepG2 or miR-1246 overexpression in LO2 HCC cells. miR-1246 deregulation was closely associated with an altered ability of HCC cells to proliferate, self-renew, invade, migrate, induce capillary tube formation in endothelial cells and initiate tumor formation in vivo. By in silico prediction, we then identified GSK3 β and AXIN2, both key players of the Wnt/ β -catenin degradation complex, as candidate downstream targets of miR-1246. Consistent with this finding, our group has also previously found β -catenin to be preferentially overexpressed in the CD133+ liver CSC subset (1). Real-time qPCR, Western blot and luciferase reporter assay further confirmed the bona fide interaction between miR-1246 and GSK3 β or miR-1246 and AXIN2. miR-1246 expression positively correlated with β -catenin. Transient overexpression of miR-1246 mimic resulted in TCF/LEF activation in both CD133-expressing Huh7 and HepG2 cells, concomitant with deregulation of downstream targets of the Wnt/ β -catenin pathway, including cyclin-D1, MMP7 and c-myc. Taken together, miR-1246 is functionally involved in driving CD133+ liver CSCs through activation of the Wnt/ β -catenin and its downstream signaling cascade.

References:

1. Ma S, Chan KW, Hu L, et al. (2007) *Gastroenterology* 132:2546-52.
2. Ma S, Tang KH, Chan YP, et al. (2010) *Cell Stem Cell* 7:697-707.