Background: Chemotherapy targets proliferating cells, not cancer stem cells (CSCs). Targeting agents, e.g., sorafenib for hepatocellular carcinoma (HCC), do not seem to target CSCs as evidenced by frequent tumor relapse and resistance after therapy. Identification and characterization of signaling pathways and biomarkers associated with CSC biology are priorities for developing new paradigms of molecular cancer therapeutics. Increase of WEE1 kinase activity through an epigenetic regulation plays an important role in the development of HCC. However, the functional role of WEE1 in HCC progression remains to be clarified.

Methods: Human HCC cell lines were transfected with WEE1 siRNA and tested for growth inhibition, apoptotic induction, molecular changes in both RNA and protein levels, and changes in CSC phenotype using various methods such as MTS, FACS, microscopic analysis, Real-time PCR, Western blotting, sphere forming assay. To find the molecular changes in response to WEE1 knockdown, global changes in gene expression were examined using RNA sequencing.

Results: We demonstrated that WEE1 siRNA silencing caused inhibition of HCC cell growth through blockade of cell cycle progression and induction of apoptosis. The anti-proliferative effects were driven by a subset of molecular alterations including the upregulation of cell cycle inhibitor p21 and the downregulation of AKT1, CEB2, cyclin B1, PARP1 and GPAM which are functionally involved in control of cell cycle, apoptosis and lipid metabolism. WEE1 silencing resulted in a strong inhibition of lipogenesis and caused a marked decrease in fat accumulation. Knockdown of WEE1 dramatically reduced the portion of liver CSC population through co-downregulation of cancer stemness genes, weakened the capacity of sphere formation and cancer cell migration. Systemic delivery of a modified WEE1 siRNA encapsulated in lipid nanoparticles inhibited human HCC growth in murine xenograft models, and increased survival.

Conclusions: Our findings suggest that the epigenetic modifier WEE1 functionally involves in HCC lipid metabolism and CSC-like phenotype maintenance and that molecular targeting of WEE1 may be an effective systemic therapy for prevention of tumor recurrence via elimination of CSCs in liver tumor microenvironment.
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