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Transcriptional Modulation of Proto-Oncogene c-Myc Promoter by α-Fetoprotein Promoter/Enhancer Driven shRNA system

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Specificity is one of the major limitations in cancer gene therapy. Hepatocellular carcinoma (HCC) is the 6th most commonly occurring cancer and 3rd leading cause of cancer related mortalities. This is as a result of its asymptomatic nature, high recurrence rate and poor prognosis. Proto-oncogene c-Myc is one of the central players of oncogenesis in HCC and most of the c-Myc transcripts are actively transcribed by its P2 promoter. Sendai virus is a parainfluenza virus which specifically interacts with the asialoglycoprotein receptors (ASGPRs) of the hepatocytes. Previously, we have shown that Sendai virosome, derived from its virus counterpart, could be efficiently utilized for liver specific delivery of therapeutic modalities both in *in vitro* and *in vivo*. dsRNA has been shown to induce long term and transmissible epigenetic changes byTranscriptional gene silencing (TGS), which is a newer toolof gene silencing. Thus, we aimed to target HCC by putting shRNA, targeting P2 promoter of c-Myc, under the transcriptional control of AFP (α-fetoprotein) promoter/enhancer fusion constructs, since AFP promoter is activated upon neoplastic transformation of hepatocytes during HCC. As the delivery of shRNA with in the target cells is often limited, we used Sendai fusion (F) virosomes for enhancingthe delivery of AFP promoter/enhancer driven TGS inducing c-Myc shRNA constructs. Post virosomal delivery, shRNA induced both methylation of specific histone tails (H3K9Me2 and H3K27Me3) and CpG islands 8,9 and 10, around the c-Myc P2 promoter locus, only in hepatoma cells. Following TGS, extensive tumor cell death was observed as evaluated by Flow cytometry and survival assays. HDACs and DNMTs were possible recruited, to the c-Myc locus, by the shRNA, since pre-treatment of cells with HDAC/DNMT inhibitor reversed epigenetic marks of TGS. Hence, AFP promoter/enhancer driven TGS inducing shRNA, along with liver specific Sendai F-virosomes, could serve as a possible model for specifically targeting HCC.