Abstract

Hepatocellular carcinoma (HCC) is one of the most prevalent causes of cancer related-mortality worldwide. Due to the expanding obesity pandemic, which represents a major risk factor for HCC, the incidence of this cancer type is predicted to further increase. Understanding the molecular mechanisms that underlie the initiation of HCC as well as investigating the associated risk factors, such as obesity, will aid in the development of novel therapeutic approaches to combat this disease. Dysregulation of m^6A modifications as well as changes in the expression of the RNA m^6A demethylase, FTO, has been linked to numerous cancers, including HCC. Furthermore, single nucleotide polymorphisms (SNPs) within the *FTO* gene are highly associated with obesity. This study aimed to investigate the direct role of hepatic FTO in the development of HCC. To this end, hepatic FTO-deficient ($FTO_{L\text{-}KO}$) lean and obese mice were subjected to the diethylnitrosamine (DEN)-HCC protocol. Interestingly, hepatic FTO deficiency increased HCC burden in lean mice without affecting proliferation or apoptosis in late stages of tumor development, suggesting that the tumor initiation phase has been altered in $FTO_{L\text{-}KO}$ mice. Indeed, FTO expression was dynamically regulated upon acute, DEN-induced liver damage in control livers. Quantitative proteomics revealed stable CUL4A protein levels in $FTO_{L\text{-}KO}$ livers upon liver damage. CUL4A is a scaffold protein of the E3 ubiquitin ligase CRL4 that regulates cell cycle and DNA damage repair and is upregulated in numerous cancers. In this study *Cul4a* mRNA has been verified as an *in vivo* downstream target of FTO by meRIP. These experiments indicate that FTO-mediated demethylation of *Cul4a* mRNA results in reduced CUL4A protein upon acute liver damage. This may lead to cell cycle arrest and DNA damage repair ultimately reducing tumor burden. Thus, FTO activity is beneficial in HCC and could be a potential target to develop novel HCC therapies.

Moreover, a novel ROSA26 locus-driven, self-destructing (s)Cas9 2A tdTOMATO mouse line has been developed, which can be activated via Cre- and Dre-mediated recombination. Cre/Dre-mediated excision of loxP- and rox-flanked stop cassettes leads to the expression of sCas9 and tdTOMATO in cells expressing both recombinases. *In vivo* delivery of an AAV carrying Cre/Dre inducible ZsGreen as well as U6-driven gRNAs against sCas9/tdTOMATO and the gene of interest, indicates Cas9 activity via a red to green switch in fluorescence, owing to self-destruction of sCas9/tdTOMATO and activated ZsGreen expression. This novel traffic light system will not only enable to report and cell type-specifically restrict sCas9-mediated gene editing, but will also limit genomic Cas9 off-target effects via self-destruction.