2017

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Background: Kinase fusion has been detected in a variety of cancer types. Approximately 3% of cancers are associated with different kinase fusions, including fibrolamellar hepatocellular carcinoma (FLC). FLC is a rare type of liver cancer that predominantly occurs in teenage population without any previous liver disease history. It is aggressive and currently surgical resection remains the only effective therapeutics. DNAJB1-PRKACA fusion is detected in at least 80% of FLC cases and is considered a potential oncogenic factor. However, the causality between DNAJB1-PRKACA fusion and FLC is not yet established.

Methods: This study aims at investigating the function of DNAJB1-PRKACA fusion in FLC oncogenesis using *Drosophila* and murine *in vivo* models. We used the Gal4/UAS system to overexpress human DNAJB1-PRKACA fusion gene in *Drosophila* eyes and used genetic and pharmaceutical inhibition of PKA activity to rescue the eye phenotype. We also used CRISPR/Cas9 genome engineering to recreate the murine chromosomal deletion equivalent to that found in FLC patients. gRNAs were designed to target murine DNAJB1 and PRKACA introns and tested for their editing efficiency. Co-transfection of a pair of effective gRNAs successfully generated a 360kb chromosomal deletion on chromosome 8 in murine hepatocytes. Multiple single cell clones were isolated, first characterized for their *in vitro* proliferation properties, and further inoculated subcutaneously *in vivo* to monitor their oncogenicity.

Results: Human DNAJB1-PRKACA fusion gene, when expressed in *Drosophila* eye progenitor cells, induced proliferation and differentiation phenotypes, including decreased eye size and abnormal eye shape. Both genetic and pharmaceutical inhibition of PKA activity rescued the fly eye phenotype. Murine hepatocyte cell lines carrying endogenous DNAJB1-PRKACA fusion gene have higher PKA activity like in FLC patients. These engineered hepatocytes formed subcutaneous tumors at faster rate *in vivo* and significantly promoted ductular metaplasia tumors.

Conclusion: DNAJB1-PRKACA fusion gene expression alone is sufficient to perturb cell growth and differentiation in different cellular contexts. *In vivo* modeling with engineered murine hepatocytes recapitulates certain prominent aspects of FLC pathological features, confirming its proposed role as a driver oncogene for FLC. Our *Drosophila* FLC model is ready as a medium throughput platform for FLC therapeutic screens.