Title: The functional roles of Tet proteins in the development of lung cancer

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Enzymatic methylation at C-5 position of cytosine (5mC) is a hallmark of mammalian epigenetic programming and is critical in regulating gene expression. Ten-Eleven Translocation (TET) enzymes oxidize 5-methylcytosine (5mC) in DNA to generate 5-hydroxymethlcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), leading to DNA demethylation. Tissue- specific DNA methylation patterns are maintained by the opposing actions of DNA methyltransferases and TET dioxygenases (TET 1-3). Expression levels of all three TET genes are sharply reduced in many different human cancers including lymphoma, breast, pancreas, prostate, and lung, with the accompanying reduced global levels of 5hmC. However, TET1 upregulation, along with aberrant increase of 5hmC levels was observed in hepatocellular carcinoma, and TET2 mutations are predictive of increased mixed-lineage leukemia patient survival. TET2 deficient myeloid neoplasms have been selectively targeted with TET inhibitors, leading to synthetic lethality and selective tumor cell targeting. These apparent contradictory results regarding the role of TET genes in cancer led us to investigate and understand the functional roles of TET proteins and their oxidized epigenetic marks (5hmC, 5fC, 5caC) in human lung cancer development. In this study, we have performed systematic analysis of TET genes in human lung cancer cell lines. We have carried out TET gene knockout, knockdown, and overexpression, followed by functional studies including cell proliferation, colony formation, RNA-seq, and global proteomics analysis. We further carried out 3D spheroid assay to study the effects of TET genes tumorigenesis in lung cancer development. Overall, our results suggest that TET proteins act as tumor suppressors in human lung cancer.