The Histone Demethylase KDM4C is a Putative Oncogene in Pancreatic Cancer

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease that is predicted to be .the second leading cause of cancer death in USA in 2030.The overall 5-year survival rate for pancreatic cancer in USA is only 11%. **Figure 1** shows the multistep progression of pancreatic cancer from normal duct to infiltrating cancer. This progression occurs through a series of histologically defined precursors called Pancreatic Intraepithelial Neoplasia (PanIN), which are precursors to pancreatic cancer (PDAC).

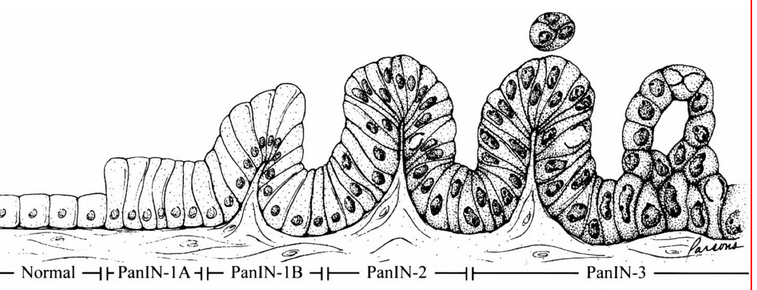

Figure 1. Progression model for pancreatic cancer[4]

In recent years epigenetic regulation including DNA methylation and histone modifications have been implicated in many cancers, apart from somatic mutations. Abnormal expression of different histone demethylases (KDMs) have been observed in many human diseases including cancers making them a potential therapeutic target. Amongst them, the Kdm4 family (Kdm4A-D) are comprised of Histone H3 lysine 9 (H3K9) demethylase enzymes that bind to their target gene promoters and remove trimethyl and dimethyl group from H3K9.

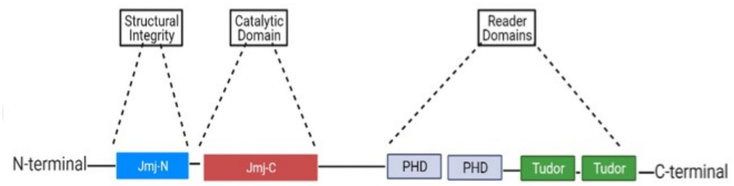

Figure 2. The structure and domains of KDM4C

Among the Kdm4s, Kdm4C is often amplified and overexpressed in various cancer including lymphoma, breast cancer, prostate cancer and lung carcinoma. **Figure 2** depicts the structure of Kdm4C and its different protein domain. The role of Kdm4C has not been studied in pancreatic cancer and therefore we determined the expression of Kdm4C in pancreas and investigated its role in signaling pathway involved in pancreatic cancer.

Hypothesis

Kdm4C is overexpressed in pancreatic cancer and may play a role in epigenetic regulation of target genes involved in cellular proliferation and other signaling pathways.

Methods

In our laboratory we use *in-vitro* and *in-vivo* model systems to study KDM4C in PDAC. The *in-vitro* models are (i) human and mouse tissues for assessment of KDM4C expression, and (ii) human and mouse pancreatic cancer cell lines with CRISPR/Cas9 edited knockout of endogenous KDM4C. For *in-vivo* analyses, we have athymic mice that are injected with PDAC cells bearing knockout of *KDM4C* versus control lines, as well as novel genetically engineered mouse models of PDAC with deregulated KDM4C expression.

Only the *in vitro* studies are described here, which were conducted by E. F. We used non-neoplastic and pancreatic cancer tissues from GEM models for immunohistochemistry to determine KDM4C expression (**Figure 3**). We then used control and CRISPR edited KDM4C KO MT4 cell lines to investigate anchorage independent growth (**Figure 5**). Finally, Western blot was performed to determine candidate effectors in the MAP kinase pathway which was previously identified on profiling studies as a target of KDM4C deregulation (**Figure 4**)

Results

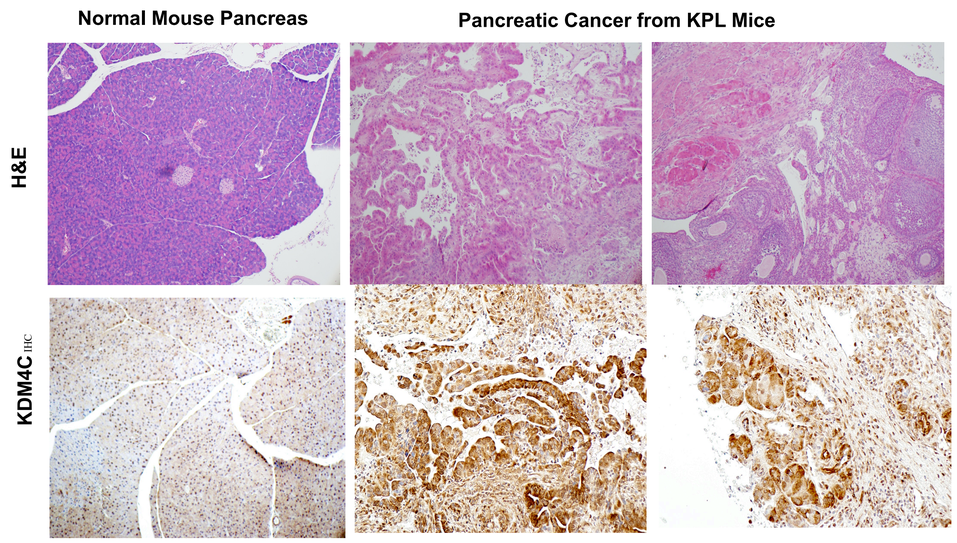

Figure 3. Immuno histochemistry of KDM4C in non-neoplastic and pancreatic cancer tissues. Upper panel shows Hematoxylin and Eosin staining of normal mouse pancreas and GEMM model of mouse pancreatic cancer (KPL) that express oncogenic Kras along with deletion of p53 and LKB1, an oncogene known to be mutated in pancreatic cancer.

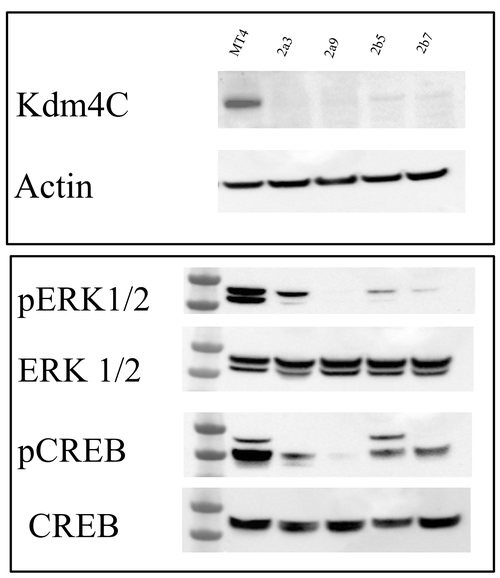

Figure 4. Western blot of control mT4 and Kdm4C KO cell lines. Upper panel shows complete absence of Kdm4C protein following CRISPR mediated gene editing in KO lines. Lower panels shows the down regulation of MAP kinase pathway proteins, phospho-ERK1/2 and phospho-CREB as compared to control MT4 while total ERK1/2 and CREB remains same.

Results (cont.)

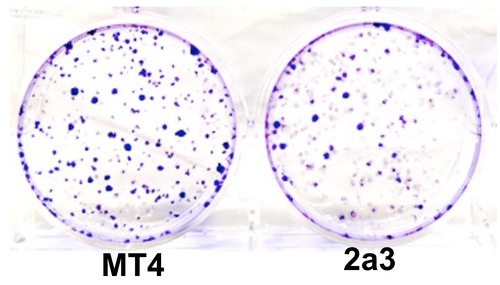

Figure 5. Colony formation assay showing absence of Kdm4C reduces anchorage independent growth in the Kdm4C KO cell line (2a3) compared to the control (MT4)

Conclusion

Kdm4C is overexpressed pancreatic cancer tissues. The downregulation of Kdm4C leads to reduction of anchorage independent growth in the mouse pancreatic cancer cell line MT4. The down regulation of MAP kinase signaling pathway by Western blot suggests that Kdm4c may be involved in direct or indirect regulation of a key effector of the Ras signaling pathway. All these data might be helpful in future studies to investigate the role of Kdm4C in regulating H3K9 methylation and expression of oncogenic transcripts involved in pancreatic cancer.

References

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