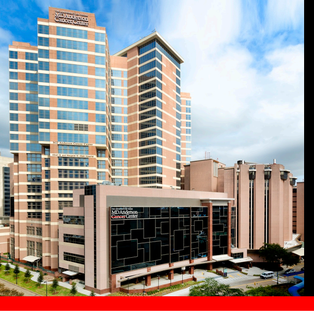

KDM4C is an Oncogenic Histone Demethylase in Pancreatic Cancer

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Background

Pancreatic cancer is the third leading cause of cancer death in both men and woman in the USA and its incidence is progressively rising. Understanding the molecular underpinnings of this lethal disease is crucial for developing potent therapies and effective biomarkers. Studies have shown that pancreatic cancer is initiated as non-invasive ductal lesions that then progress to infiltrating pancreatic adenocarcinoma through the accumulation of genetic alterations. 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). They are precursors to pancreatic cancer, which is also known as pancreatic ductal adenocarcinoma (PDAC). In addition to genetic alterations, recent studies have shown how epigenetic modifications play an important role in cancer progression as well. Unlike genetic modifications, epigenetics do not involve changes in DNA sequence, but rather affects gene expression through reversible DNA or chromatin modifications, such as DNA methylation and histone acetylation. Epigenetic regulation takes place through a variety of different enzymes also known as the writers, readers, and erasers (Figure 2). The writers add a variety of chemical groups onto DNA or histone amino acids, for example histone lysine methylases can add up to three methyl groups onto one lysine. One of the well characterized lysine modifications is H3K9me3, a mark for closed chromatin (heterochromatin), which means it is inaccessible for gene expression i.e. gene expression is suppressed. The modifications that writers lay are not permanent, and can be reversed by another group of epigenetic modifiers known as erasers. Lysine demethylases like KDM4C remove methyl groups from methylated lysines, resulting in open chromatin formation (euchromatin) and allowing gene expression to occur. An example is the histone demethylase KDM4C, an eraser in the Jumonji (JMJ) family. The Structure of KDM4C is shown in Figure 3, and it consists of two JMJ family domains, one of which is responsible for the structural integrity and the second, which is the catalytic domain. Recent studies suggest that the altered regulation of these epigenetic tools plays a key role in tumorigenesis. Since these modifications are reversible and induced by deregulated enzymes, therapeutic approaches targeting these alterations are areas of great interest in preclinical therapeutics. KDM4C overexpression has been reported in lung, prostate, breast and brain cancers. KDM4C has not been previously studied in pancreatic cancer. Therefore, we interrogated the expression of KDM4C in pancreatic cancer and its effect on tumor growth.

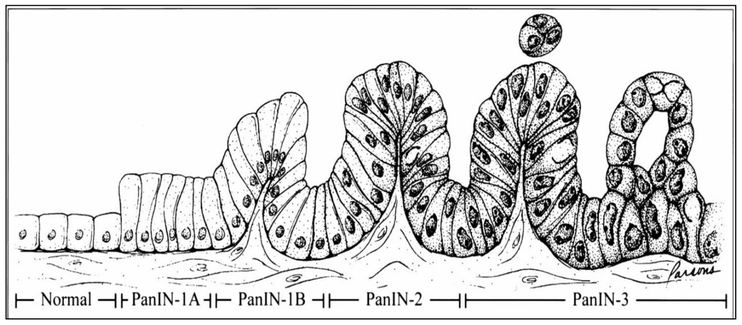

Figure 1. Multistep progression model of pancreatic cancer

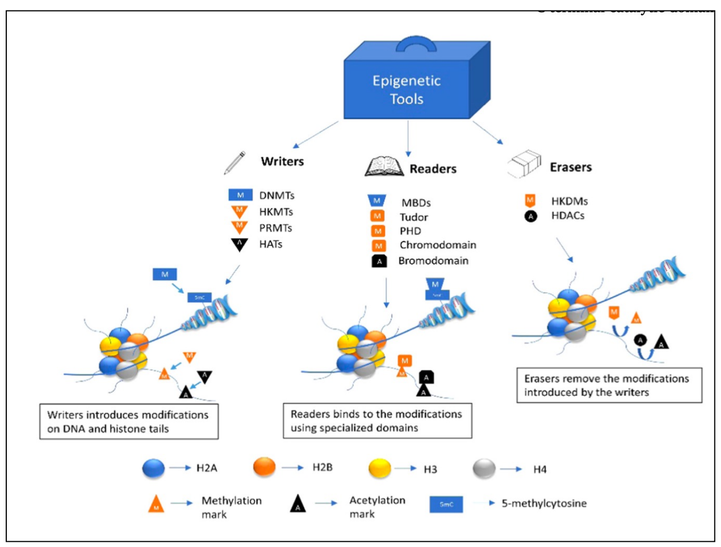

Figure 2. Epigenetic modifiers - writers, readers and erasers

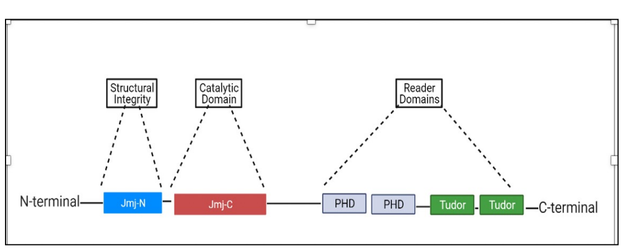

Figure 3. KDM4C structure and domains

Methods

In our laboratory we use orthogonal *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 pancreatic cancer cell lines with CRISPR/Cas9 induced 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 L.B. We first sectioned human and mouse tissues and used multiplex IF technology to stain for KDM4C expression, including the epithelial marker EpCAM and neoplastic marker CK19 in the multiplex panel. Confocal microscopy was used to evaluate expression and photomicrographs obtained. We then used CRISPR/Cas9 and guide RNAs against *KDM4C* to knockout the endogenous protein in AsPC1 pancreatic cancer cell line. We conducted real time PCR and western blot analysis for quantitative assessment of KDM4C expression. Finally colony formation assays were conducted to evaluate the impact of KDM4C loss on AsPC1 anchorage independent growth.

Results

KDM4C is Overexpressed in Pancreatic Cancer Tissues Compared to Non-neoplastic Mouse and Human Pancreas

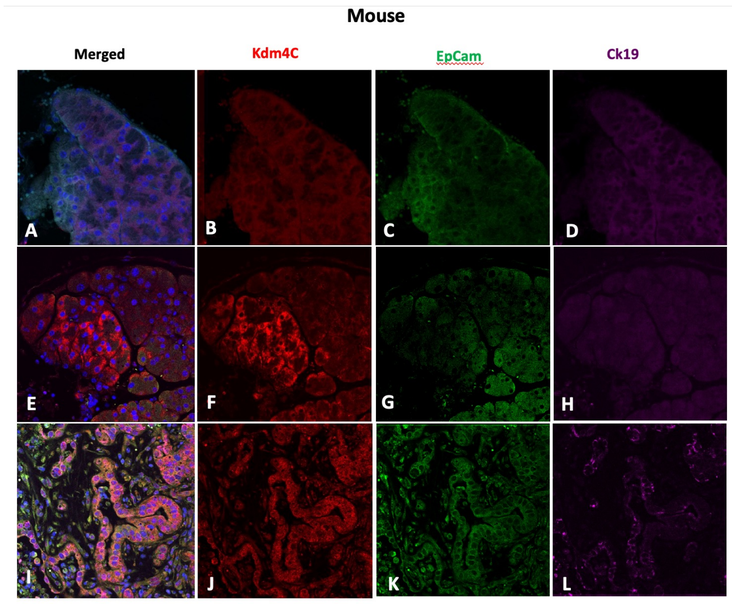

Figure 4. Multiplex **i**mmunofluorescence of normal pancreas (A-D) and pancreatic ductal adenocarcinoma (I-L) showing overexpression of Kdm4C protein (red) in a Kras;p53 mouse model of pancreatic cancer. EpCam (green), an epithelial marker and CK19 (magenta), a marker of PDAC are also overexpressed. E-H, shows elevated expression of Kdm4C in regions of acinar ductal metaplasia (ADM) compared to histologically normal pancreas.

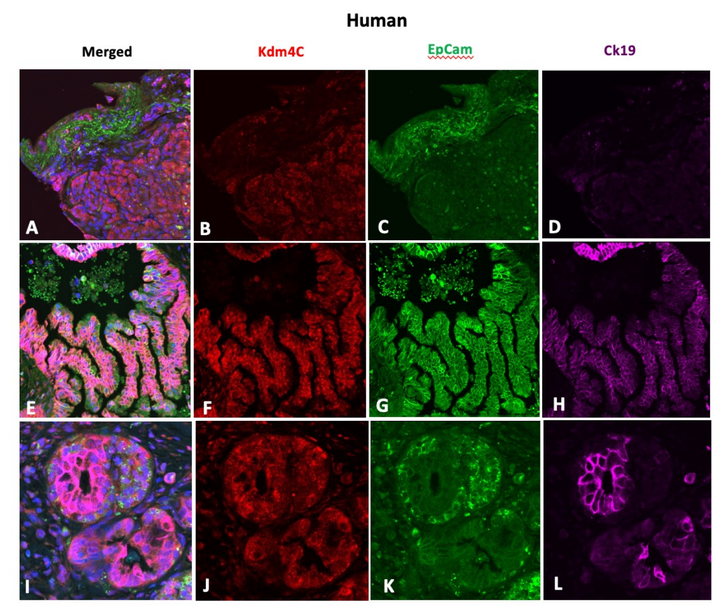

Figure 5. Immunofluorescence of Kdm4C (red), EpCam (green) and Ck19 (magenta) expression in resected normal (A-D) and cancer-bearing regions (E-L) of a representative patient-derived pancreatic cancer sample. Note the elevated expression of Kdm4C 9 in the PDAC region compared to the normal pancreas of the same patient. Localization of Kdm4c protein within the neoplastic epithelium is confirmed by co-localization of EpCAM and CK19 expression.

KDM4C Protein Levels Are Depleted After CRISPR Knockouts

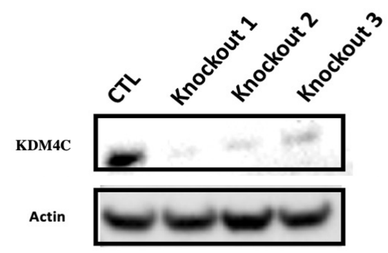

Figure 5. Western Blot showing low tp absent Kdm4c protein levels in single cell clones of AsPC1 cell line with CRISPR-induced KDM4C knockout, versus parental AsPC1 line. Actin is used as a loading control.

KDM4C Relative Gene Expression in Control Cell Line vs Knockouts

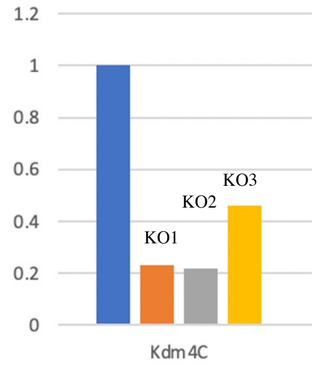

Figure 6. qRT-PCR shows depleted levels of *Kdm4c* transcripts in the three AsPC1 knockouts vs control cell line.

Loss of KDM4C Reduces Anchorage Independent Growth

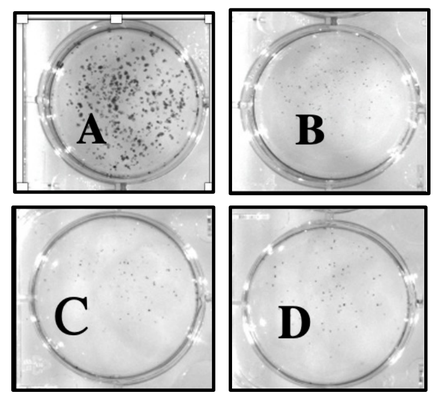

Figure 8. Colony Formation Assay. Well A is AsPC1 control cell line. Wells BB,C, and D are the CRISPR knockouts KO1, KO2, and KO3 respectively.

Conclusions

KDM4C is overexpressed in human and mouse pancreatic cancer tissues relative to normal pancreatic tissue. The downregulation of KDM4C expression significantly impedes anchorage independent growth of AsPC1 cells. These foundational data form the basis for future studies that will examine the downstream effector pathways of aberrant KDM4C expression in pancreatic cancer, including transcripts whose expression is altered as a result of changes in the H3K9me levels in the corresponding regulatory regions. We anticipate these future studies will also identify potential actionable pathways that might form the basis for reversing the oncogenic effects of *KDM4C* in pancreatic cancer.

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