Loss of SIRT6 Reactivates the Oncofetal Protein Lin28b to Drive Pancreatic Cancer

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Changes in chromatin dynamics are a fundamental process of cellular differentiation. In pluripotent cells, chromatin is hyperdynamic and globally accessible. During normal differentiation, however, certain regions of the genome are silenced by heterochromatin formation, thereby allowing only certain genes to be expressed and determining cell fate. Dysregulation of the epigenome endows cancer cells with the plasticity to override normal differentiation and reactivate early developmental programs. Although chromatin remodeling proteins are frequently dysregulated in human cancer, little is known about how they control tumorigenesis.

The sirtuins are a family of 7 highly conserved, nicotinamide adenine dinucleotide (NAD)-dependent enzymes involved in cellular lifespan regulation. SIRT6 is a nutrient sensor that reprograms the epigenome in response to nutrient stress and shows downregulation of expression in human pancreatic ductal adenocarcinoma (PDAC) relative to normal tissue. At the Mostoslavsky Laboratory at the MGH Cancer Center, led by Raul Mostoslavsky, MD, PhD, researchers have focused on exploring the role of SIRT6 in PDAC by determining how the loss of this chromatin modifier influences PDAC formation, progression, and metastasis.

Using NAD as a cofactor, SIRT6 removes acetyl groups from H3K9 and H3K56 to convert actively transcribed open chromatin to silent closed chromatin. When SIRT6 is lost, however, chromatin remains hyperacetylated and accessible for HIF and Myc to bind and drive expression. Loss of SIRT6 expression appears to characterize PDAC cells. An analysis of public SIRT6 expression datasets showed that 63% of pancreatic cancer cell lines demonstrate copy-number loss of SIRT6. Similarly, an analysis of primary human PDAC tissue samples showed that the vast majority had lost SIRT6 expression compared to normal pancreas tissue.

To determine whether SIRT6 loss could influence the onset and progression of PDAC in vivo, researchers crossed SIRT6 conditional knockout (KO) mice into a well established model of pancreatic cancer in which oncogenic KRAS is expressed in the pancreas. Loss of SIRT6 appeared to accelerate the progression of PDAC, irrespective of p53 status. The SIRT6 KO mice had aggressive, highly disseminated disease that was metastatic to the liver and the lung, suggesting that SIRT6 is a potent tumor suppressor in PDAC.

SIRT6 also appears to control the growth of both murine and human PDAC cells. In murine PDAC cell lines established from the tumors of wild-type (WT) and KO mice, the KO cells showed a greater propensity to form tumor spheres in low-attachment conditions. The KO cells also demonstrated an increase in H3K56Ac and H3K9Ac bulk chromatin levels. Restoring WT SIRT6 expression suppressed murine PDAC growth both in vitro and in vivo. In human PDAC cell lines, SIRT6 expression decreased global H3K56 and K9Ac levels and reduced growth. Conversely, removal of SIRT6 in normal human pancreatic ductal epithelial cells led to an increase in H3K56Ac levels and increased cell growth.

To determine the mechanism by which SIRT6 suppresses PDAC growth and progression, the next series of experiments identified Lin28b as the most hyperacetylated gene in PDAC cells. Lin28 is an RNA-binding oncofetal protein that directly inhibits the let-7 family of tumor suppressor miRNAs. Although normally silenced in healthy adult tissues, Lin28 is reactivated in approximately 15% of all cancers, including cancers of the colon, breast, lung, liver, kidney, ovary, cervix, as well as CML and germ-cell tumors. To date, however, Lin28b expression had never been studied in PDAC.

Among murine PDAC cell lines, the SIRT6 KO cells were universally positive for Lin28b expression. In a panel of human PDAC cell lines, Lin28b expression showed a clear inverse correlation with SIRT6 expression. In both murine and human PDAC cells, restoration of WT SIRT6 expression reduced Lin28b expression at the RNA and protein levels. In human PDAC cells, SIRT6 loss results in hyperacetylation of H3K9 and H3K56 at the Lin28b promoter, Myc
recruitment, and pronounced induction of Lin28b and of downstream Lin28b/let-7 targets. This epigenetic program defines a distinct subset of human PDAC characterized by reduced SIRT6 expression and heightened dependence on Lin28b activation for tumor growth.

**Summary**

Findings to date support a model in which SIRT6 acts as an epigenetic barrier to suppress Lin28b expression in normal healthy adult tissue. Loss of SIRT6 creates a permissive hyper-acetylated chromatin state, allowing for Myc-dependent transactivation of Lin28b. The reactivation of Lin28b in turn drives PDAC through the activation of multiple oncogenic targets (e.g., IGF2BP) involved in PDAC growth and progression. This work not only provides new insights into the epigenetic mechanisms governing the reactivation of oncofetal proteins in cancer, but also defines a specific PDAC subset that may benefit from novel therapeutic approaches aimed at targeting components of the LIN28B/let-7 pathway.

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**References**