FOXA1 control of MYC-driven metabolic adaptations in aggressive prostate cancer

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Background: Lifestyles-related factors such as obesity and excessive saturated fat intake (SFI) increase the risk of developing lethal prostate cancer (PCa). We showed that high SFI-induced obesity boosts the oncogenic c-MYC (MYC) transcriptional program and gives rise to tumour glycolytic features that create an immunosuppressive and protumourigenic microenvironment. Upon MYC overexpression or under SFI-induced obesity conditions, the enhancement of the MYC transcriptional program creates new epigenetic and metabolic liabilities. However, the mechanisms underpinning these liabilities thus far has been uncharacterized.

Methods: We leveraged murine models of MYC-driven PCa that recapitulates several molecular features of human prostate adenocarcinoma. We performed CRISPR/Cas9 knock-out (KO) screens *in vitro* using custom libraries targeting epigenetic-related genes in murine PCa cellular models driven by MYC overexpression, loss of *Pten* alone or combined with loss of *Rb1*. We generated stable KO cells together with doxycycline-inducible re-expression and performed *in vitro* (*e.g.*, proliferation, Seahorse) and *in vivo* characterization under conditions of either a control diet (CTD; 10% kCal fat) or SFI-induced obesity (60% kCal fat) using next-generation sequencing-based experiments and analyses (*e.g.*, RNA-seq, ATAC-seq, ChIP-seq).

Results: Our *in vitro* CRISPR/Cas9 KO screens identified the pioneer transcription factor forkhead box A1 (FOXA1) as a top candidate unique in promoting MYC-driven PCa cellular proliferation. This was confirmed by independent genetic KO. Gene set enrichment analysis identified FOXA1 as a positive regulator of the glycolysis transcriptional signature. Supporting this finding, FOXA1 KO clones demonstrates reduced compensatory glycolysis following mitochondrial inhibition as well as reduced ATP production. Importantly, SFI enhances chromatin accessibility to binding sites of the FOX transcription factor family in MYC-overexpressing prostates in comparison to CTD-fed animals. Critically, SFI was not able to sustain the same growth rate in FOXA1 KO allografts or induce glycolysis in the absence of FOXA1. Along this line, we found that FOXA1 controls the expression of hexokinase 2 (HK2), the rate-limiting enzyme that catalyzes the irreversible conversion of glucose to glucose-6-phosphate.

Conclusions: Our studies revealed FOXA1 as a mechanistic link underlying metabolic adaptation in MYC-driven PCa and provides the basis to counteract SFI-associated and FOXA1-mediated PCa progression by interfering with glycolysis.

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