Stony Brook University
The Graduate School

Doctoral Defense Announcement

Abstract

Functional Characterization of PKM Splice-switching Antisense Oligonucleotides in Hepatocellular Carcinoma

By

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Pyruvate kinase M2 (PKM2) is a *PKM* isoform that is preferentially expressed in nearly all cancers. It primarily functions as the last enzyme in glycolysis, where it converts phosphoenolpyruvate to pyruvate. Notably, PKM2 has relatively low enzymatic activity, which can result in accumulation of upstream glycolytic intermediates that can be used for macromolecule synthesis. Additionally, PKM2 has a variety of non-canonical functions, which includes recruiting transcription factors to oncogenes, as well as phosphorylating proteins. Our lab previously developed antisense oligonucleotides that disrupt alternative splicing of *PKM* pre-mRNA (PKM-ASOs), resulting in a PKM2-to-PKM1 isoform switch in hepatocellular carcinoma (HCC), which reduces HCC growth *in vitro* and *in vivo*. The PKM1 isoform has higher enzymatic activity than PKM2, which potentially drives metabolism away from macromolecule synthesis, and may explain decreased HCC proliferation upon PKM-ASO treatment. However, since PKM-ASOs also reduce PKM2 levels, the effect of *PKM* splice-switching on HCC proliferation has an unclear mechanism. Therefore, I characterized the individual consequences of altering PKM1 or PKM2 protein levels in HCC, and observed that reducing PKM2 alone was sufficient to decrease HCC proliferation, whereas overexpressing PKM1 had no effect on proliferation. Moreover, increasing PK activity via small-molecule PKM2 activators had no effect on HCC proliferation, suggesting that PKM-ASOs target PKM2 functions other than metabolism. Indeed, stable-isotope tracing with ^{13}C-glucose in HCC cells treated with PKM-ASOs showed increased glycolytic flux into the TCA cycle, without significant alterations in upstream macromolecular synthesis. However, RNA-sequencing of HCC cells treated with either siPKM2 or PKM-ASO revealed upregulation of dual-specificity phosphatases (DUSPs), which act directly on ERK1/2 in the MAPK signaling pathway. I also observed significantly decreased phosphorylation of ERK1/2 following treatment of HCC cells with either siPKM2 or PKM-ASO. Notably, the receptor tyrosine kinase inhibitor lenvatinib is a second-line therapy for HCC that indirectly reduces ERK1/2 phosphorylation. Co-treatment with PKM-ASOs and lenvatinib significantly reduced proliferation in lenvatinib-resistant HCC cells, compared with either treatment alone. Interestingly, PKM splice-switching also depleted dimeric PKM2 via inducing heterotetramerization with PKM1. Given that PKM2's non-canonical functions require dimeric PKM2, these results demonstrate a novel mechanism by which PKM-ASOs impact PKM2 dependency in HCC.

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