Assessing Novel RIPK2-MKK7 Interaction Inhibitors in Prostate Cancer Cell Lines

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Metastatic prostate cancer (mPC) stands second in cancer-related mortality among men. As current treatment options are largely palliative, there is a dire need for novel therapeutic targets in mPC. Prior research has identified receptor-interacting protein kinase 2 (RIPK2) as a promising drug target in mPC, acting through a non-canonical RIPK2/MKK7/c-Myc signaling pathway. Selectively targeting the protein-protein interaction between RIPK2 and MKK7 has strong potential to inhibit RIPK2-driven PC metastasis while minimizing side effects, as this approach spares disrupting the canonical RIPK2 signaling pathway, crucial for immune response and wound healing. In this study, nine candidate small-molecule RIPK2-MKK7 inhibitors were tested in two prostate cancer cell lines: PC3 and 22Rv1. Western blotting was used to assess their effects on reducing phosphorylated c-Myc (S62) levels, a key oncoprotein and readout for RIPK2/MKK7/c-Myc pathway activity. Among the nine proprietary candidates (A-I), inhibitor H decreased p-c-Myc-S62 levels in both cell lines: 24% in PC3 cells (P=0.043) and 39% in 22Rv1 cells (P=0.042). Similarly, inhibitor F decreased p-c-Myc-S62 expression by 31% in PC3 cells (P=0.002). To determine whether the top inhibitors (F and H) reduce RIPK2-MKK7 interactions, a Duolink® proximity ligation assay was conducted, which allowed for the fluorescent detection of RIPK2-MKK7 interactions in situ. Inhibitors F and H significantly decreased RIPK2-MKK7 interactions: H resulted in a 46% reduction in PC3 and 38% in 22Rv1 while F resulted in a 38% reduction in both cell lines. This study represents an important first-step in the investigation of a protein-protein interaction as a novel target in mPC.

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