Utilizing Ligand Structuring Metaservers to Model Pathogenic p16 Mutation Effects on Binding Sites of Cell Signaling Pathways

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Melanoma, a tumor arising from melanocytes, is responsible for most skin cancer deaths. Familial melanoma is linked to germline mutations in the CDKN2A (p16) tumor suppressor gene, which regulates cell proliferation. A previously characterized novel p16 mutant L117P appeared to compromise a potential adenosine monophosphate (AMP)-binding pocket. AMP binding by p16 may contribute to regulation of intracellular AMP levels. Greater AMP levels feed into activation of the AMPK pathway which functions in cell proliferation regulation. As part of intracellular AMP levels, p16 binds inosine monophosphate dehydrogenase (IMPDH), related to rate-limiting purine nucleotide synthesis. To gain insight into how 12 known pathogenic mutations in p16 affect interaction with AMP, structural modeling was employed to investigate effects on the AMP-binding pocket. p16 3D structures for wild-type and mutant were input into protein modeling metaservers RaptorX and BSP-SLIM targeting binding probability at prospective AMP-binding locations. IMPDH binding activity to p16 was characterized by immunoprecipitation (IP). Expression of pAMPK was analyzed using Western Blot. RaptorX indicated 10 of 12 (83%) mutations disrupt the AMP-binding pocket. BSP-SLIM visual models indicated shifts in AMP-binding pocket to different domains of the p16 structure. IP data shows 3 mutations losing IMPDH binding capacity to p16. Mutations losing AMP binding showed changed pAMPK expression. These changes observed in the AMP-binding pocket suggest important roles for p16-binding in AMP-mediated signaling and indicate compromise of p16-AMP interaction may relate to melanoma predisposition. This study emphasizes the importance of understanding p16 mutations, identifying new therapeutic targets for melanoma such as AMP.

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