Efficacy of Mutant HPV-16 E6 Proteins in p53 Degradation

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Background and Objective:
High-risk human papilloma viruses (HPV) cause the majority of anal, cervical, vaginal, vulvar, penile, and oropharyngeal cancers with an annual incidence of 630,000 cases worldwide. HPVs can cause dysplasia that increases neoplasia risk. HPV's encode eight major viral proteins with the E6 protein being crucial for replication. E6 binding to ubiquitin ligase E6AP initiates polyubiquitination of p53, targeting the protein for proteasomal degradation. We are taking a novel approach to inhibit HPV infection by designing inhibitors targeting the E6-E6AP binding pocket, thereby preventing p53 degradation and restoring its tumor suppressor function. To affirm interaction with the targeted binding site, we generated E6 point mutations that were designed to disrupt interaction with the compound. To ensure their suitability for our studies, we are herein characterizing their capacity to bind E6AP and degrade p53.

Methods:
E6 mutants were generated by site-directed mutagenesis and confirmed by sequencing. H1299 cells were transfected with GFP, wild-type (WT) E6, or mutant HPV-16 E6 plasmids +/- WT p53 plasmid. After 48 hours, cells were lysed and 16E6 immunoprecipitated. Proteins bound to 16 E6 were separated by SDS-PAGE and subjected to western blot. Binding to E6AP was analyzed and presence of E6 was confirmed by immunoblotting. To test for p53 degradation, H1299 cells were transfected with firefly luciferase (transfection control) and a p53-luciferase fusion gene along with WT and mutant E6, or empty LXSN plasmid. 48 hours later p53-luciferase was measured with Dual-Glo Luciferase Assay.

Results:
Western blot of immunoprecipitation lysates revealed that the 16E6(Y32F) mutant retained E6AP binding capacity. p53 degradation assay showed p53 degradation comparable to WT for both 16E6(Y32F) and 16E6(C51S) mutant proteins.

Conclusion:
Our findings indicate the 16E6(Y32F) mutant will be acceptable for use in future compound studies, and p53 degradation ability of 16E6(C51S) implies that this also retains E6AP binding.