Characterization of a conserved cysteine residue in the papillomavirus E2 protein

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Background:
Human papillomaviruses (HPVs) are DNA tumor viruses that infect cutaneous and mucosal epithelium. While most infections are self-limiting, a small subset that infects the mucosal epithelium progresses to cancer. All papillomaviruses encode the protein E2 which regulates viral transcription and replication; a highly conserved cysteine residue in the DNA contact helix of E2 plays an unknown role. Previous research suggests the residue is not necessary for replication or binding to DNA. We hypothesize that post-translational modification of this conserved cysteine residue leads to release of viral DNA during packaging of progeny virions.

Methods:
Mutations of the murine papillomavirus conserved E2 C307 residue to serine and phenylalanine were used to investigate its role in E2 function. C33A, HPV negative cervical cancer cells, were transfected with an E2-responsive luciferase reporter and either wild type or mutant C307 E2 vectors; luciferase assays were performed 48 hours post-transfection to assess transcriptional activity. Whole cell lysates from overexpressed C307 mutants were separated by SDS-PAGE and immunoblotted to assess expression levels relative to wild type. To examine protein localization, C33A cells were transfected with equal amounts of wild type or mutant E2 and fixed 48 hours post-transfection for immunofluorescence.

Results:
C307S and C307F mutants are both capable of weakly activating transcription. Overexpression of the mutants resulted in a dose dependent increase in transcriptional activity. Both mutants are expressed at levels comparable to wild type E2 and are correctly localized to the nucleus.

Conclusion/Impact:
The deficient transcription function displayed by the C307 mutants cannot be explained by poor expression or mislocalization. Continued study of this conserved cysteine will help to further understanding of papillomavirus biology and may offer insight into novel avenues for treatment or prevention of HPV-associated cancers.