

Overexpression of homologous repair proteins in human papillomavirus positive head and neck squamous cell carcinoma by immunohistochemistry analysis. A potential target for molecular therapies.



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INTRODUCTION

The incidence of human papillomavirus (HPV) positive squamous cell carcinoma (SCCA) is rising rapidly, predominately in oropharyngeal tumors.¹ HPV positive oropharyngeal SCCA has distinct pathophysiologic differences from HPV negative SCCA. HPV oncogenes E6 and E7 contribute to tumorigenesis through inactivation of the tumor suppressor gene p53² and cell cycle regulator pRB.³ This contributes to the accumulation of DNA damage. E6 and E7 have recently been shown to alter repair mechanisms for double stranded DNA breaks (DSB's) through inhibition of homologous repair (HR) and translesion synthesis (TLS). HPV oncogenes increase HR proteins in affected cells, however these oncogenes also inhibit HR foci function.⁴ This study compares expression of HR and TLS proteins in HPV positive and negative SCCA through immunohistochemistry (IHC) staining of a tissue microarray (TMA) derived from surgical specimens of head and neck tumors.

METHODS

- Paraffin embedded patient tissue collected after approval from the Institutional Review board through the University of Kansas Cancer Center, Biospecimen Repository Core.
- TMA created from twenty-seven HPV positive and nine HPV negative oropharyngeal SCCA surgical specimens
- TMA slides stained with antibodies to three HR proteins (BRCA-1, BRCA-2, and RAD51) and four TLS proteins (PCNA, RAD6, RAD18, RPA70)
- IHC analysis performed by two independent pathologists
- Staining intensity and percentage of nuclear staining measured
 - Composite score derived by multiplying percent positive by intensity
- Computer-based analysis using Aperio software of images of TMA slides performed using a standardized algorithm for calculation of staining percentage and intensity
- Composite scores compared using a Mann-Whitney U test
- SPSS software was used for all statistical analyses (version 24; IBM Corp)

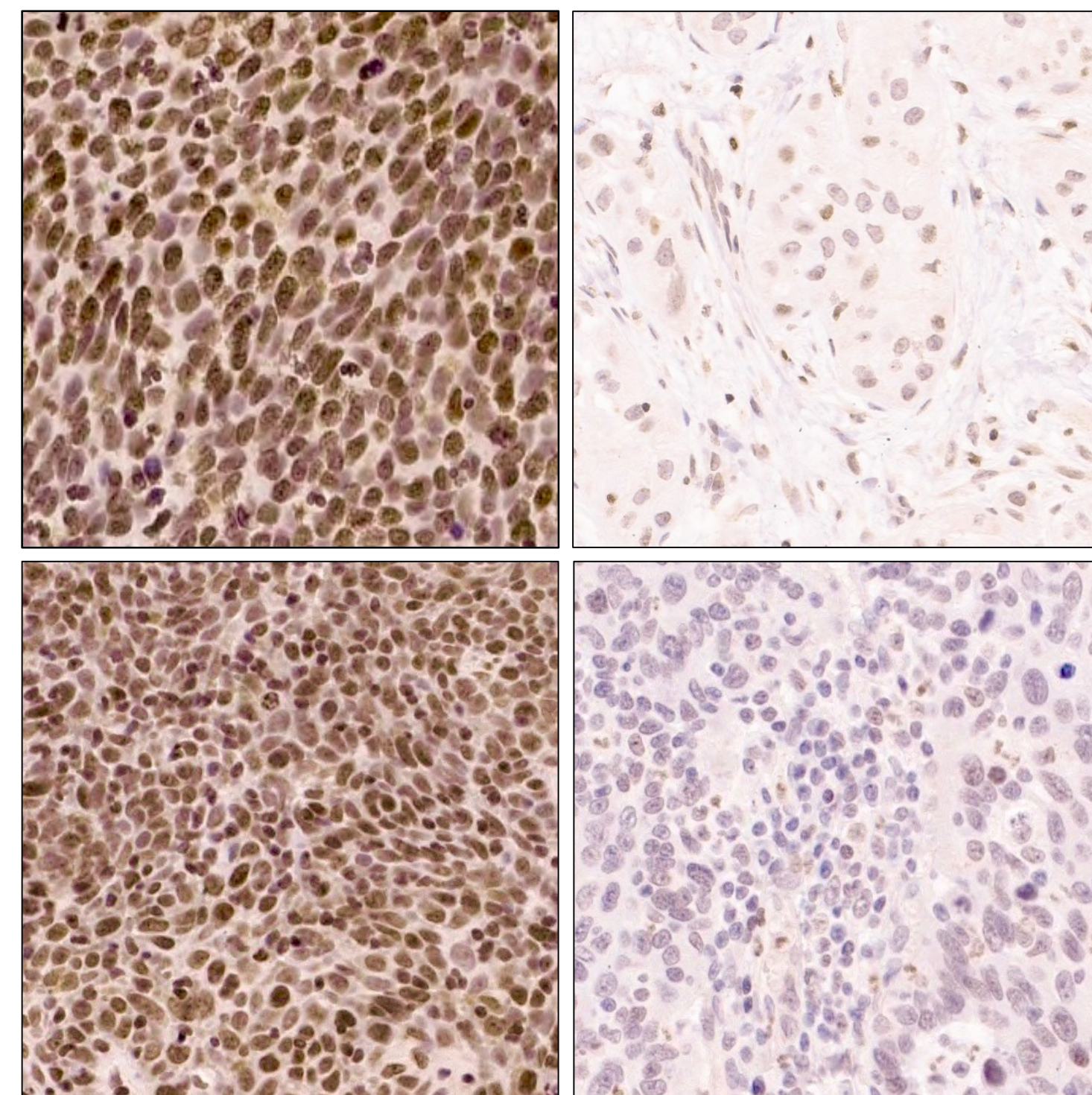


Figure 2: High magnification (20x) images of TMA slides. The top row shows strong (left) and weak (right) staining samples for BRCA-1, and the bottom row shows strong (left) and weak (right) staining samples for RAD51

RESULTS

- Differential staining identified between groups for two HR proteins
 - BRCA-1 - Mean composite scores for HPV positive and negative groups were 1.04 and 0.63 respectively ($p=0.07$)
 - RAD51 - Mean composite scores for HPV positive and negative groups were 2.06 and 0.76 respectively ($p=0.002$)
- TLS proteins were not differentially expressed, nor was BRCA-2
- Inter-rater reliability between pathologists was excellent
 - Intra-class correlation coefficient 0.901
- Computer based analysis yielded similar results
 - Pearson correlation coefficient 0.679

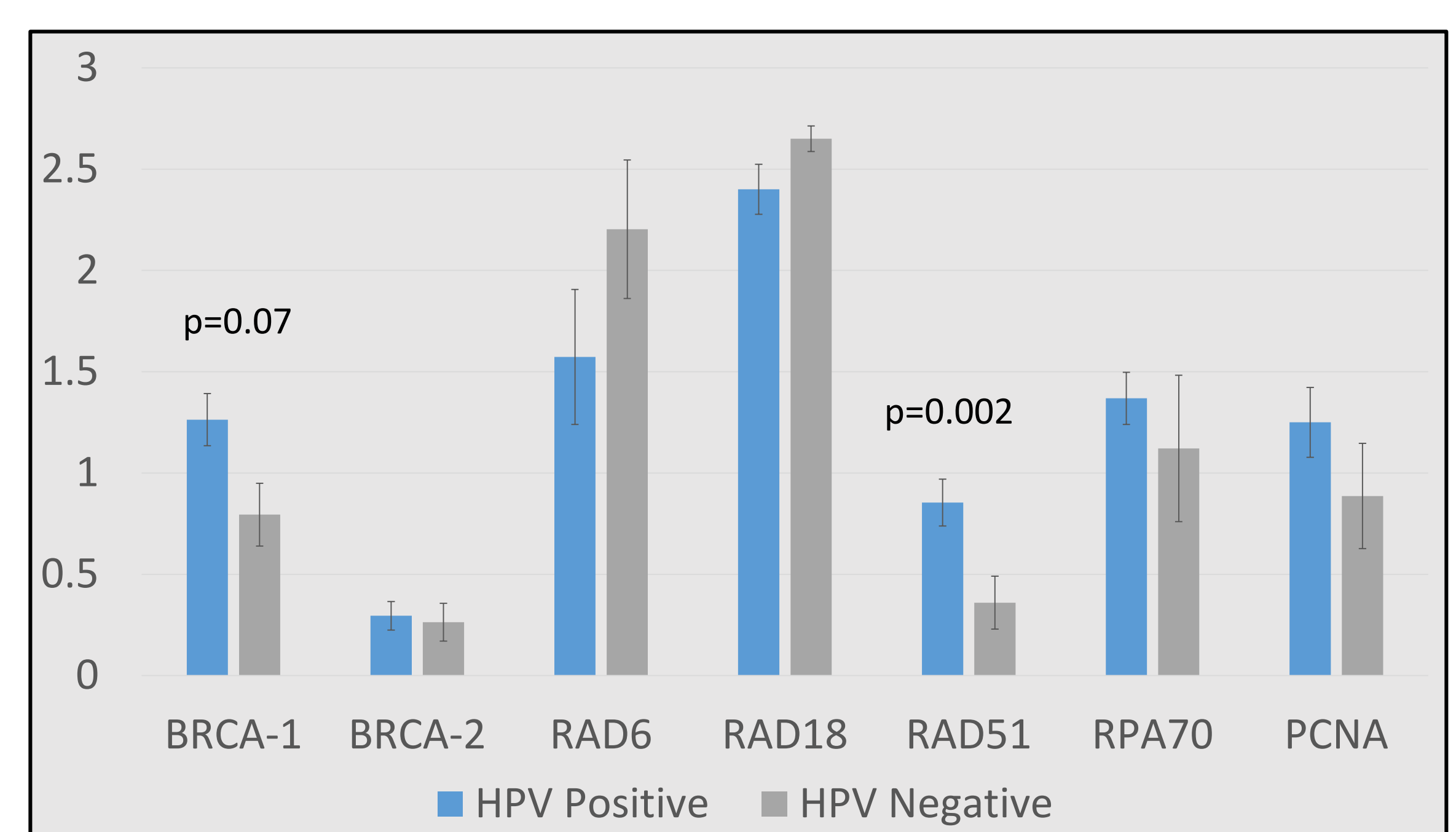
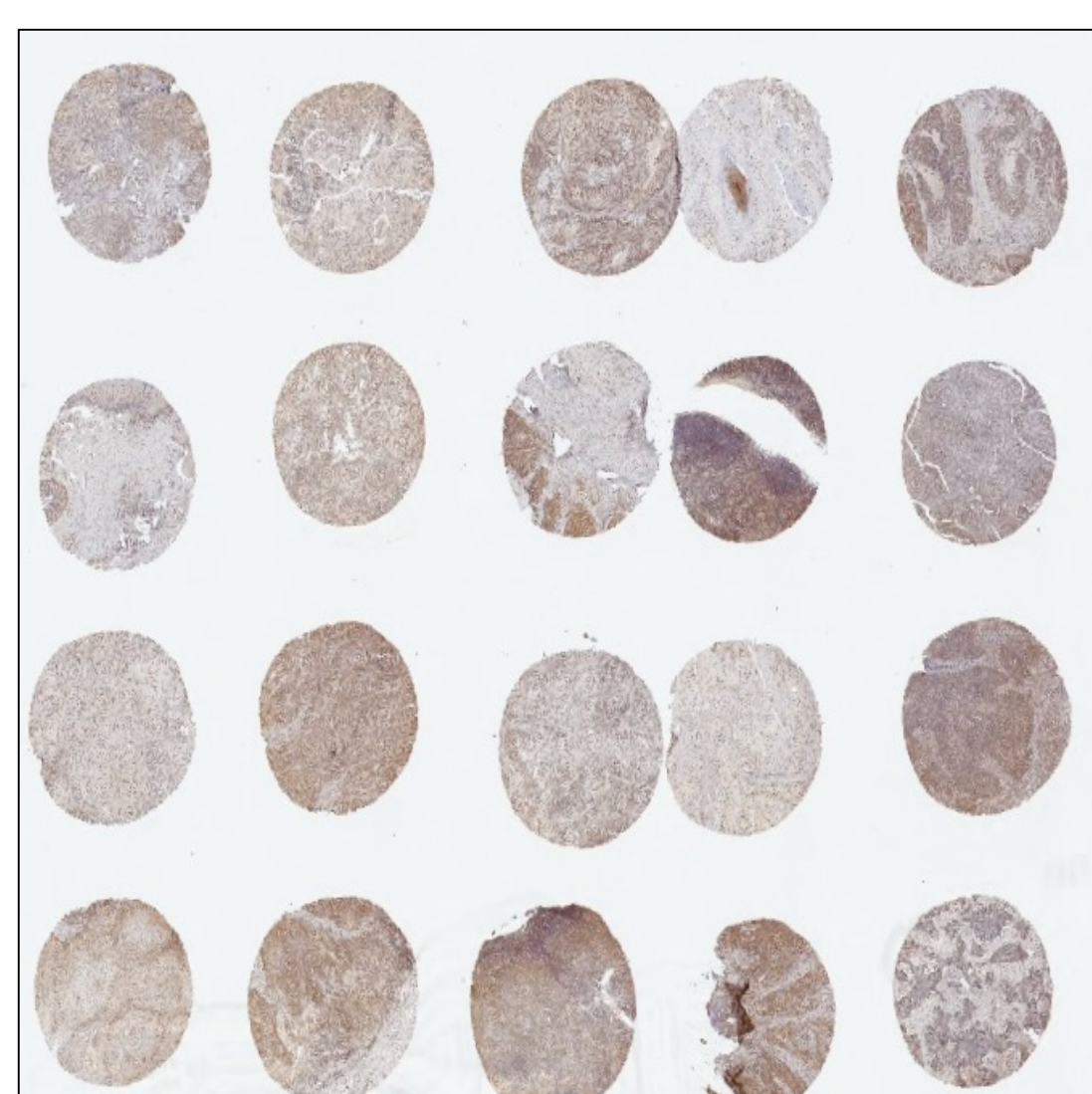


Table 1: Composite scores compared between HPV positive and negative SCCA

Figure 1: Example of TMA. Created from 2mm punch biopsies of surgical specimens placed in a paraffin block, TMA slides are thinly sliced and stained, allowing testing of multiple antibodies on large numbers of patient samples.



DISCUSSION

- DSB's contribute to genomic instability and tumorigenesis
 - HR repair foci utilize sister chromatids as templates for repair of DSB's⁵
 - TLS is a DNA damage tolerance process that allows cells to replicate past DNA lesions and reduce formation of DSB's⁶
 - DSB's facilitate viral episomal DNA integration into the host genome⁷
- HR protein overabundance has been linked to HPV oncogenes⁴
 - Overabundance of HR proteins found in HPV related cervical cancers
 - Expression of E6 and E7 in human keratinocytes increases HR proteins
- Despite overabundance of HR foci, DSB repair is inhibited by HPV oncogenes through multiple mechanisms⁴
 - HPV oncogenes alter timing of HR foci formation, creating foci that lack homologous templates for repair
 - Localization of HR foci to DSB's is impaired predominately by E6
- Our study demonstrates increased expression of HR proteins BRCA-1 and RAD51 in HPV positive SCCA relative to HPV negative SCCA
- These findings validate prior evidence in cell culture models using samples from surgically removed tumor specimens

CONCLUSION

These study findings support the role of HPV oncogenes in alteration of DNA repair mechanisms and further differentiates HPV positive from HPV negative head and neck SCCA. Further studies may address the specific importance of BRCA-1 and RAD51 relative to other HR proteins and may explore specific molecular targets that could alter the interactions between HPV oncogenes and HR proteins.

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