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“The Study of HPV Integration as a Means for Discovery of Driver Genes in HNSCC”

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James B040
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VITA

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ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) is sixth in cancer incidence worldwide. Human papillomavirus (HPV) accounts for ~5% of the world’s cancer burden, including a distinct subset of oropharyngeal HNSCCs rising in incidence in numerous developed countries. The transforming ability of HPV is primarily attributed to the viral oncoproteins E6 and E7 which inactivate the tumor suppressor proteins p53 and pRb, respectively. While E6 and E7 expression are sufficient for immortalization, poorly defined, secondary genetic events are necessary for cellular transformation. Whole genome sequencing of HPV-positive HNSCC cell lines and primary tumors demonstrated a direct association between HPV integrants and focal host genomic instability, including amplification, rearrangement, deletion, and translocation. HPV integrants frequently disrupted expression of host genes with established roles in cancer pathogenesis. We hypothesized that host genomic alterations caused by HPV integration are critical secondary genetic events necessary for the pathogenesis of HPV-caused cancers and that identification of HPV integration sites can serve as a tool for discovery of driver genes targetable for HNSCC therapeutics.

To test this hypothesis we extensively characterized two integration events at TP63 and PIM genes in UM-SCC-47 and
UPCI:SCC090, respectively. Both genes were candidates due to their extensive links to cancer. In UM-SCC-47, HPV integration resulted in disruption of wild-type p63 transcripts and detection of unique fusion transcripts that served as a template for translation of a novel truncated p63 protein (p63T). Although morpholino mediated knockdown of p63T did not affect UM-SCC-47’s growth and viability, p63T displayed novel localization in the cytoplasm indicative of possible new protein interactions not previously characterized. Furthermore, its stability was shown to be modulated by p53 and the proteasome.

In UPCI:SCC090, HPV integration resulted in a 16-fold amplification of the \( PIM1 \) oncogene. This gene amplification was accompanied by high levels of \( PIM1 \) transcripts and protein as detected by RNAseq and Western blot, respectively. Genetic and pharmacologic knockdown of PIM1 using CRISPR technology, shRNA, and pan-PIM inhibitors greatly compromised the growth of UPCI:SCC090 cells demonstrating that the HPV mediated \( PIM1 \) amplification is a driver of this cell line’s growth and viability. High \( PIM \) expression was also observed in other HNSCC cell lines and primary tumors. \( PIM \) expression is regulated through the JAK/STAT, PI3K-AKT, and NFKβ pathways, which are frequently activated in HNSCCs. Pan-PIM inhibitor studies extended to other cell lines demonstrated sensitivity in the low \( \mu \)M range and synergistic cell death in combination with epidermal growth factor receptor (EGFR) inhibitors. PIM kinases are rational
and promising new targets for development of HNSCC therapeutics.

Combined together, our work demonstrates that HPV is a direct insertional mutagen that significantly alters gene structure, expression, and function. Comprehensive understanding of the functional consequences of HPV integration will expand our understanding of HPV malignancies and provide insight on the design of novel therapeutics for all HNSCCs.
RECENT PUBLICATIONS


**Broutian TR**, Hennessey B, Gillison ML. Characterization of a Novel HPV Mediated Truncated p63 Protein. *PLOS1* [manuscript in preparation].


Pickard RK, Xiao W, **Broutian TR**, He X, Gillison ML. (2012). The prevalence and incidence of oral human papillomavirus


*Authors contributed equally to this work.*

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