High-risk Human Papillomavirus Oncoproteins Dysregulate Interleukin-1 Signaling

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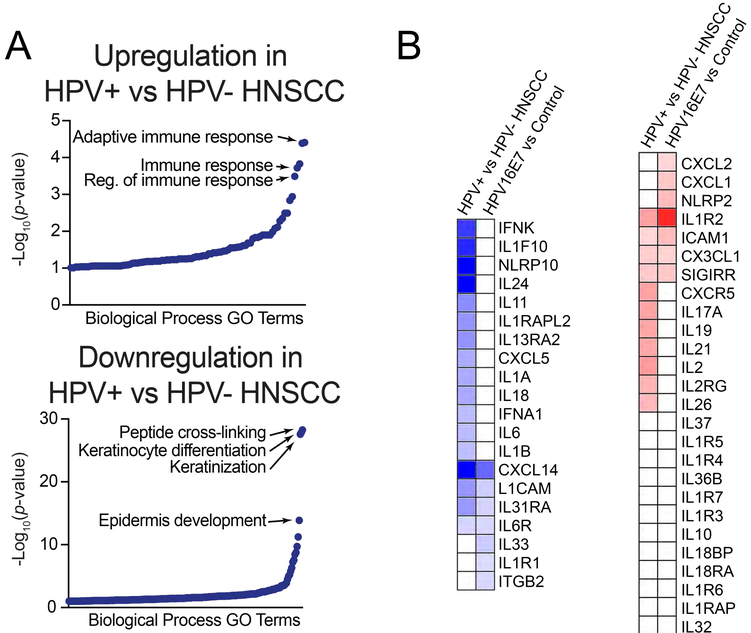
BACKGROUND

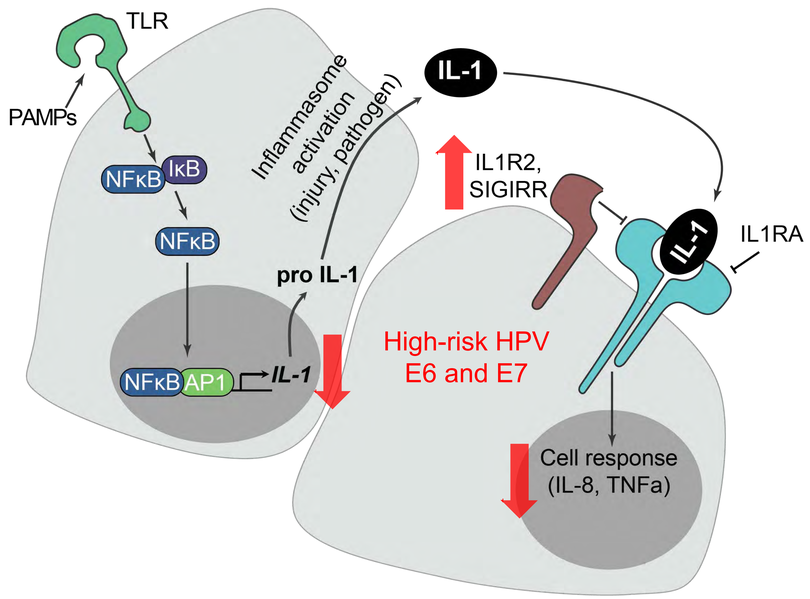
High-risk human papillomavirus (HPV) infection causes cervical, anogenital, and oropharyngeal cancers that account for ~5% of cancer cases worldwide. These malignancies are classified as AIDS-defining (cervical) or non-AIDS-defining (anal, oropharyngeal) cancers and their incidence among HIV-infected individuals is increasing. A mutation in one of several components of the interleukin-1 (IL-1) signaling pathway predisposes individuals to develop HPV-associated malignancies, suggesting that IL-1 signals restrict HPV infection or development/ progression of HPV-positive neoplasia. IL-1β is a pro-inflammatory cytokine and the IL-1 pathway is subject to complex regulation. The purpose of the study was to define the effects of high-risk HPV E6 and E7 oncoproteins on IL-1-related gene expression and signaling.



(A) 520 HNSCC samples from The Cancer Genome Atlas were analyzed for the presence or absence of HPV DNA (Nulton et al. (2017) Oncotarget 8:17684-17699). Genes differentially expressed in HPV+ vs HPV- HNSCC were analyzed by DAVID to determine Gene Ontology (GO) terms enriched among the up- and down-regulated gene lists. (B) Human foreskin keratinocytes (HFK) were transduced with a retroviral vector encoding HPV16 E7 or with a control retrovirus. PolyA-selected RNA from HFK-16E7 and HFK-Control was subjected to RNA-seq. Heat maps display differential expression data from the two data sets (HPV+ vs HPV- HNSCC, HFK-16E7 vs control) for selected genes related to interleukin signaling.

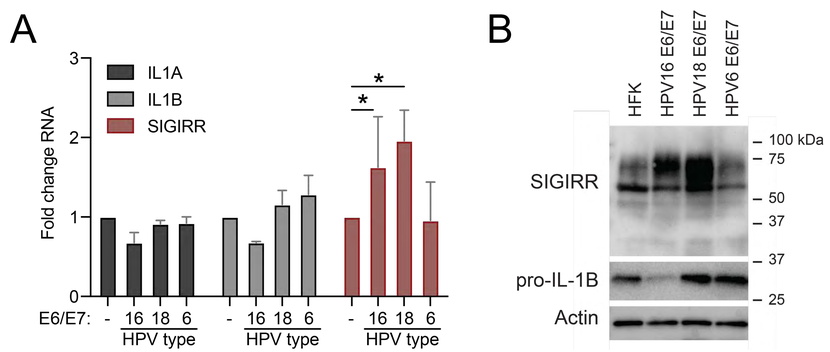
METHODS

We used several models to assess HPV oncoprotein-dependent changes in the IL-1 pathway. In several experiments we compared human keratinocytes engineered to express high-risk HPV E6/E7 to matched negative controls. We also employed a panel of HPV-positive and HPV-negative patient-derived xenografts (PDX). In each model we measured the levels of IL-1 signaling-related transcripts and the response of cells to IL-1 treatment.

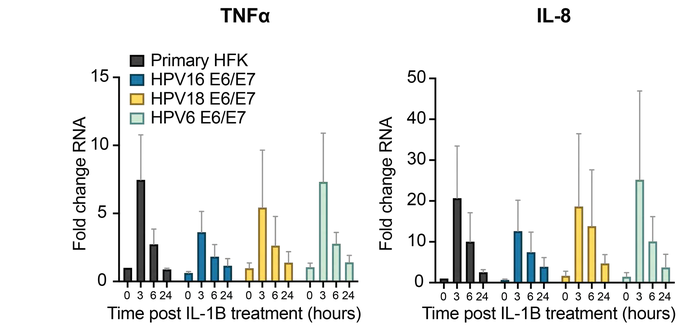


RESULTS

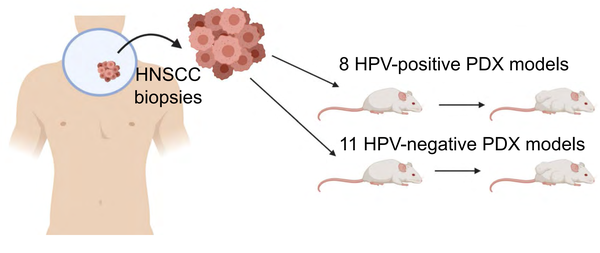
In cell lines and PDX, the presence of high-risk HPV oncogenes was associated with decreased expression of *IL1B* (for HPV16 E6/E7) and increased expression of *SIGIRR*, a negative regulator of IL-1 signaling (for both HPV16 and HPV18 E6/E7). High-risk E6/E7-expressing cells were less responsive to stimulation with IL-1β as assessed by the transcription of *IL8* and *TNFA*.

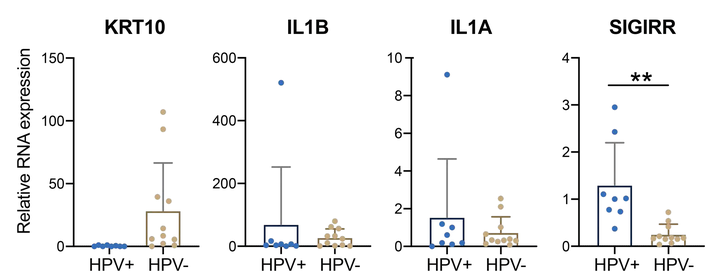


HFK were engineered to express the E6 and E7 oncogenes from high-risk HPV16 or HPV18 genotypes or from the low-risk HPV6 genotype. (A) RNA from HFK-E6/E7 was isolated, reverse transcribed, and analyzed by qRT-PCR for *IL1A*, *IL1B*, and *SIGIRR*. SIGIRR is a negative regulator of IL-1 signaling and its expression is upregulated in the presence of the high-risk but not low-risk HPV E6 and E7 oncoproteins. *, p<0.05. (B) Protein from HFK-E6/E7 was separated by SDS-PAGE and Western blotted with antibodies to SIGIRR, pro-IL1B and actin. SIGIRR protein is upregulated in the presence of high-risk but not low-risk HPV E6 and E7 oncoproteins.



HPV E6/E7 expressing cells were treated with 1 ng/ml IL-1β and harvested for RNA isolation at 0, 3, 4, 6 and 24h post-treatment. RNA was isolated, reverse transcribed, and analyzed by qRT-PCR for *TNFA* (left) and *IL8* (right). High-risk HPV E6/E7 expressing cells exhibit an impaired response to IL-1β treatment.





RNA was isolated from eight HPV-positive and eleven HPV-negative patient-derived xenografts (PDX) (Facompre et al., bioRxiv 652537; doi: https://doi.org/10.1101/652537). RNA was reverse transcribed and analyzed by qRT-PCR for *KRT10*, *IL1B*, *ILA*, and *SIGIRR*. **, p<0.01.

CONCLUSIONS

High-risk HPV E6/E7 oncoproteins induced changes in the IL-1 pathway consistent with reduced activity. Some changes were recapitulated in a PDX panel consisting of 11 HPV-negative and eight HPV-positive models. HPV oncoprotein-expressing cells upregulated fewer genes related to inflammation upon treatment with IL-1β and were impaired in the production of IL-1β following nigericin treatment. Future studies will aim to determine the mechanisms underlying this dysregulation and to assess its role in HPV-mediated carcinogenesis.

ADDITIONAL KEY INFORMATION

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