

# La Nian-A-a-lai

# HPV<sup>+</sup> Head and Neck Squamous Cell Carcinoma-Derived Exosomes Mediate Neutrophil Attraction via Activation of Fibroblasts in the Tumor Microenvironment

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### Abstract

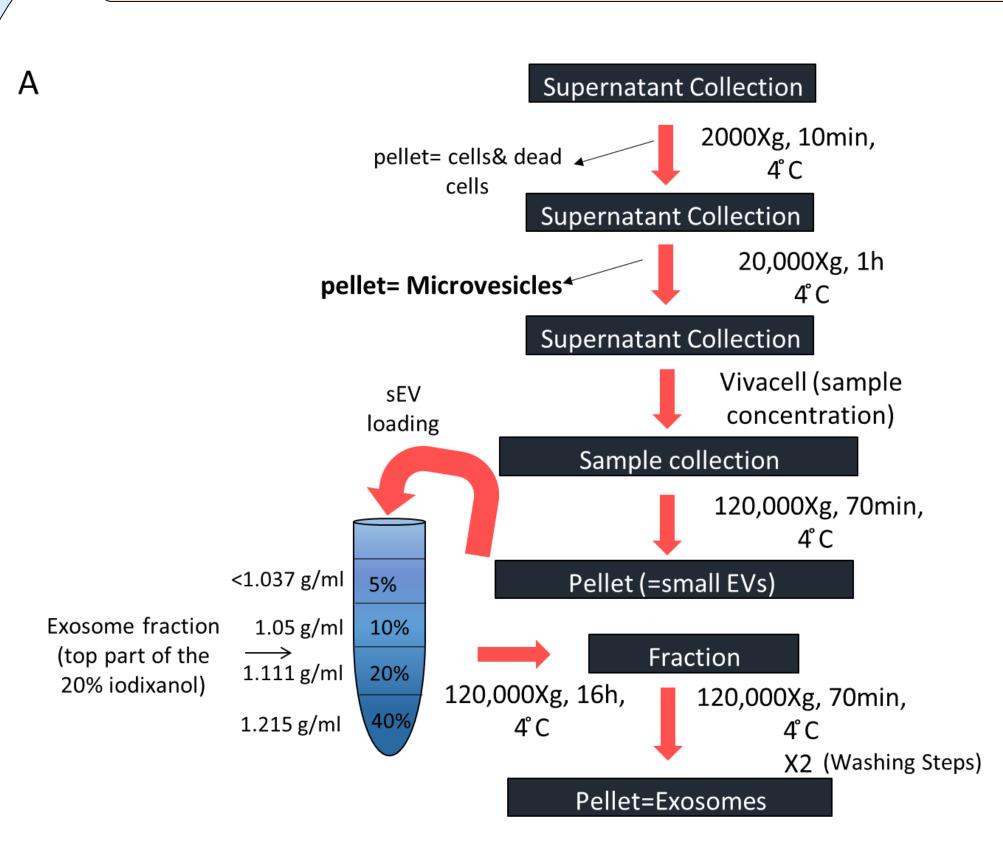
Head and Neck Cancer Squamous Cell Carcinoma (HNSCC) accounts for more than 90% of the cancer of the head and neck. Human Papilloma Virus (HPV) infection has been implicated in the pathogenesis of 25% HNSCC cases in the United States.

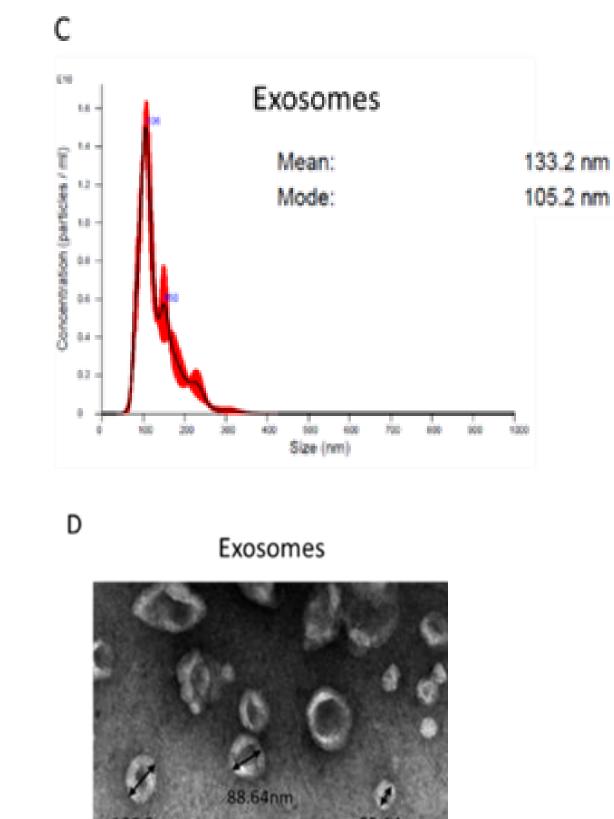
The impact HPV has on the tumor microenvironment (TME) is in its infancy. Cancer-Associated Fibroblasts (CAFs) in the TME play a major role in tumor progression by mediating inflammation and ECM modifications. Understanding pathways of communication between tumor cells and fibroblasts is an important step in deciphering the challenging phenomenon of cancer progression. To address this question, we analyzed the miRNA content of HPV<sup>+</sup> and HPV<sup>-</sup> HNSCC-derived exosomes, and tested their impact on fibroblast activation.

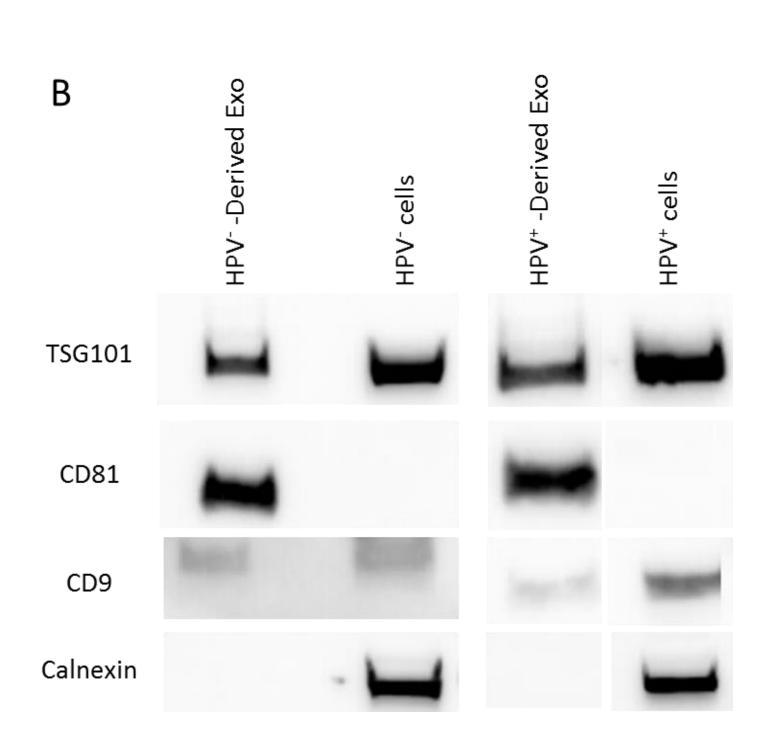
We found that HPV<sup>+</sup> and HPV<sup>-</sup> exosomes derived from HNSCC cells had distinct effects on CAF gene expression towards pro-inflamamtory or ECM modulating activities. This is reflected in enhanced neutrophil recruitment in the blood of HPV<sup>+</sup> patients, and higher αSMA levels and collagen secretion in HPV<sup>-</sup> tumors. Using this new understanding of TME may lead to exciting novel therapeutic approaches.

### Results

#### 1. Exosome Isolation from Human HPV<sup>+</sup> and HPV<sup>-</sup> HNSCC Cell Lines

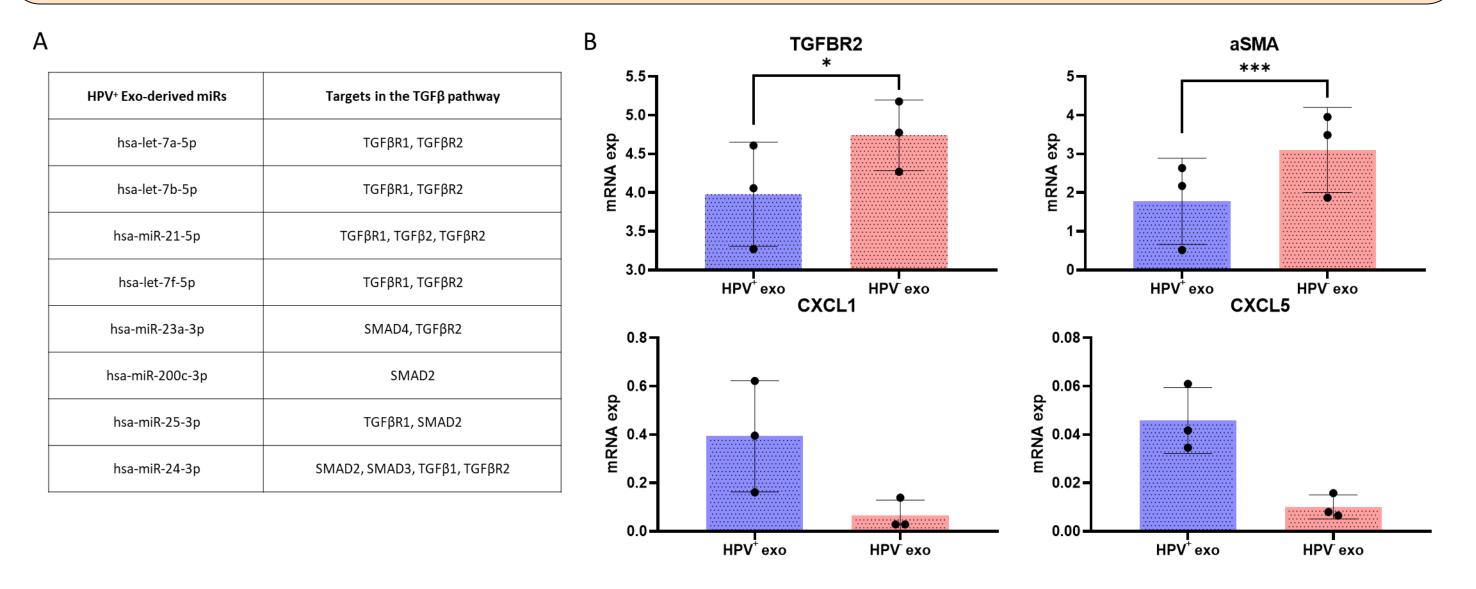






- (A) Flow chart for exosome isolation procedure by a series of ultracentrifugation steps, density gradient ultracentrifugation and concentration with Vivacell.
- (B) Western blot for exosomal markers
  (TSG101, CD9, CD81). Calnexin was used as a negative marker for ER-derived EVs.
- (C) Mean and mode particle size distribution of exosomes by NTA.
- (D) Representative TEM images of the isolated exosomes. The diameters of the vesicles are indicated in the image.

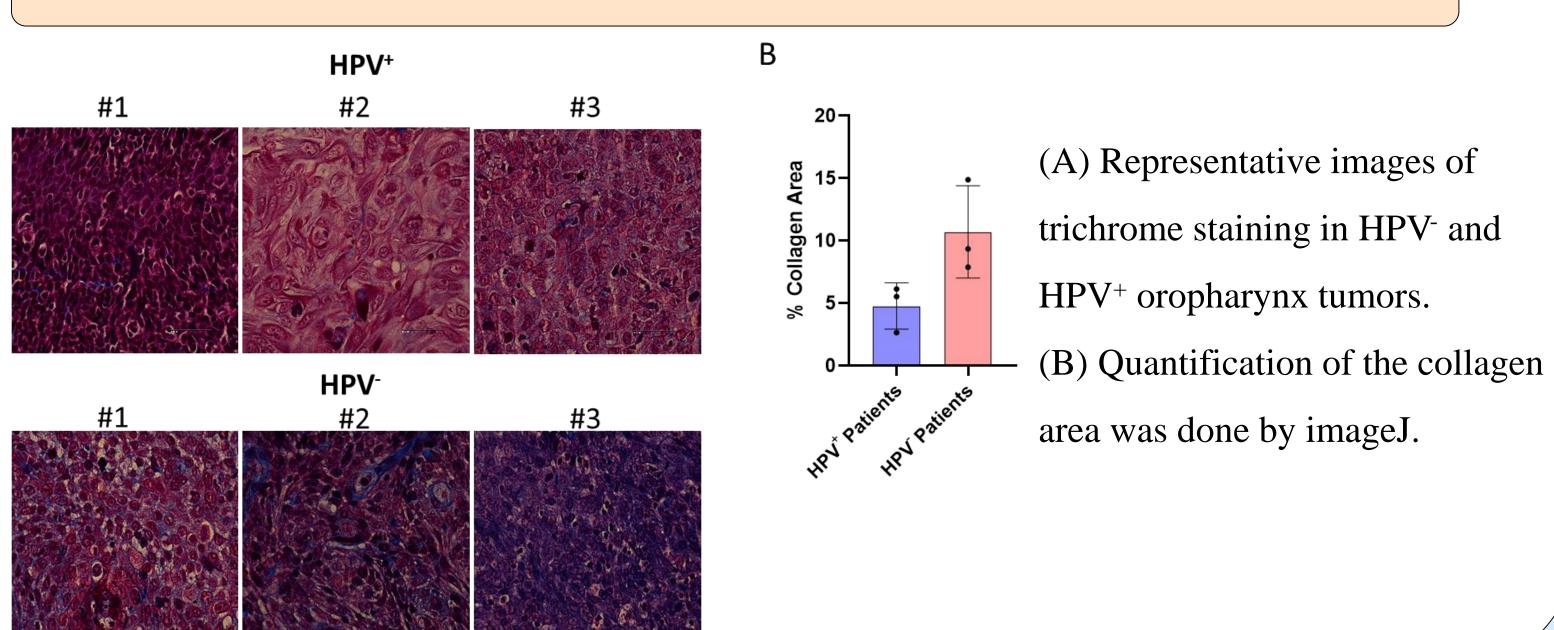
## 2. HPV+ Exosome-Derived miRs Downregulate TGFβR2 and αSMA mRNA Levels and Elevate CXCL1 and CXCL5 in Human Oral Fibroblasts



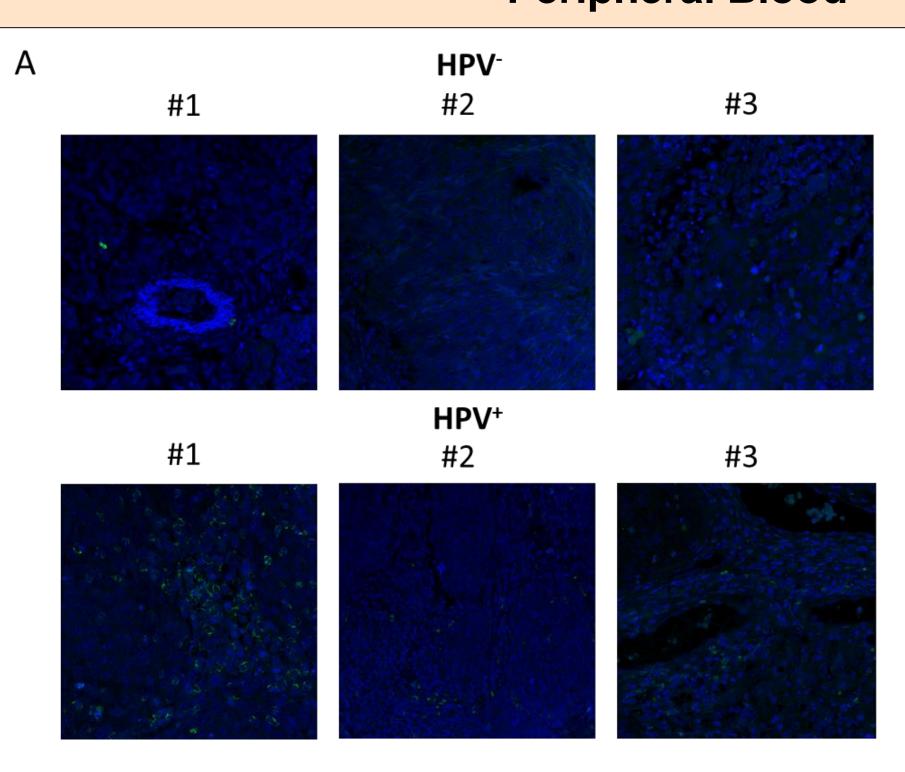
(A) miRs enriched in HPV<sup>+</sup> vs. HPV<sup>-</sup> exosomes, which have common targets in the TGF $\beta$  pathway. (B) qRT-PCR analysis of TGFBR2,  $\alpha$ SMA, CXCL1 and CXCL5 gene expression in human oral fibroblasts, treated with

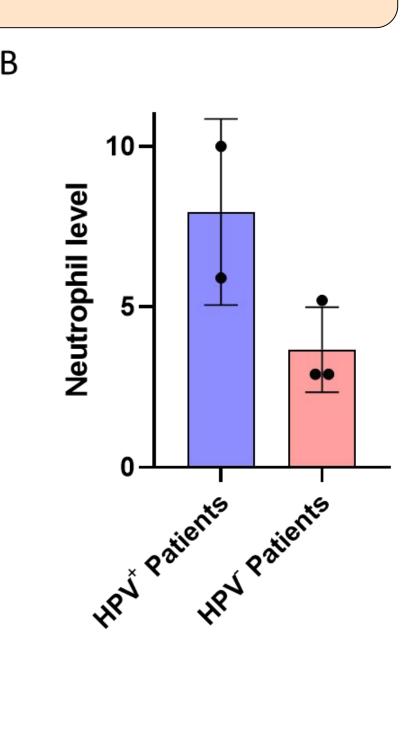
HPV<sup>+</sup> and HPV<sup>-</sup> exosomes.

### 3. Higher Collagen Secretion in Human HPV Oropharynx Tumors



### 4. Higher Neutrophil Levels in HPV+ Oropharynx Tumors and Peripheral Blood

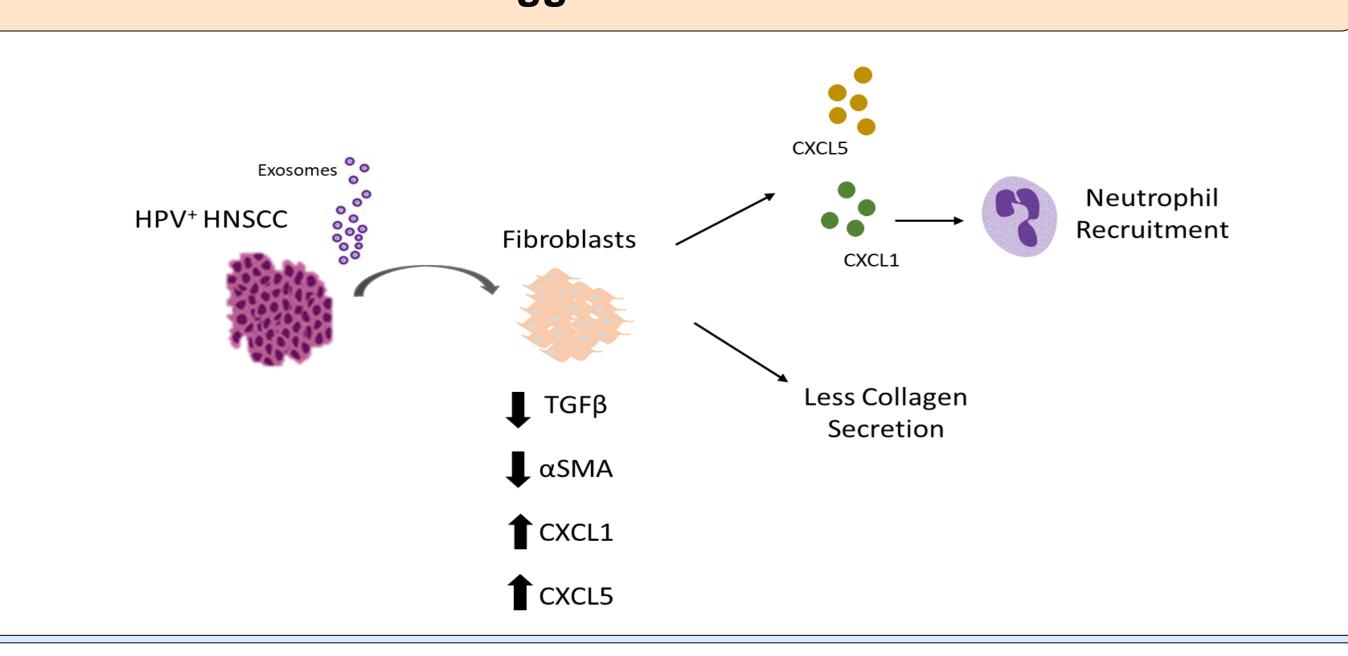




(A) Representative images of neutrophil staining in HPV<sup>-</sup> and HPV<sup>+</sup> oropharynx tumors.

(B) Neutrophil levels in peripheral blood of HPV<sup>+</sup> and HPV<sup>-</sup> oropharynx tumors.

### Suggested Model



### Summary

- $HPV^+$  HNSCC-derived exosomes contain miRs against targets in the  $TGF\beta$  pathway. .
- Fibroblasts in the TME of HPV<sup>+</sup> tumors uptake the exosomes, which cause downregulation of TGFBR2, thus downregulation in aSMA and elevation in CXCL1 and CXCL5 gene expression.
- CXCL1 and CXCL5 act as neutrophil chemoattractants, thus higher levels of neutrophils in HPV<sup>+</sup> vs HPV<sup>-</sup> tumors.