



# HPV-induced Epigenetic Modifications in the Initiation of Oropharyngeal Cancer

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## Background

Among the cancers that routinely afflict the aerodigestive tract, oropharyngeal squamous cell carcinoma is of particular importance due to its higher rate of increasing incidence. It is evidenced that this annual 2.5% increase is due in part by the adjunct infection with Human papillomavirus (HPV) which induces oncogenic and molecular modifications. An epigenome-wide association (EWAS) study was recently performed on DNA methylation in peripheral blood leukocytes in 20 pairs of HPV (+) and HPV (-) OPSCC cells. It showed leukocyte DNA in HPV (+) patients to have a hypomethylated pattern in comparison to HPV (-) patients. In contrast, a non-coding RNA, nc886, was most significantly hypermethylated in HPV (+) patients. However, it remains undetermined the distinct epigenetic exchange occurring between suspect methylation patterns and the presence of nc886. The aim of this study is to decode the genetic interplay potentially involved between DNA methylation and HPV-induced oropharyngeal carcinogenesis.

## Hypothesis

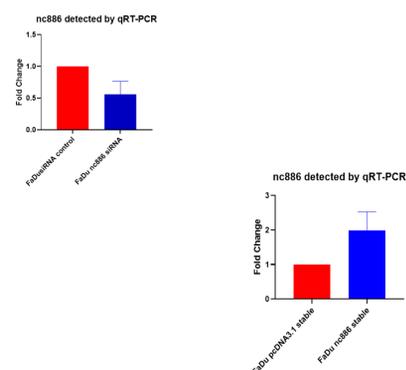
If the novel non-coding RNA, nc886, is involved in HPV-induced OPSCC etiology such that there is an epigenetic mechanism occurring between HPV oncogenes and nc886, then overexpression and gene knockout of nc886 will affect the propensity of OPSCC cell proliferation, migration, and metastasis via its down regulatory relationship with Protein Kinase R phosphorylation.

## Methods

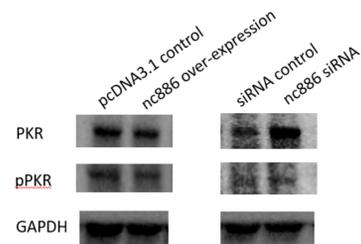
- HPV negative OPSCC cells of the FaDu cell line were transfected with HPV E6/E7 (oncogenic proteins) via lentiviral transfection
  - Transfected cells were isolated via ultracentrifugation and filtration
- CRISPR/Cas9 along with single guide RNA (sgRNA) technology was applied to knockout nc886 in targeted OPSCC cells
  - siRNA technology was utilized to knockdown gene expression
- Methylation sequencing, qRT-PCR, flow cytometry, and Western blot were utilized to analyze the phenotypic effects of nc886 silencing and overexpression on cell growth, proliferation, and PKR phosphorylation

## Results

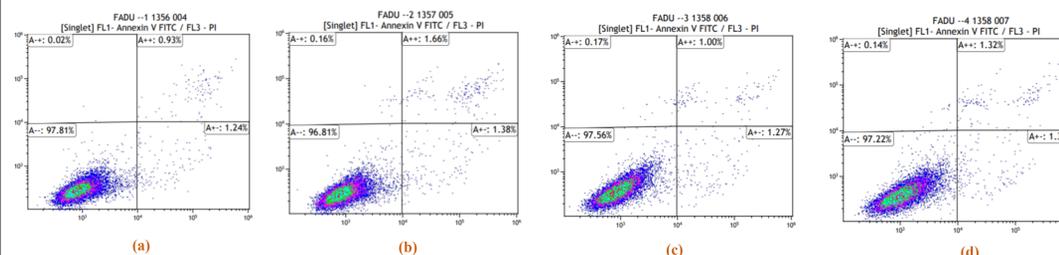
**Figure 1.** qRT-PCR analysis of forced nc886 expression



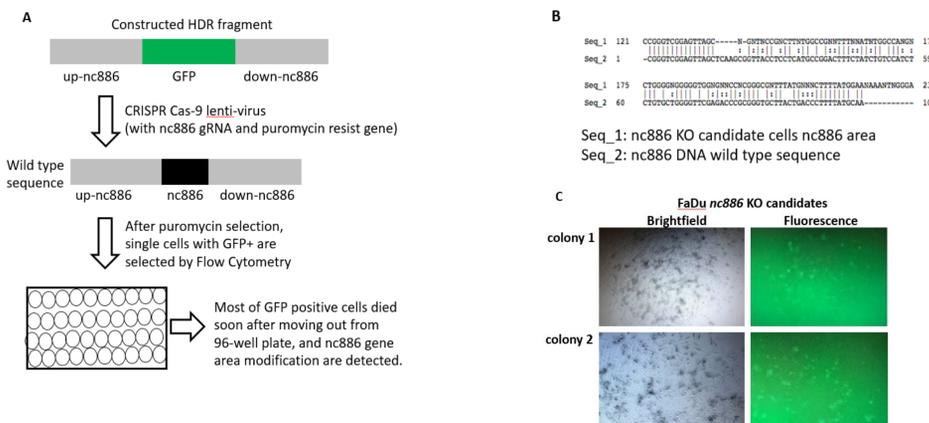
**Figure 2.** Western Blot analyses of PKR expression in over-expressed nc886 cells. Equal proportions of protein concentrations were added to all wells, and the presence of PKR was detected with monoclonal antibody GAPDH. GAPDH was detected as a loading control. Comparison of PKR and pPKR demonstrates differential kinase activity patterns



**Figure 3.** Flow cytometry data obtained from a representative sample is shown. Cells of the FaDu cell line were first gated based on apoptosis (Annexin V-FITC). Necrotic cells in the whole blood sample were detected by Propidium Iodide (PI) staining. (a) pcDNA3.1 control FaDu. (b) High expression of nc886 FaDu. (c) siRNA control FaDu. (d) nc886 and siRNA FaDu.



**Figure 4.** nc886 knockout leads to cell death in FaDu. (A) The flow chart of CRISPR/Cas9 homology directed repair (HDR). (B) Gene modification detected in nc886 sequence area of GFP+ cells. Those cells died soon after moving out from 96-well plate. (C) GFP detection of the FaDu nc886 KO candidates by fluorescence microscopy.



## Conclusions/Implications

- Differential nc886 expression patterns in patient samples may be indicative of cell stability, apoptotic resistance, and relevant to cancer prognosis and intervention.
- PKR expression and kinase activity may be directly correlated to levels of nc886 present, potentially linking phosphorylation and cell proliferation to signature markers of cancer etiology.
- Function of nc886 may influence cell survival and cancer regulatory mechanisms as implicated via gene knockout of nc886 in GFP (+) cells.
- The implications of this study should yield the first evidenced event that two prominent epigenetic events (DNA methylation and non-coding RNA regulation) can synchronize to initiate HPV-induced modulations in the onset and progression of oropharyngeal cancers.

## Acknowledgements

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## Responsible Conduct of Research

The integrity and authenticity of this research project was ensured through the adherence to institutional guidelines as prescribed by MD Anderson. Data transference was officially permitted and obtained solely through MD Anderson secured databases.

## References

1. Hyun-Sung Lee et al (2014). *Epigenetic silencing of the non-coding RNA nc886 provokes oncogenes during human esophageal tumorigenesis*
2. Eun Kyung Lee et al(2016). *nc886, a non-coding RNA and suppressor of PKR, exerts an oncogenic function in thyroid cancer*
3. Nawapol Kunkeaw et al(2019). *Mechanism mediated by a noncoding RNA, nc886 in the cytotoxicity of DNA-reactive compound*