HPV Oncogenes E6/E7 Promote Cervical Dysplasia Progression Through Suppression of miR-4488 and Activation of Wnt Signaling

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Objective

This study aims to identify mechanisms involved in progression of low-grade cervical dysplasia to ultimately aid in early intervention and treatment of low-grade cervical dysplasia to prevent progression to cancer.

Background

Cervical Dysplasia

Each year, approximately 3 million women in the United States are diagnosed with Human Papillomavirus (HPV)-associated low grade cervical intraepithelial neoplasia (cervical dysplasia).[1] Low grade cervical dysplasia is a precancerous lesion that, over the course of months to years, has the potential to develop into cancer. HPV oncogenes E6 and E7 aid in progression of cervical dysplasia. Despite HPV's oncogenic potential, most individuals with low-grade cervical dysplasia will clear it naturally, but a few will progress to high-grade dysplasia which increases their risk of developing cervical cancer.[2]

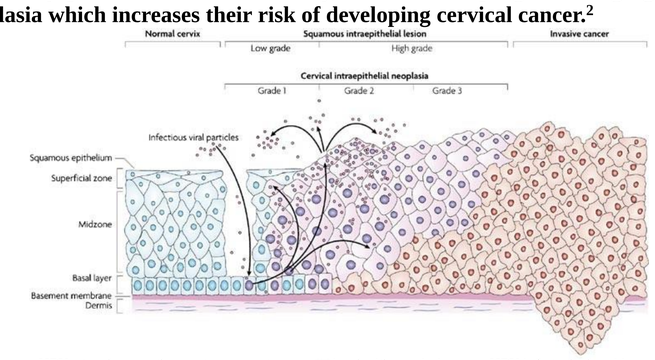

Figure 1. HPV 16 and 18 are the most common types associated with cervical cancer. HPV infects the basal layer of epithelial tissue and induces abnormal cellular proliferation (purple nucleated cells). During the infection, cells become more dysregulated, and the number of abnormal cells correlates with the severity of the dysplasia diagnosis. Without treatment, some women develop cancer.[3]

MicroRNA-4488

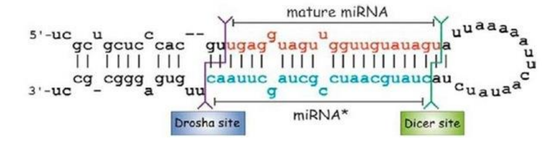

Figure 2. A generic depiction of a primary microRNA(miRNA) and the cut sites that convert the miRNA into mature miRNAs.

- MicroRNAs (miRNAs) are single-stranded non-coding RNA molecules
- Contain approximately 22 nucleotides
- Help control gene expression at RNA level

We specifically focused on microRNA-4488 since it has been previously found to be downregulated in women with progressive cervical dysplasia, as seen in Figure 3.

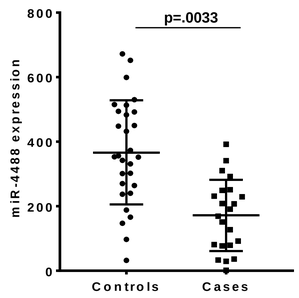

Figure 3. Differential expression of miR-4488 in low grade cervical dysplasia biopsies from individuals with progressive cervical dysplasia (cases) compared to individuals whose low-grade cervical dysplasia resolved naturally (controls).

Wingless and INT-1 (Wnt) Signaling

- Wnt signaling is a cancer pathway that promotes cell migration, survival, and proliferation[4]
  - Wnt signaling activation steps:
    - Wnt agonist binds to receptors
    - The β-Catenin destruction complex is inactivated
    - Accumulation and translocation of β-Catenin in the nucleus
    - β-Catenin associates with TCF/LEF transcription factor (TF complex)
    - TF complex promotes Wnt responsive gene expression
- Has been shown to be activated in cervical cancer[5]

MiR-4488 is predicted to target genes within the Wnt signaling pathway

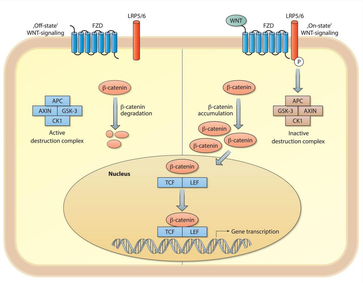

Figure 4. Inactivated Wnt signaling (left) pathway and activated Wnt signaling (right) pathway shown side-by-side.[6]

We hypothesize that HPV oncogenes E6 and E7 promote cervical dysplasia by downregulating miR-4488 and inducing Wnt signaling.

Results

HPV Oncogenes E6/E7 Downregulate miR-4488

- In primary ectocervical cells, E6/E7 expression reduced miR-4488 expression 2-fold compared to GFP controls
- Confirms expression data of progressive cervical dysplasia
- Potential early infection phenotype

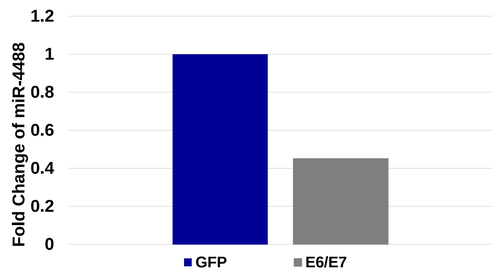

Figure 5. Primary Ectocervical cells expressing HPV 16 oncogenes E6/E7 downregulated miR-4488 compared to GFP expressing controls.

HPV Oncogenes Enhance Wnt Signaling

- Wnt responsive luciferase reporter assay
- Performed in the HPV negative cervical cancer cell line C-33A
  - Transduced to express HPV-16 E6/E7 oncoproteins
  - Vector controls maintained with Geneticin (G418)

HPV-16 oncogenes E6 and E7 promote Wnt signaling.

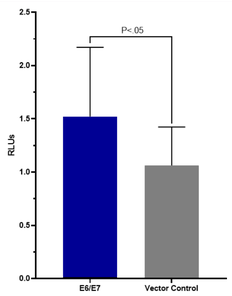

Figure 6. Results of a Wnt Responsive Luciferase Reporter Assay in the presence of HPV-16 oncogenes E6/E7. Data presented is 3 pooled experiments. Relative Light Units (RLU).

Methods

- MiR-4488 Gene Expression
  - Measured in primary ectocervical cells expressing HPV-16 E6/E7, compared to GFP expressing controls
  - HEK 293 cells stimulated with Wnt Agonist 1 (APE x Bio)
    - Extractions occurred at timepoints (0 hours – 72 hours)

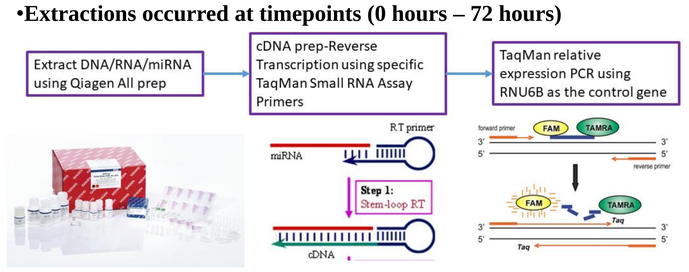

Figure 7. A general schematic of extracting RNA and measuring miR-4488 expression.

- Wnt Responsive Luciferase Reporter Assay

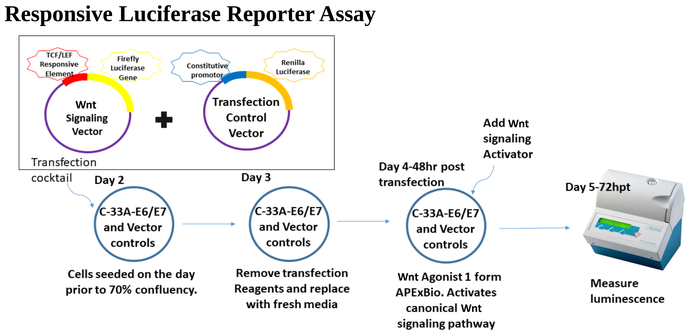

Figure 8. Schematic of the TCF/LEF Reporter kit (BPS Biosciences) performed in C-33A cells expressing either HPV-16 E6/E7 or Vector Controls. Wnt agonist 1 was obtained from APE x BIO. Results were measured using the Dual Luciferase Firefly-Renilla Assay System (BPS Biosciences).

Results

Wnt Activation Decreases miR-4488 Expression

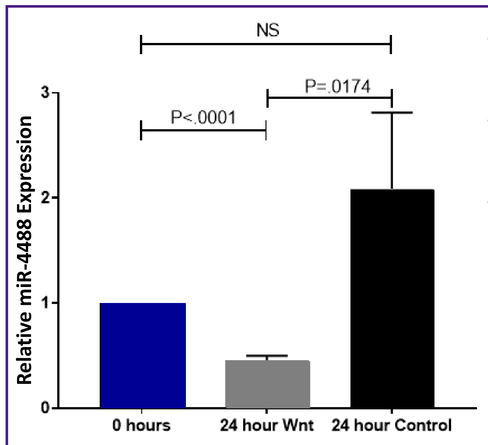

Figure 9. At 24 hours post Wnt activation, miR-4488 expression was measured and normalized to RNU6B. Timepoint controls were taken without activation of Wnt signaling. Data is presented as a relative fold expression as calculated by the ΔΔCt method. Data presented is 3 pooled experiments.

- MiR-4488 expression was analyzed 24 hours post Wnt signaling activation
- MiR-4488 expression decreased 2-fold following activation
- Expression of miR-4488 was significantly decreased compared to controls at 24 hours post Wnt activation

Early activation of Wnt signaling may decrease miR-4488 expression in progressive cervical dysplasia

Conclusions

- HPV oncogenes E6/E7 downregulate miR-4488 expression while enhancing Wnt signaling
- Progressive cervical dysplasia promotes downregulation of miR-4488 and was confirmed as an early indicator of HPV oncogene expression
- Wnt signaling activation decreases miR-4488 expression
- Both downregulation of miR-4488 and activation of Wnt signaling may be early indicators of oncogenic HPV E6/E7 overexpression and cervical dysplasia progression.

Future Work

- Examine putative targets of miR-4488 within the Wnt signaling pathway
- Examine the effects of downregulation and overexpression of miR-4488 on Wnt signaling activation
- Examine low-grade cervical dysplasia for markers of activated Wnt signaling
- Ultimate goal: to find a way to screen for these pathways and expressions in order to determine who will clear the cervical dysplasia on their own and who will progress to cancer.

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