In Vitro Comparison of Oral Squamous Cell Carcinoma HPV-positive and HPV-negative Invasiveness and Voltage-Gated Sodium Channel Isoforms Expression

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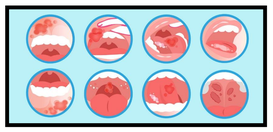
Introduction

Oral Cancer and Human Papillomavirus (HPV)

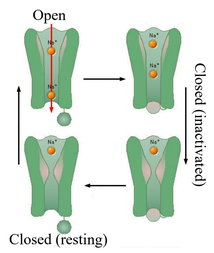
- Oral squamous cell carcinomas (OSCCs) are cancers that are primarily caused by alcohol use, tobacco use, poor dental hygiene, excessive sun exposure, and human papillomavirus (HPV).
- HPV is the most common sexually transmitted disease, and it is estimated around 14 million people in the U.S. are infected.
- HPV has no cure; however, it can be prevented with a vaccine.



- As cancer metastasizes, the prognosis becomes progressively worse. OSCCs have a 30% five-year survival rate in their advanced stages.
- Recent studies have shown that despite HPV infection being a significant cause of increased incidences of cancer, HPV-positive OSCCs have demonstrated a 53% improved overall survival rate compared to HPV-negative OSCCs.

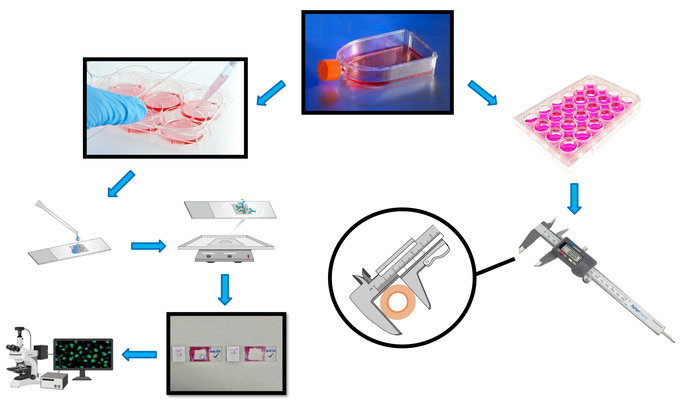
Voltage-gated Sodium Channels (VSGCs)

- VSGCs are transmembrane proteins that allow for the flow of sodium ions across the cell membrane.
- Their function is well known to propagate action potentials along nerve and cardiomyocytes muscle cells.
- The dysfunction of VGSCs can cause illnesses such as epilepsy, myopathies, and cardiac arrhythmias.
- The increased expression of VGSCs is associated with increased cell migration and invasion in cancer metastasis in various types of cancer.
- VGSCs have at least nine distinct sodium channel isoforms found in mammals: Nav1.1, Nav1.2, Nav1.3, Nav1.4, Nav1.5, Nav1.6, Nav1.7, Nav1.8, and Nav1.9.
- In recent studies, three isoforms have been found to be possibly associated with the increased expression of VSGC in cancer cells: Nav1.5, Nav1.6, and Nav1.7.



- Nav1.5 is expressed in cardiac muscles.
- Nav1.6 is expressed in the peripheral and central nervous system.
- Nav1.7 is expressed in the peripheral nervous system.

Methods



Immunofluorescence Staining

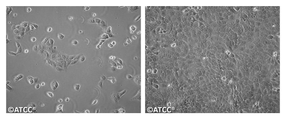
- Fixative
- Indirect staining (Primary and secondary antibodies)
- Imaging

Invasiveness Assay

- Incubated for 7 days
- Measured with caliber

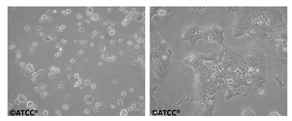
Cell Lines

Cal-27



- HPV-negative
- Found on the middle of the tongue
- Taken from a 56-year-old, white male in 1982

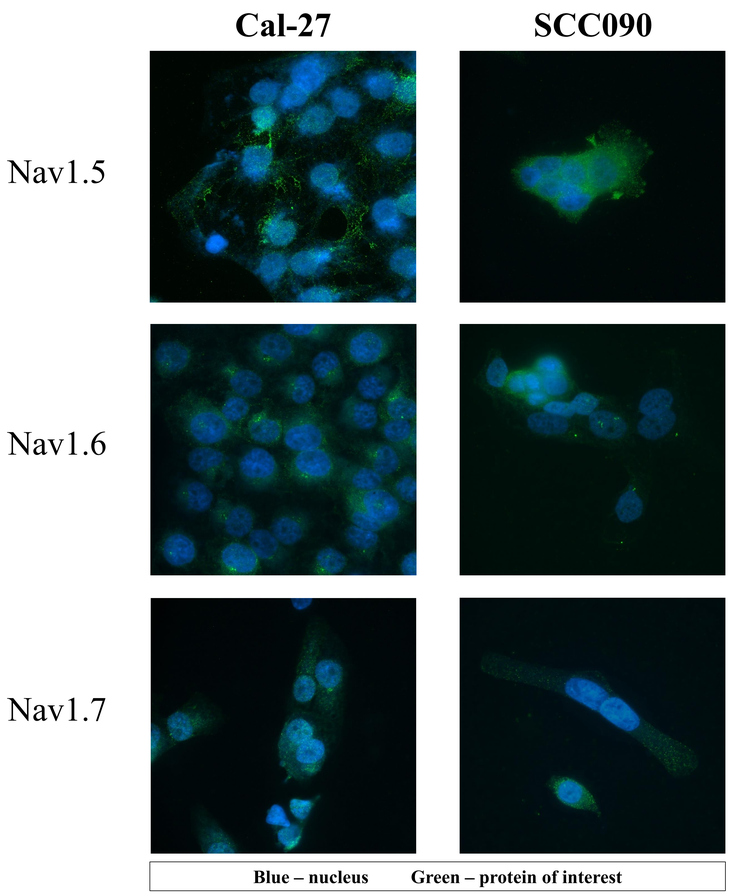
SCC090



- HPV-positive
- Found on the base of the tongue
- Taken from a 46-year-old, white male in 1994

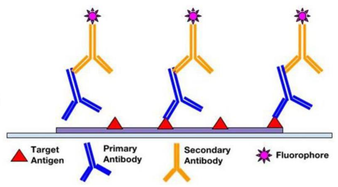
Comparing Cal-27(HPV-) and SCC090(HPV+)	Significance p<0.05
MTT Proliferation/Viability Assay after 24 hours	No
Cell Growth after 24 hours	No
Cell Growth after 48 hours	Yes
Motility after 24 hours	Yes

Staining

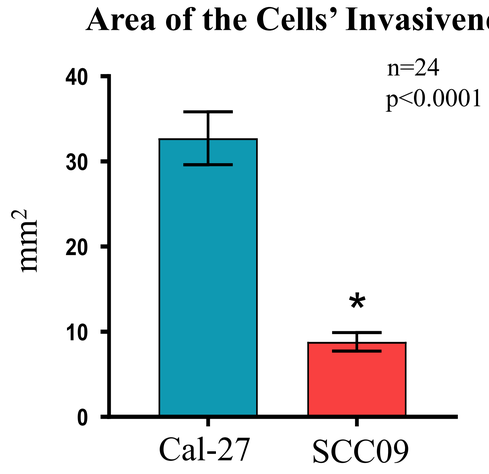


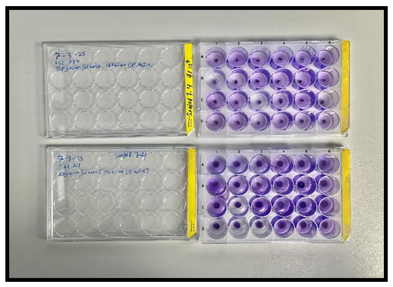
Immunofluorescence Staining

- Fixed with 1% Paraformaldehyde (PFA)
- Blocked with 3% Bovine Serum Albumin (BSA) and 5% goat serum
- Indirect staining was performed
- Primary antibodies: host rabbit, 1:200 dilution
  - Anti-Nav1.5
  - Anti-Nav1.6
  - Anti-Nav1.7
- Secondary antibody: goat anti-rabbit IgG 488 Alexa, 1:1200 dilution
- Slides mounted with ProLong™ Gold antifade reagent with DAPI



Invasiveness





Invasiveness Assay

- Cells from the two cell lines were embedded within an 1% agarose matrix and incubated for 7 days.
- The Bio-Rad ChemiDoc Imaging System was used to capture images of the plates.
- The areas of the cell migrations were manually measured from the images and calculated using the formula of a circle ($radius_1 \times radius_2 \times \pi$).

Conclusion

Quantitative

- There was a significant difference in the average of the mean area between the Cal-27 (HPV-) cells and SCC090 (HPV+) cells, with the Cal-27 (HPV-) cells migrating farther in the agarose matrix when compared to the SCC090 (HPV+) cells.
- Similarly, in previous experiments, the Cal-27 (HPV-) cell growth and motility were also greater than the SCC090 (HPV+) cell growth and motility.

Qualitative

- There was no observable difference in the expression patterns of the three sodium channel isoforms Nav1.5, Nav1.6, and Nav1.7 when comparing cell types Cal-27 (HPV-) to SCC090 (HPV+).
- However, there was an observable difference in the expression patterns when comparing isoforms. Nav1.6 had the most distinct expression pattern when compared to Nav1.5 and Nav1.7.

Future Directions

Experiments to support this qualitative data with quantitative data:

- A reverse transcription quantitative polymerase chain reaction (RT-qPCR) is a technique used to determine the number of messenger RNA in a sample. Messenger RNA are significantly involved in protein synthesis.
- A western blot identifies the specific number of proteins in a sample.

This research project was supported through the LSU Health Sciences Center, School of Graduate Studies.