**In Vitro Characterization of Oral Squamous Cell Carcinomas with Cell Migration**

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**Introduction**

As the 6th most common cancer, there are extremely high incidences of oral and pharynx cancer in Louisiana. Risk factors - tobacco use, excessive alcohol use, sun exposure, oral HPV, and poor nutrition - are omnipresent. In fact, oral and pharynx cancers have a reoccurrence rate of ~76% after two years. Metastasis is the development of secondary tumors in a separate location from the original tumor, but the origins and parameters of metastasis are poorly understood. Metastasis is the primary cause of death in most cases. The epithelial-mesenchymal transition (EMT) process has a major role in metastasis. EMT is the conversion of epithelial cells into mobile cells that can invade, resist stress, and disseminate. Cell motility and migration assays are a hallmark of the way metastasis is studied via cell culture. Cell signaling events cause various molecules to affect the rate of cell migration. For example, tumor necrosis factor-alpha (TNFα) is a cytokine produced during acute inflammation that participates in a cell signaling cascade that leads to necrosis or apoptosis. TNFα aids in cancer resistance and infections. Similarly, nerve growth factor beta is a protein that stimulates nerve growth and the differentiation of sympathetic and sensory neurons. Studies have shown that tumors may grow towards neurons/havers, so NGF-β is added to the cell medium to create a microenvironment mimicking an area with high nerve activity to determine if oral squamous cell carcinomas react. Tumor microenvironment, which includes cytokines TNFα and NGF-β, can influence cancerous development. Previous studies show the correlation between both inflammation and perineural invasion to cancer severity. In this experiment, a Wound Healing Assay is used as the primary technique to determine whether an inflammatory cytokine, TNFα, or neural signaling molecule, NGF-β, will affect the rate of motility in oral squamous cells. CAL-27, I hypothesized that the cells treated with TNFα will have a greater rate of motility than the cells without treatment, and I hypothesized that the cells treated with NGF-β will have a greater rate of motility than the cells without treatment.

**Background**

- Studies show that oropharynx tumors are linked to HPV infections, so tumors and cells are classified by HPV status. The HPV+ have a better prognosis. We thawed CAL-27, an HPV negative cell line, and UPC- SCC090, an HPV positive cell line.
- Inflammation is the immune response that cells emit after injury, stimulus, foreign substance, etc. The cells release cytokines and macrophages to mediate the current situation and prevent further damage.
- Epithelial-mesenchymal transition (EMT)
- TNF-α - a cytokine that is produced during acute inflammation that participates in the cell signaling cascade that leads to necrosis or apoptosis.
- NGF-β - a protein that stimulates nerve growth and the differentiation of sympathetic and sensory neurons.

**Model System**

**In Vitro Cell Culture**

CAL 27 - Epithelial squamous cell carcinoma, extracted from the middle of tongue, HPV negative

- 14.14ng/mL TNF-α – the concentration was previously used in various experiments
- 2ng/mL NGF-β – the concentration was taken from a published dose response curve

**Experimental Design**

- **Motility Rate of TNF-α treated CAL-27 cells Over Time Intervals**
- **Motility Rate of TNF-α treated CAL-27 cells Over 24hrs**

**Results**

- **Figure 1:** This graph shows the TNF-α-mediated motility rate of CAL-27 over multiple time intervals. There is no significance for the standard average of the mean in the motility rate between 0-6 hours or 6-18 hours. However, there is a trend in the 6-18 hour time point, suggesting more experimentation is needed for confirmation.

**Conclusion & Future Experiments**

- The data suggests that inflammatory mediators modulate the in vitro motility rate of oral cancer squamous cell lines. This correlates with previous research because nicotine, an inflammatory agent, is a very prevalent ingredient in tobacco products. Tobacco products are a major risk factor oropharynx tumors.
- At this concentration of NGF-β, there was no effect on motility over 24-hour period.
- Immunofluorescence for the receptors of NGF-β and Voltage-Gated Sodium Channels (VGSC)
- Wound Healing Assays with with different cytokines and immune molecules
- Concentration curve experiments to further characterize the effect of NGF-β on the cells

This research project was supported by Award Number: DBI-2051440 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program