In Vitro Characterization of Oral Squamous Cell Carcinomas with Cell Migration

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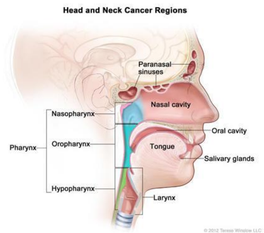
Introduction

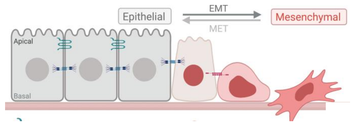
As the 6th most common cancer, there are extremely high incidences of oral and pharynx cancer in Louisiana. Risk factors - tobacco use, excessive alcohol use, sun exposure, oral HPV, and poor nutrition – are omnipresent. In fact, oral and pharynx cancers have a reoccurrence rate of 76% after two years. 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/nerves, so NGF-ß 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-ß, 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-ß, 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-ß will have a greater rate of motility than the cells without treatment.

Background

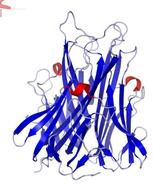
- Studies show that oropharynx tumors are linked to HPV infections, so tumors and cells are classified by HPV status. The HPV+ have a better prognosis. We thawed CAL 27, an HPV negative cell line, and UPCI: SCC090, an HPV positive cell line.



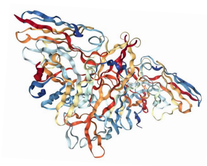
- Inflammation is the immune response that cells emit after injury, stimulus, foreign substance, etc. The cells release cytokines and macrophages to mediate the current situation and prevent further damage.
- Epithelial-mesenchymal transition (EMT)



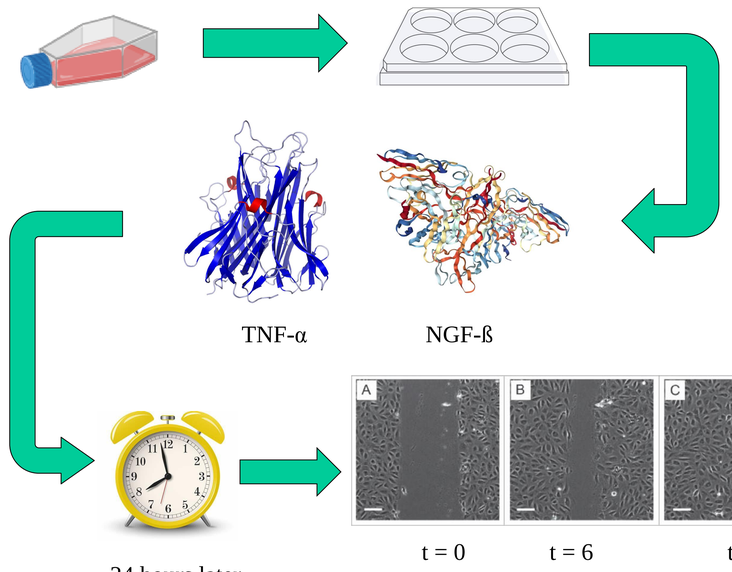
- TNF-α - a cytokine that is produced during acute inflammation that participates in the cell signaling cascade that leads to necrosis or apoptosis.



- NGF-ß - a protein that stimulates nerve growth and the differentiation of sympathetic and sensory neurons.



Experimental Design



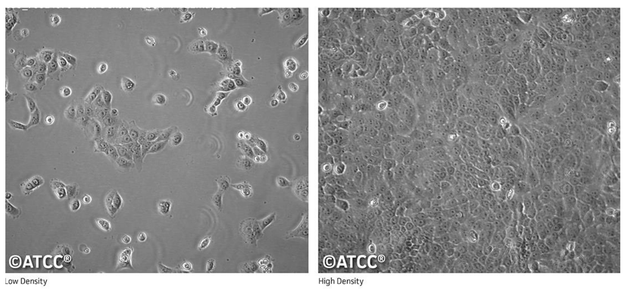
$$R_M = \frac{w_I - W_i}{t}$$

R_m = rate of cell migration (µm/hr)
W_i = initial wound width (µm)
W_f = final wound length (µm/hr)
t = duration of migration (hr)

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-ß – the concentration was taken from a published dose response curve

Results

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

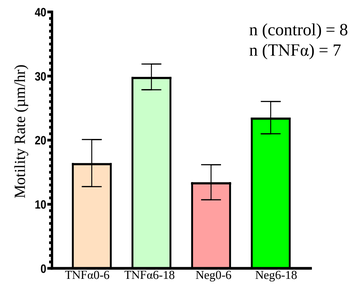


Figure 1: This graph shows the TNF-α-mediated motility rate of CAL-27 over multiple time intervals. There is no significance for the standard average of the mean in the motility rate between 0-6 hours or 6-18 hours. However, there is a trend in the 6-18 hour time point, suggesting more experimentation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

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

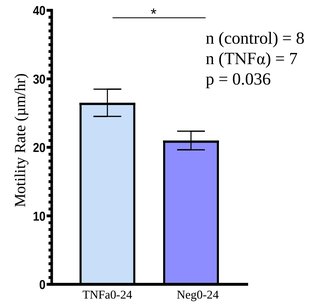


Figure 2: This graph shows the TNF-α-mediated motility rate of CAL-27 over 24 hours. There is a significant increase in the motility rate of TNF-α treated CAL-27 cells over 24 hours.

Motility Rate of NGF-ß treated CAL-27 cells Over Various Time Intervals

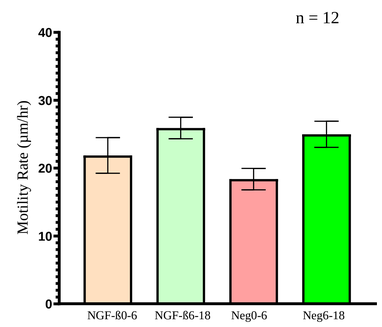


Figure 3. This graph shows the NGF-ß-mediated motility rate of CAL-27 over multiple time intervals. There is no significance for the standard average of the mean in the motility rate between 0-6 hours or 6-18 hours.

Motility Rate of NGF-ß treated CAL-27 cells Over 24hrs

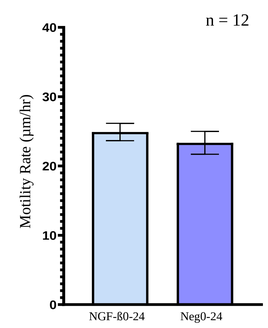


Figure 4. This graph shows the NGF-ß-mediated motility rate of CAL-27 over multiple time intervals. There is no significance for the standard average of the mean in the motility rate over 24 hours.

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-ß, there was no effect on motility over 24-hour period.
- Immunofluorescence for the receptors of NGF-ß and Voltage-Gated Sodium Channels (VGSC)
- Wound Healing Assays withs with different cytokines and immune molecules
- Concentration curve experiments to further characterize the effect of NGF-ß on the cells

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