IL-8 Promotes Cancer Cell Proliferation and Migration in Cutaneous Squamous Cell Carcinoma

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Background

Cutaneous squamous cell carcinoma (cSCC) is one of the most common cancers worldwide and has the highest disease incidence in the head and neck. Currently, surgery is the mainstay of therapy with the addition of adjuvant radiation and/or immunotherapy for aggressive disease. In recent years, there is increasing interest to develop targeted therapies capable of halting cSCC tumor progression or even trigger cancer regression. A potential area to target is the tumor microenvironment, which comprise of a rich milieu of cancer-associated support cells and signaling molecules that promote cancer survival and progression.

Methods

An established human cSCC (A431) was cultured. Culture media was harvested on day 2 and a cytokine array was performed. One highly expressed cytokine (IL-8) was chosen for further investigation. To study cell proliferation, A431 cells were plated on a 96-well plate and 3 different concentrations of IL-8 were added. CellTiter-Glo assays were used to assess cell viability at hours 24 and 48. Two migration assays were performed. For the scratch migration assay, A431 cells were placed on a 24-well plate and allowed to grow to confluency. A scratcher comb was used to make uniform gaps in the cell monolayer. 3 concentrations of IL-8 were added to the wells, and the plates were incubated in a live-imaging microscope and imaged every 12 hours for 48 hours. For the transwell migration assay, a monolayer of cells was added to transwell inserts, which were fully submerged in media with or without IL-8. Cells were allowed to migrate for 12, 24, or 48 hours, stained with DAPI, and cells were counted using fluorescence microscopy.

Conclusion

- cSCC secrete high levels of IL-8 into the tumor microenvironment
- IL-8 promotes cancer cell chemotaxis, migration, and proliferation
- Further investigation into IL-8 and the tumor microenvironment may provide insight into cSCC progression and reveal novel therapeutic targets

Results

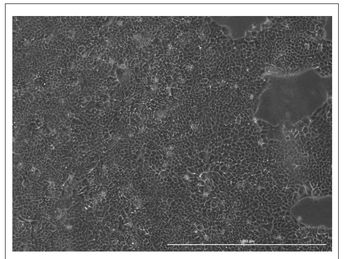

Figure 1. Phase Contrast Microscopy of Cell Line Representative image of human cutaneous squamous cell carcinoma cells (cSCC-A431) at 4x magnification.

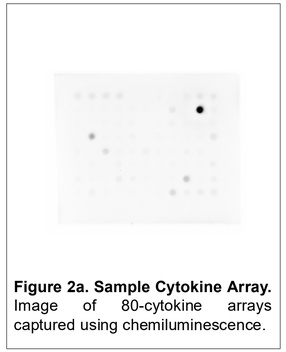

Figure 2a. Sample Cytokine Array. Image of 80-cytokine arrays captured using chemiluminescence.

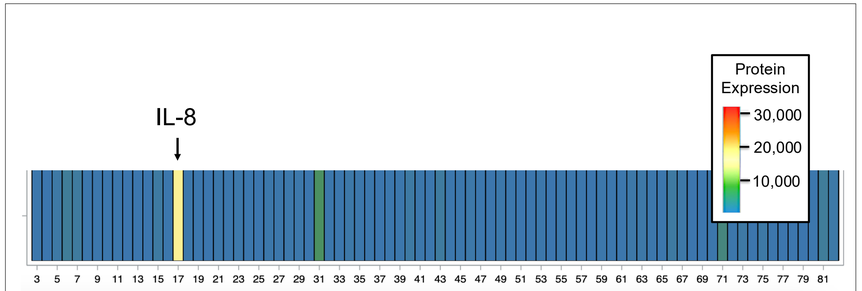

Figure 2b. Cytokine Expression of A431 cells. cSCC-A431 cells secreted several cytokines into the conditioned media. The highest expressing cytokine was interleukin-8 (IL-8).

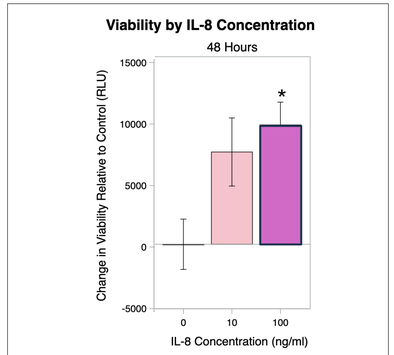

Figure 3. IL-8 promotes cell proliferation. Addition of 100 ng/ml of IL-8 significantly increased cSCC-A431 cell proliferation at 48 hours.

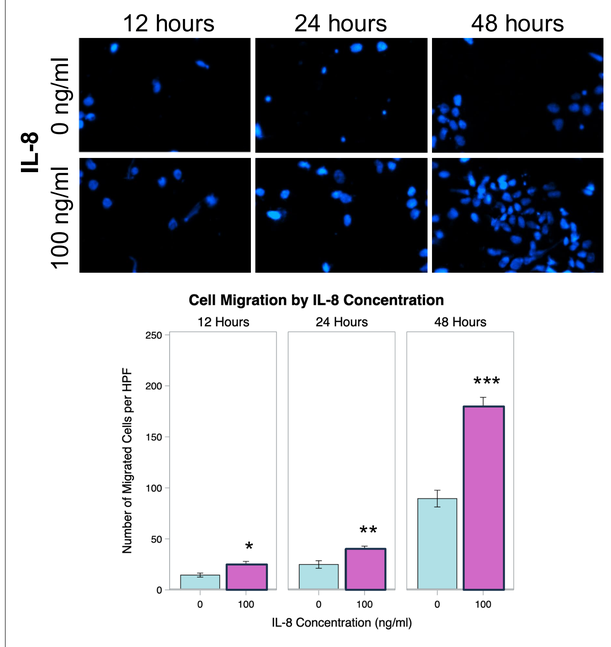

Figure 3. A431 Cell Migration by IL-8 Concentration. *Left* Represenatitive images of transwell migration assay. Compared to the negative control, there was more cancer cell migration in the IL-8 group at all 3 timepoints.

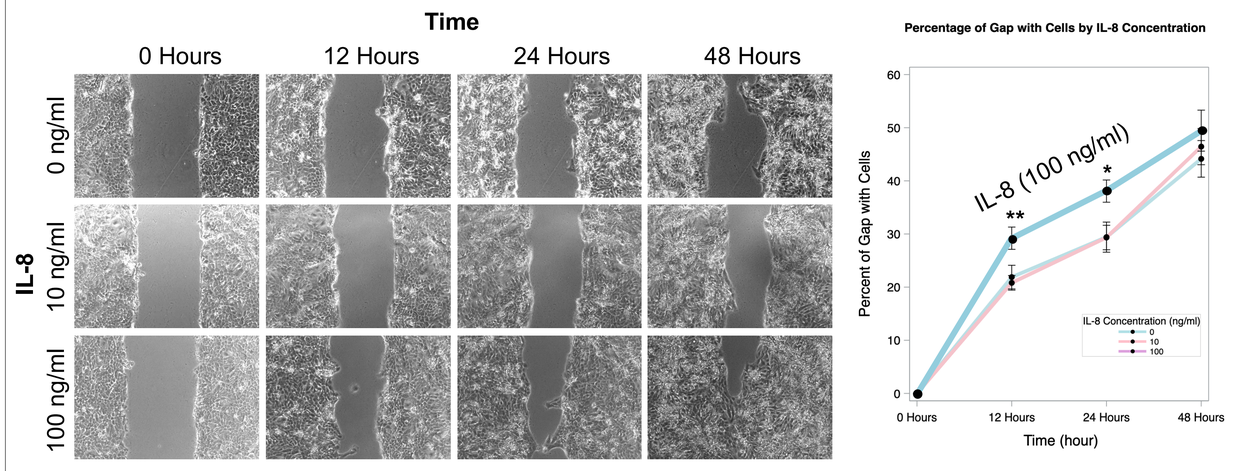

Figure 4. Scratch Migration Assay of A431 With and Without Addition of IL-8. (*Left)* Representative images of scratch migration assay of cells treated with 0, 10, and 100 ng/ml of recombinant IL-8 imaged at 0, 12, 24, and 48 hours. The gaps closed significantly more at all timepoints in the 100 ng/ml group compared to the negative control. (*Right)* Cells treated with 100 ng/ml of IL-8 had significantly faster gap closure at 12 and 24 hours compared to other groups, indicating IL-8 promotes cancer cell migration.

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