3111: Investigation of the Tumor-Initiating Cell Microenvironmental Niche in Recurrent Head and Neck Squamous Cell Carcinoma Using a Novel Microenvironment Microarray Platform

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Objective

•To determine which microenvironmental proteins promote recurrence after radiation in head and neck squamous cell carcinoma (HNSCC).

•To determine if there are differences between primary and lymph node microenvironments.

Background

•Recurrent HNSCC carries a dismal prognosis, similar to that of distant metastatic disease, of 10.1 months with first line chemotherapy.

•Salvage therapies including re-irradiation are associated with significant morbidity.

•The underlying mechanisms driving recurrent disease and associated treatment resistance are unknown.

•One proposed mechanism is the persistence of CD44-expressing treatment resistant “tumor initiating cells” or TICs.

•Targeting of both TICs and microenvironmental proteins has been associated with improved chemo- and radio-sensitivity in pre-clinical models.

Hypothesis

Surgery, chemotherapy and radiation lead to reduction in tumor mass but may not be effective in killing primitive TICs that are responsible for recurrence.

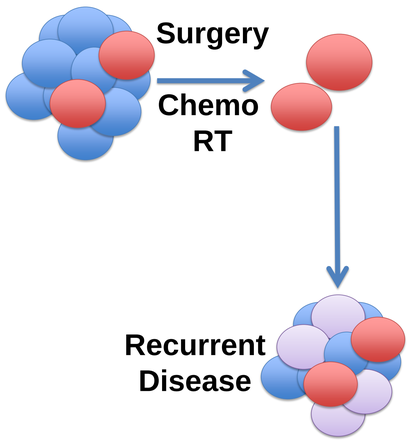

Figure 1. Definitive HNSCC therapies lead to a fibrotic microenvirnoment that may be a “niche” for regrowth of TICs.

We hypothesize that specific interactions between CD44+ TICs and the recurrent tumor microenvironment provide a nurturing bed for HNSCC re-growth.

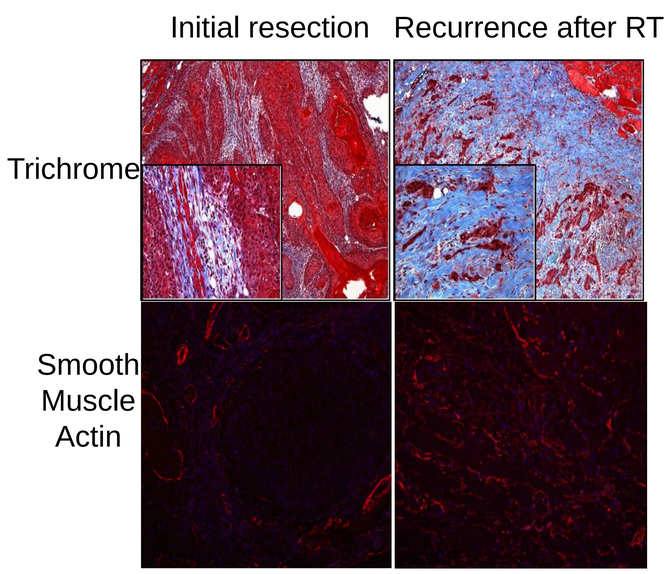

Figure 2. Example images of trichrome and smooth muscle actin immunostains from an initial oral cavity resection and a recurrence after radiation highlighting increased collagen deposition and cancer-associated fibroblasts.

Microenvironment Microarrays

•Novel screening platform to determine extracellular matrix molecules and growth factors important for a queried phenotype.

•HNSCC cell lines UMSCC-10a and 10b were incubated on micorarrays for 5 days both with and without exposure to radiation (8Gy x 1 dose).

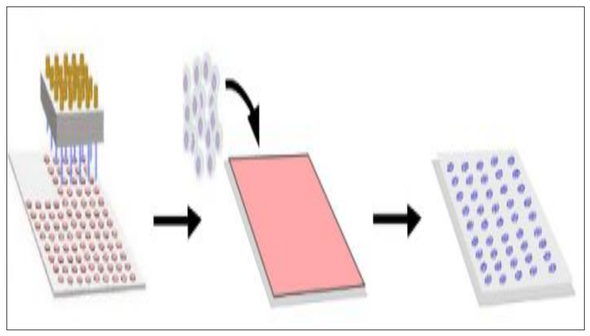

Figure 3. Proteins are spotted in combinatorial format on the array located in a multi-well cell culture dish. Cells are incubated over the array and preferentially bind to certain spots.

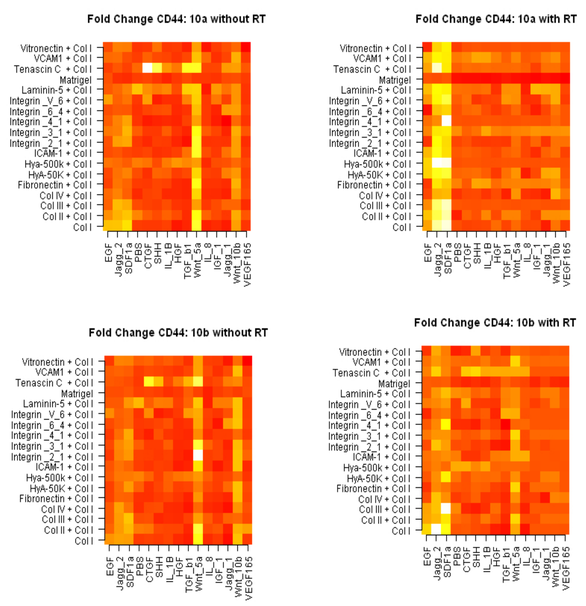

Figure 4. Heat map showing immunofluorescent signal fold change for CD44/cell mask (to normalize for cell number) for cell lines 10a (larynx primary) and 10b (metachronous LN met).

Conclusions

•Wnt 5a and Wnt 10b are associated with increased relative CD44 expression in primary and LN lines.

•Jagged2 and SDF1a are associated with increased relative CD44 expression after exposure to radiation.

Validation

•Targets showing increased CD44 expression are currently being validated for various endpoints using IncuCyte in vivo imaging.

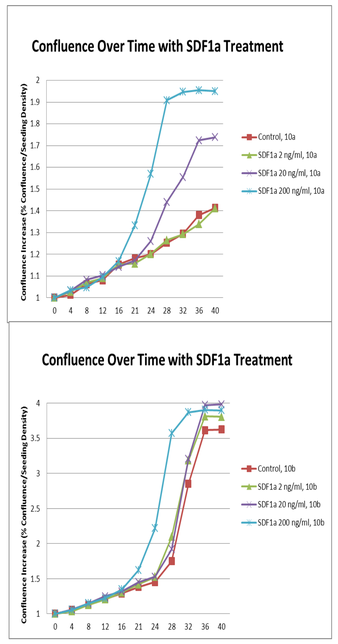

Figure 5. Confluence change over time for cell lines 10a and 10b with SDF1a treatment. Primary line 10a shows dramatic dose-dependent proliferation.

Future Directions

•Clonogenic survival assays and CD44 flow cytometry after incubation with growth factors of interest: SDF1a, Jagged2, Wnt5a, Wnt10b

•Inhibitor assays

•In vivo validation using xenograft models

•Validation in human HNSCC tissue arrays

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