Cancer Stem Cell Specific MicroRNA Aberrations in a Model of Recurrent Squamous Cell Carcinoma: Implications for Targeted Radiosensitization

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Disclosure

I have no conflicts of interest to disclose
Cancer Stem Cells in Recurrence and Metastasis

Cancer Stem Cells (CSC):

- Rare subpopulation
- Pluripotent
- Generate tumor heterogeneity
- Characteristics of normal stem cells
- Predicted roles in metastasis, and tumor recurrence

http://sarcomahelp.org/research_center/MPNST_progression.html
Cancer Stem Cells are Radioresistant

Complete treatment response
Tumor recurrence or metastasis

CSC targeted radiosensitizing agents

Complete treatment response
Keratin 15 expression is restricted to the hair follicle bulge.

The hair follicle bulge contains a subset of epithelial stem cells.
Mouse Model of Squamous Cell Carcinoma from Stem Cell Targeted Mutations

K15

K15.CrePR1

Cre ΔPR

Kras$^{G12D}$

G12D

E1

stop

loxP

loxP

Smad4f/f

E8

loxP

loxP

RU486
Identification of Two Distinct CSC Populations from Stem Cell Derived SCC

SP$^+$/CD34$^-$/CD49f$^-$

SP$^-$

CD34$^+$/CD49f$^+$

SP$^+$/CD34$^+$/CD49f$^+$

SP$^+$/CD34$^-$/CD49f$^-$

Hoechst Blue

Hoechst Red

1x10^4 SP

1x10^4 non-SP/CD34$^-$/CD49f$^-$

1x10^4 CD34$^+$/CD49f$^+$

3 Weeks after grafting
Recurrent Tumors are Less Differentiated and More Metastatic

Metastasis: 20%

Epithelial Predominance

Metastasis: 80%

Mesenchymal Predominance

Well Differentiated

Moderately Differentiated

Poorly Differentiated

SPCC
SP Size is Increased in Metastatic Tumors

- * p = 0.009

**SP size (%)**

- Red: Average SP size
- Blue: Individual tumor SP size

**CD34+/CD49f+ population size (%)**

- Green: Average CD34+/CD49f+ size
- Purple: Individual tumor CD34+/CD49f+ size

Metastasis
Differentially Expressed miRNAs in Cancer Stem Cells

- Primary SP
- Passage 1 SP
- Primary CD34+/CD49f+
- Passage 1 CD34+/CD49f+

miRNAs:
- mmu-miR-155
- mmu-miR-9
- mmu-miR-132
- mmu-miR-214
- mmu-miR-199a-3p
- mmu-miR-155
- mmu-miR-214
- mmu-miR-574-3p

Expression levels range from -3.00 to 3.00.
Loss of miR-9 Inhibits Metastasis

B931-GFP
Control

B931-Zip9
miR-9 Knockdown

# Metastatic Lesions

* P = 0.04
MicroRNAs Regulate Behavior of Distinct CSC Populations and are Potential Radiosensitizing Targets

• The Side Population and the CD34+/CD49f+ CSC populations are distinct and have unique microRNA expression fingerprints
• The SP may play a role in mediating metastasis of recurrent tumors
• MicroRNA-9 regulates metastasis in this population
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Identification of miRNAs Overexpressed in CSC Populations from Metastatic Tumors
miR-9 is a Marker of SCC Lymph Node Metastasis

<table>
<thead>
<tr>
<th>miR-9 Positive</th>
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<tbody>
<tr>
<td>Normal Head &amp; Neck Tissue (n=25)</td>
<td>0%</td>
</tr>
<tr>
<td>Head &amp; Neck SCC (n=67)</td>
<td>21%</td>
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<tr>
<td>SCC Lymph Node Metastasis (n=72)</td>
<td>78%</td>
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miR-9 Expression in LN Metastasis