“Tumor Initiating Cells in Human Cutaneous Squamous Cell Carcinoma”

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Introduction

Human cutaneous squamous cell carcinomas (SCC) exhibit a heterogeneous morphology with a developmental hierarchy of proliferating and differentiating cells may be maintained by a distinct population of cancer stem cells or tumor initiating cells (TIC).

In vitro tissue culture assays and in vivo animal models that can accurately recapitulate the human cancer were developed to identify and characterize TIC.

Demonstrate that a small subset of human SCC cells (~1%) expressing a prominin-1 (CD133) epitope are highly enriched for TIC in human SCC.
Squamous cell carcinomas and basal cell carcinomas represent more than $10^6$ cases per year, about 25% SCC

Etiology due to DNA damage secondary to sun and environmental exposure

High incidence of SCC metastasis in transplanted and immunocompromised patients

Proliferating dysplastic keratinocytes invade locally as a mass with finger-like tumor projections invading into tissues
SCC continue to differentiate with Ki67+ proliferating cells located at the periphery of SCC tumor projections.

Normal Skin: K5 & Involucrin

Squamous cell carcinoma Ki67+, K14 and Involucrin
Isolation and characterization of tumor initiating cells in SCC

I. Isolation of single cell suspension
II. Separation of cells based on cell surface markers
III. Assess for growth/tumor formation

In vitro assay

In vivo tumor initiation assay
Human SCC form spheroid tumor cell colonies in culture

Bulk Human Squamous Cell Carcinoma Cell Suspension

Tissue Culture Plate with Irradiated 3T3 feeder layer

Normal Human Keratinocytes

Human Squamous Cell Carcinoma
SCC spheroid colonies could be serially passaged without increase or decrease in colony numbers noted.

Whole plate trypsinized and passaged

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<thead>
<tr>
<th>Input</th>
<th># 1° colonies</th>
<th># 2° colonies</th>
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<tr>
<td>$10^5$</td>
<td>49</td>
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Cloning cylinders used to trypsinize and passage individual spheres.
Successful xenografts of human SCC cell suspensions required extensive “humanization” of the graft site.

Establishing this in vivo assay required 140 separate human SCC samples and 155 individual mouse xenografts over a 3 year period.
Xenograft SCC tumor growth rate and histology was similar to original SCC tumor

**Tumor Xenograft Growth**

- **SCC1** – Poorly Differentiated
- **SCC2** – Moderately Differentiated
- **SCC3** – Well Differentiated

**Xenograft Tumor**

- SCC1
- SCC2
- SCC3

**Original Tumor**

- SCC1
- SCC2
- SCC3
Isolation and characterization of tumor initiating cells in SCC

1. **Human SCC**
   - Isolation of single cell suspension

2. **Separation of cells based on cell surface markers**

3. **Assess for growth/tumor formation**
   - *In vitro* assay
   - *In vivo* tumor initiation assay
Potential Cell Surface Markers for Tumor Initiating Cells

1. Previously, panels of cell surface markers were identified for human keratinocyte stem cells in hair follicles (CD200^{hi}24^{lo}34^{lo}71^{lo}146^{lo})

2. The CD44 and CD24 cell surface markers have been used to isolate tumor initiating cells from fresh human cancer specimens, including breast, head and neck, and pancreas. The glycosylated CD133 cell surface marker has also identified tumor initiating cells in primary human cancer, including brain (medulloblastomas and glioblastomas), colon, and pancreas.
CD133 was expressed on scattered cell clusters in the proliferating layer of the human SCC tumor projections.
**CD133+ cells represent a rare subset of human SCC cells**

Mean 0.81% +/- 0.86% n=31
Summary of Cell Surface Markers in SCC

Proliferating SCC periphery
CD71-hi (CD24- and CD146-)

Differentiating inner SCC
CD24-hi and CD146-hi (CD71-)

CD133-Hi

CD200+ cells are not present in SCC and CD44+ cells were CD45+
Isolation and characterization of tumor initiating cells in SCC

I. Isolation of single cell suspension

II. Separation of cells based on certain characteristics

III. Assess for growth/tumor formation

*In vitro assay*  *In vivo tumor initiation assay*
CD133+ cells isolated from SCC are enriched for spheroid colony formation.
Isolation and characterization of tumor initiating cells in SCC

I. Human SCC
   1. Isolation of single cell suspension
   2. Separation of cells based on certain characteristics
   3. Assess for growth/tumor formation

   - In vitro assay
   - In vivo tumor initiation assay
Tumor growth was dependent on the number of unsorted human SCC cells xenotransplanted

82 Total Xenographs into Nude Mice

TIC frequency = 1 / 1,400,000 Total SCC cells
Xenotransplanted CD133+ SCC cells are highly enriched for TIC

42 total xenografts from 28 different human SCC specimens

TIC frequency = 1 / 483 CD133+ cells
The number of implanted CD133+ SCC cells can determine xenograft size (at 12 weeks)
CD133+ SCC can be serially transplanted - demonstrating the stem cell properties of self-renewal and tumor reconstitution.

Primary SCC Xenografts

FACS analysis of CD133+ / CD45- cells in primary SCC xenograft = 0.7% (n=11)

Serial transplants of CD133+ from 1’ xenografts

Secondary SCC Xenografts
Human CD133+ SCC cells are enriched for TIC when serially transplanted into mice

14 total serial xenografts from 8 different human SCC specimens

TIC frequency = 1 / 863
Xenotransplanted CD133+ SCC cells recreates the original tumor morphology
Different SCC histological grades had equivalent rates of tumor formation.
Different SCC histological grades had equivalent percentages of CD133+ cells.
CD133+/CD45- cells were relatively quiescent

CD45-CD133+

CD45-CD133-
Conclusions

A discrete small sub-population (1%) of human CD133+ SCC cells are highly enriched for tumor-initiating cells (TIC) SCC in an in vivo SCC xenograft model.

The CD133+ cells could recapitulate the histology and hierarchy of the original SCC tumor.

The percentages of CD133+ TIC in SCC tumors of different histological grades were equivalent.
Future Considerations

Future studies will focus on how normal skin developmental programs have been altered in SCC by analyzing TIC for:

- Global gene expression profiles
- Genetic and epigenetic changes
- Stromal microenvironment or “niche” influences on TIC behavior

Other SCC tumors, such as the highly aggressive SCC in renal transplant patients also need to be studied.

Enriched TIC also represent potentially valuable targets for therapeutic strategies that can selectively inhibit their growth and self-renewal.
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