“Tumor Initiating Cells in Human Cutaneous Squamous Cell Carcinoma”

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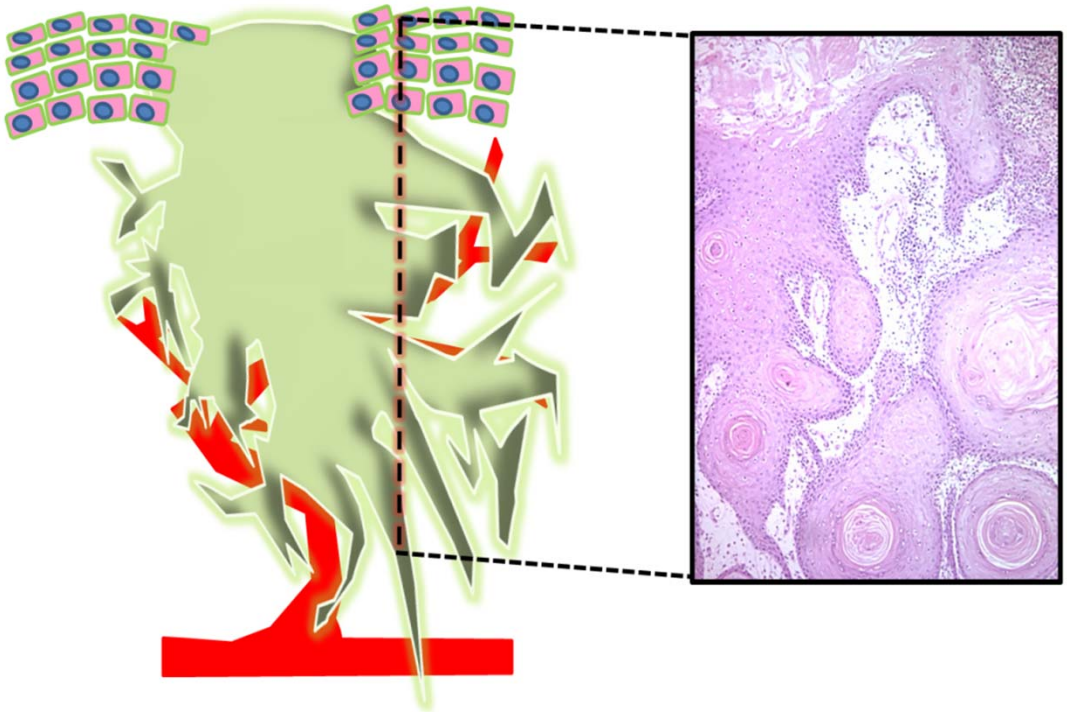


Introduction

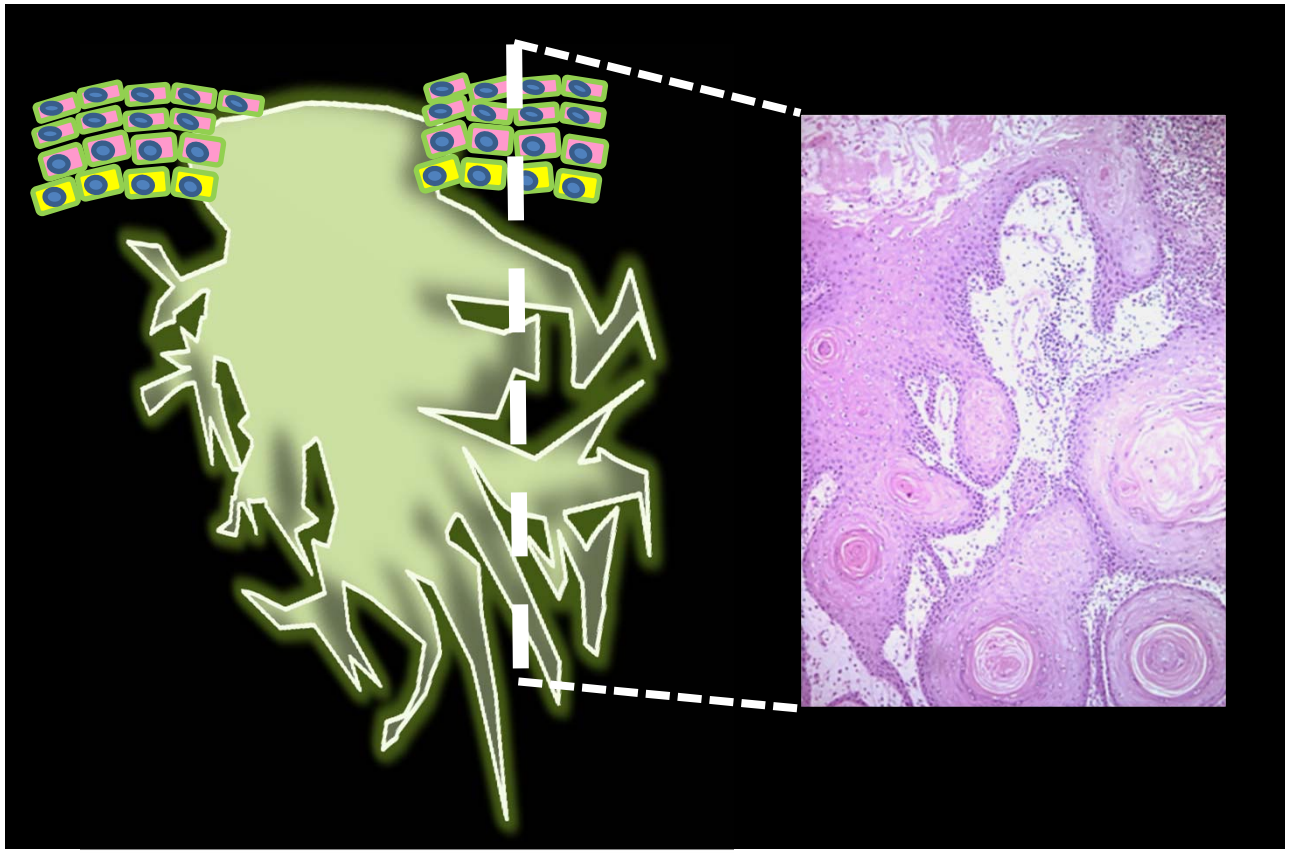
Cancers that exhibit a heterogeneous morphology with a developmental hierarchy of proliferating and differentiating cells may be maintained by a distinct population of cancer stem cells or tumor initiating cells (TIC), that possess stem cell properties of self-renewal and long-term reconstitution of the tumor.

To identify, isolate, and characterize these cells, in vitro tissue culture assays and in vivo animal models that can accurately recapitulate the human cancer are needed.

TIC are valuable targets for understanding alterations in the normal developmental processes that can lead to cancer.

Demonstrate that a small subset of human SCC cells (~1%) expressing a prominin-1 (CD133) epitope are highly enriched for TIC

Human Squamous Cell Carcinoma (SCC)



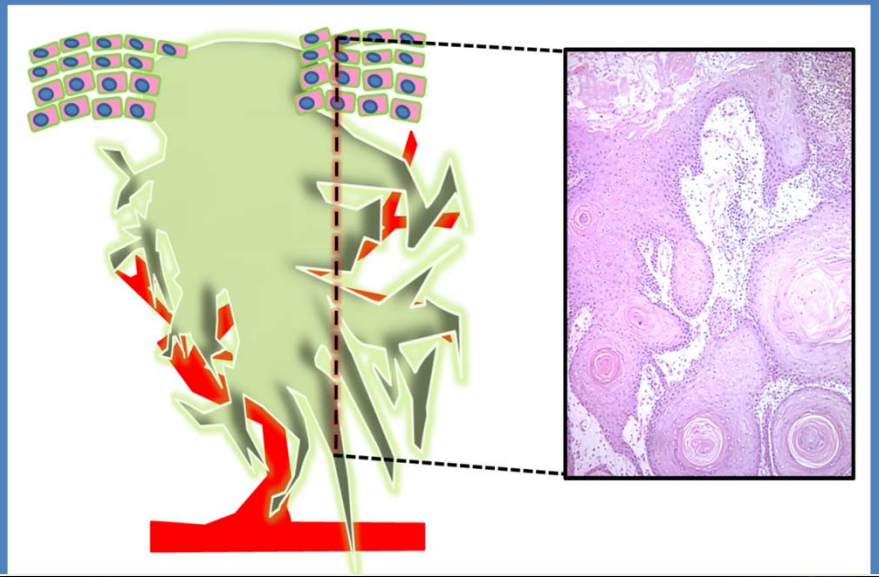
Squamous cell carcinomas and basal cell carcinomas represent more than 10^6 cases per year, about 25% SCC

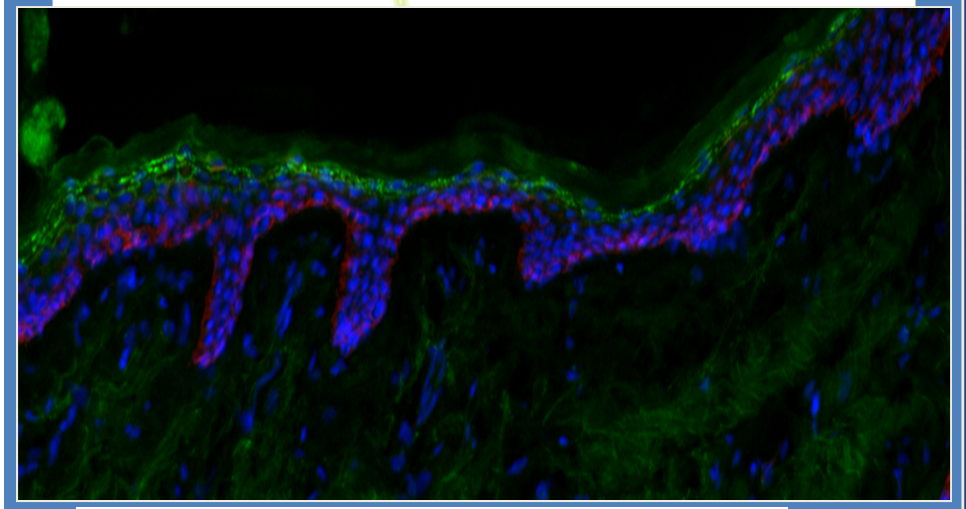
Etiology due to DNA damage secondary to sun and environmental exposure

High incidence of SCC metastasis in transplanted and immunocompromised patients

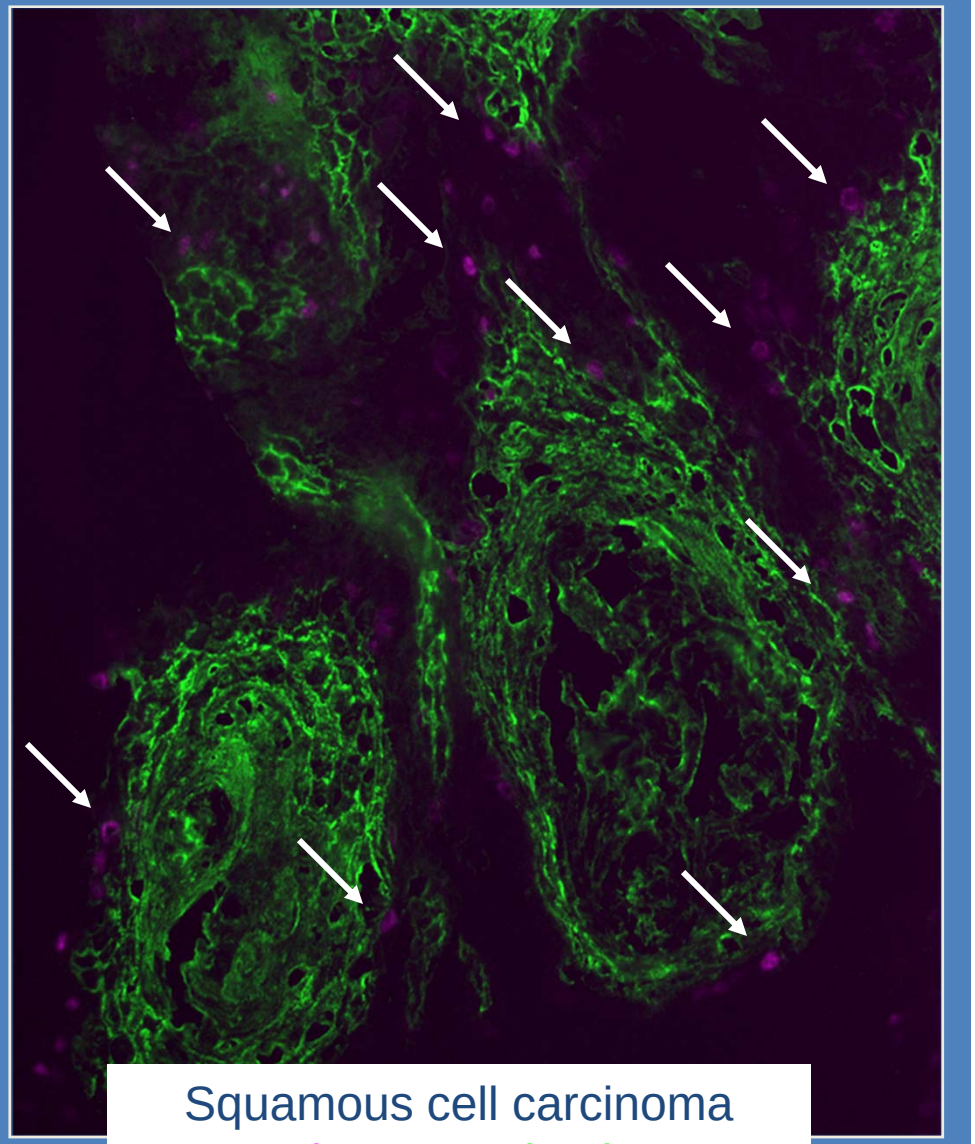
Proliferating dysplastic keratinocytes invade locally as a mass with finger-like tumor projections invading into tissues

SCC continue to differentiate with Ki67+ proliferating cells located at the periphery of SCC tumor projections





Normal Skin: K5 & Involucrin



Squamous cell carcinoma
Ki67 & Involucrin

Isolation and characterization of tumor initiating cells in SCC

Human SCC

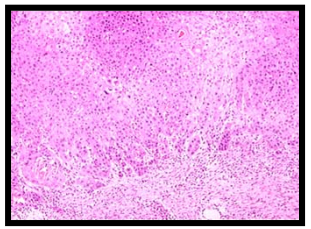
I Isolation of single cell suspension

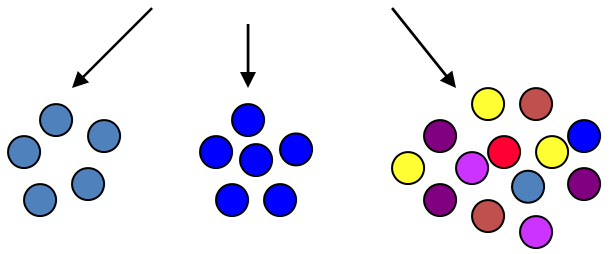
II Separation of cells based on cell surface markers

III Assess for growth/tumor formation

In vitro assay

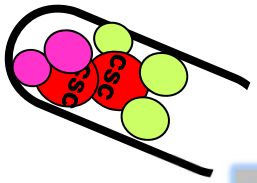
In vivo tumor initiation assay

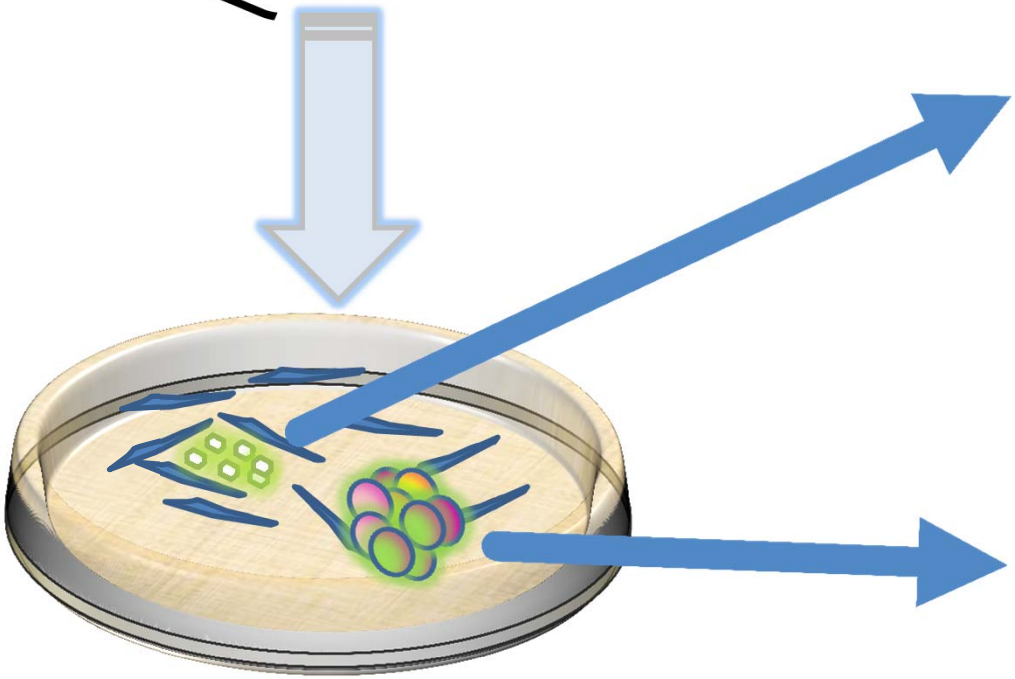




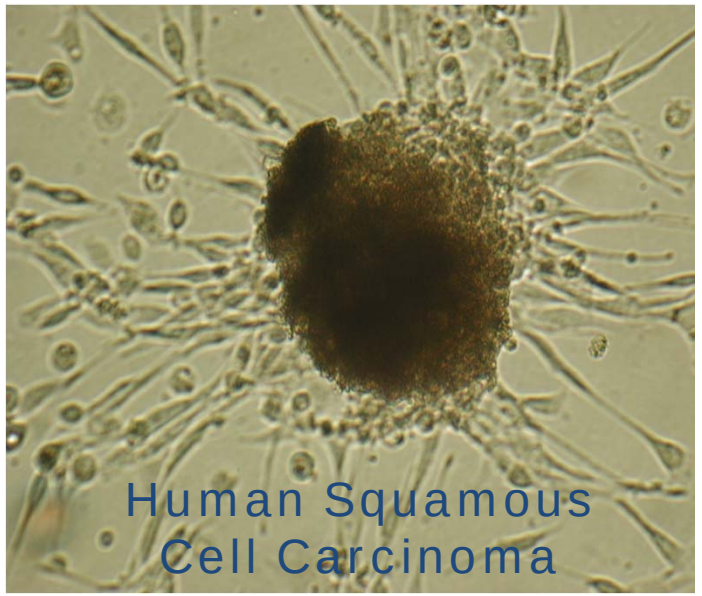
Human SCC form spheroid tumor cell colonies in culture

Bulk Human Squamous Cell Carcinoma Cell Suspension



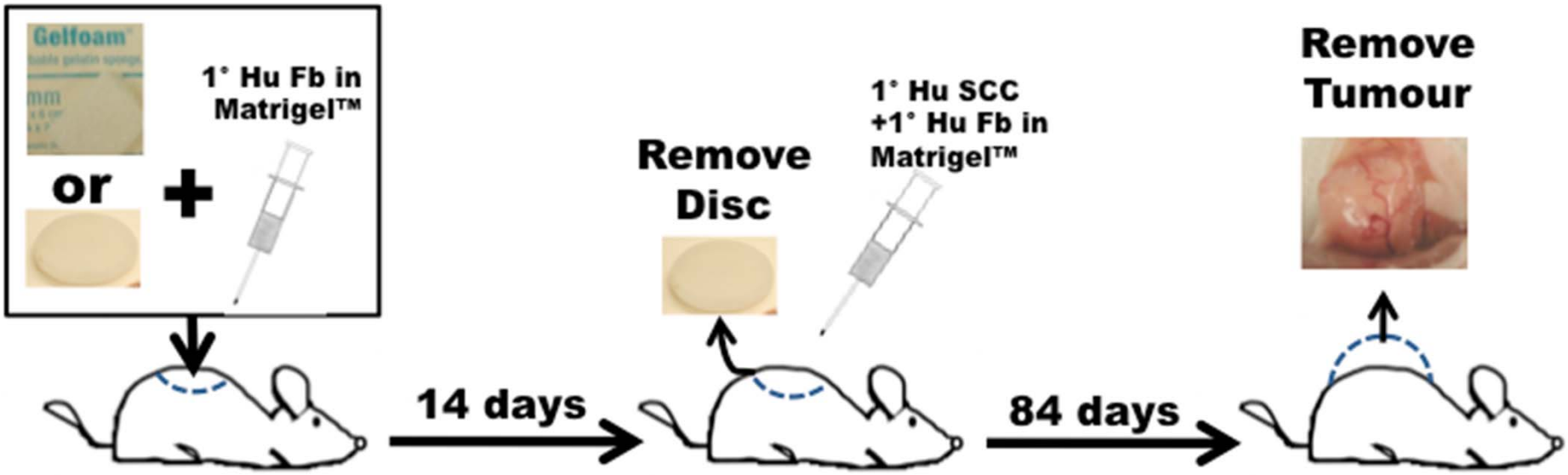


Tissue Culture Plate with Irradiated 3T3 feeder layer



In Vivo Model for Human SCC Initiation

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

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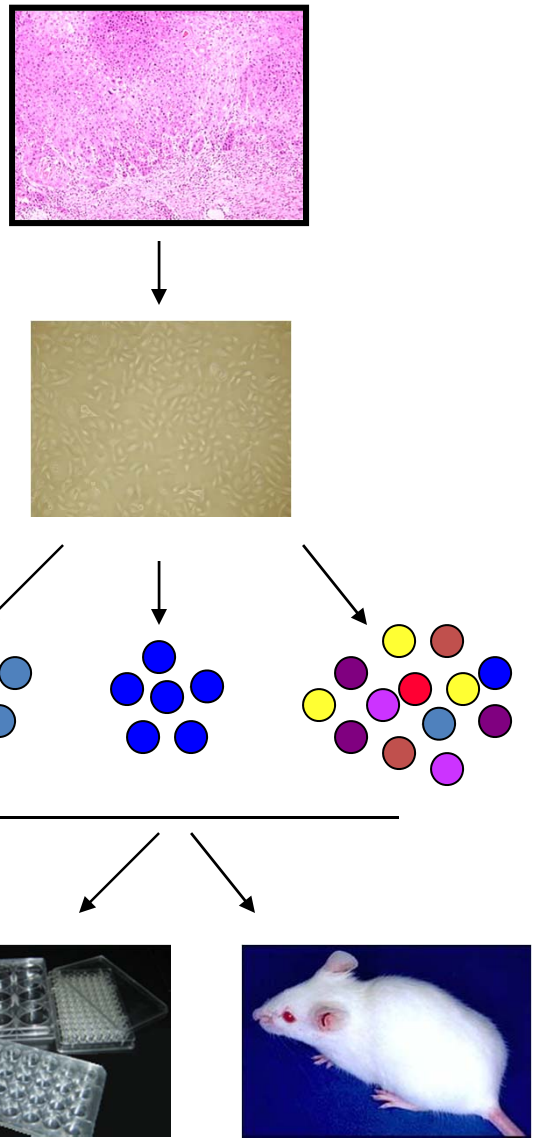
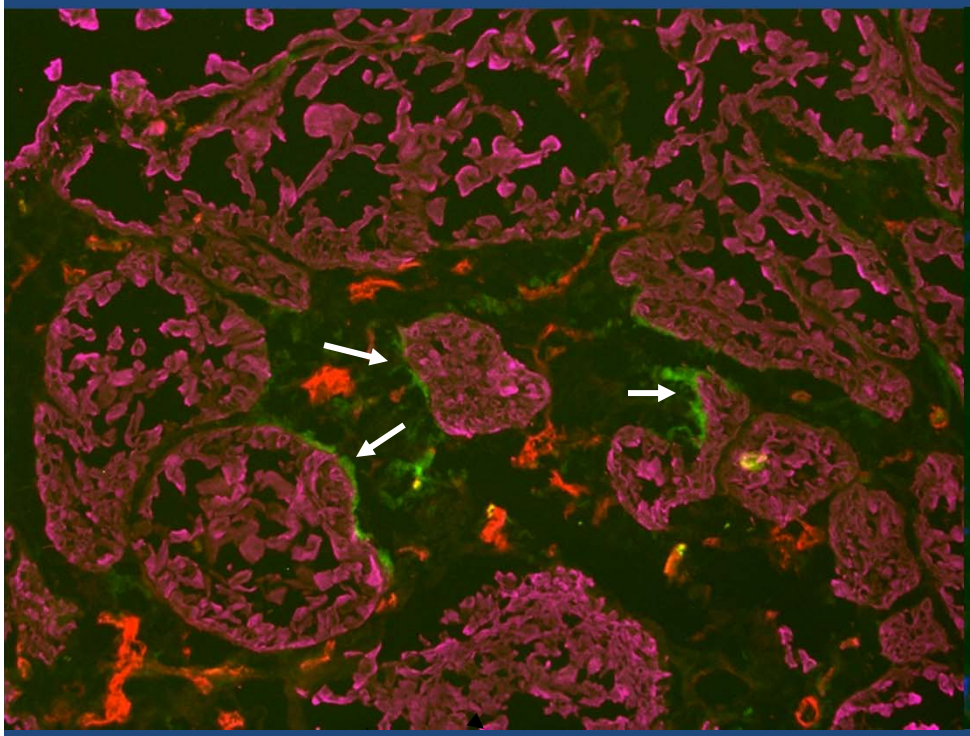
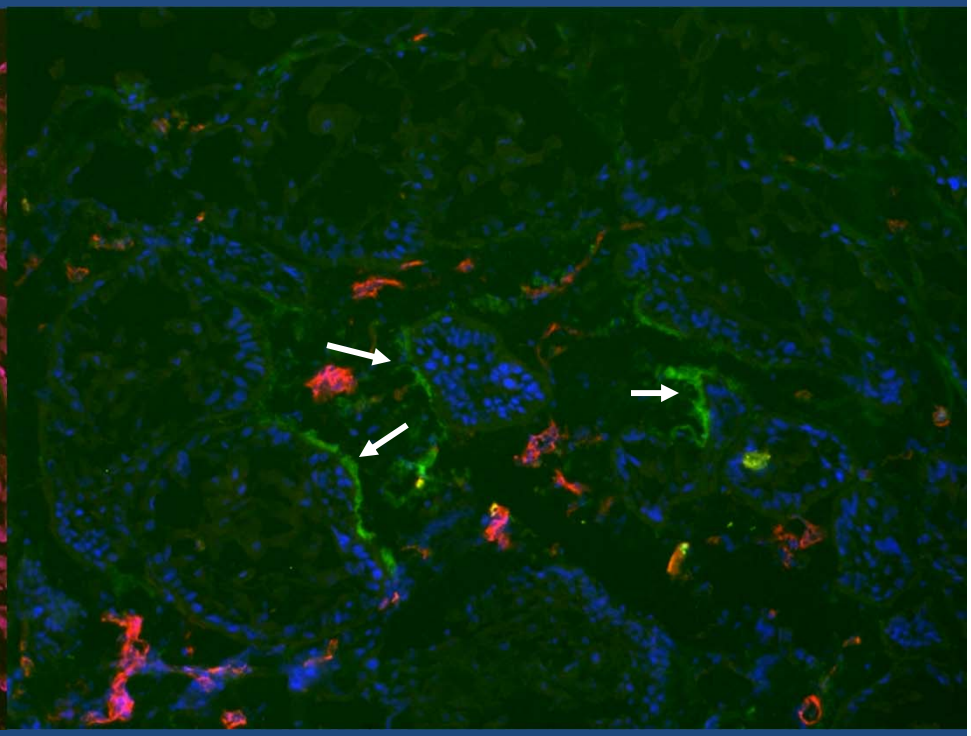


CD133 was expressed on scattered cell clusters in the proliferating layer of the human SCC tumor projections



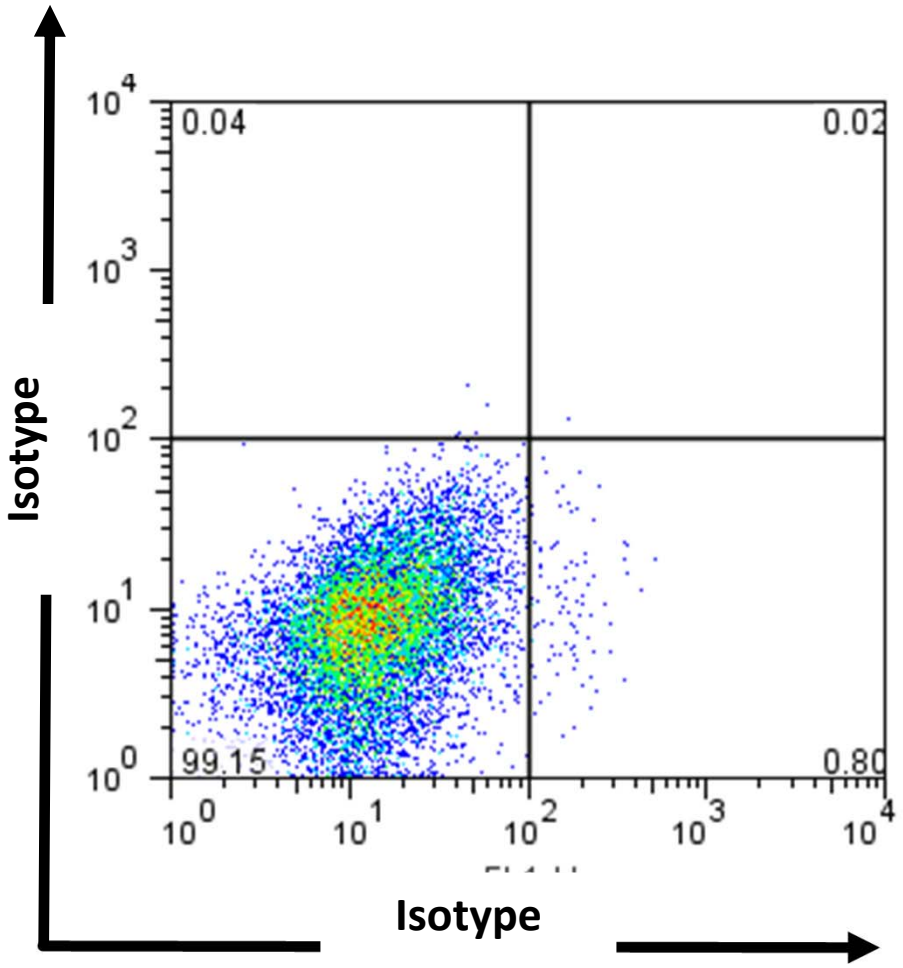
CD31 (pecam)
CD133 (promin1)
Keratin 14

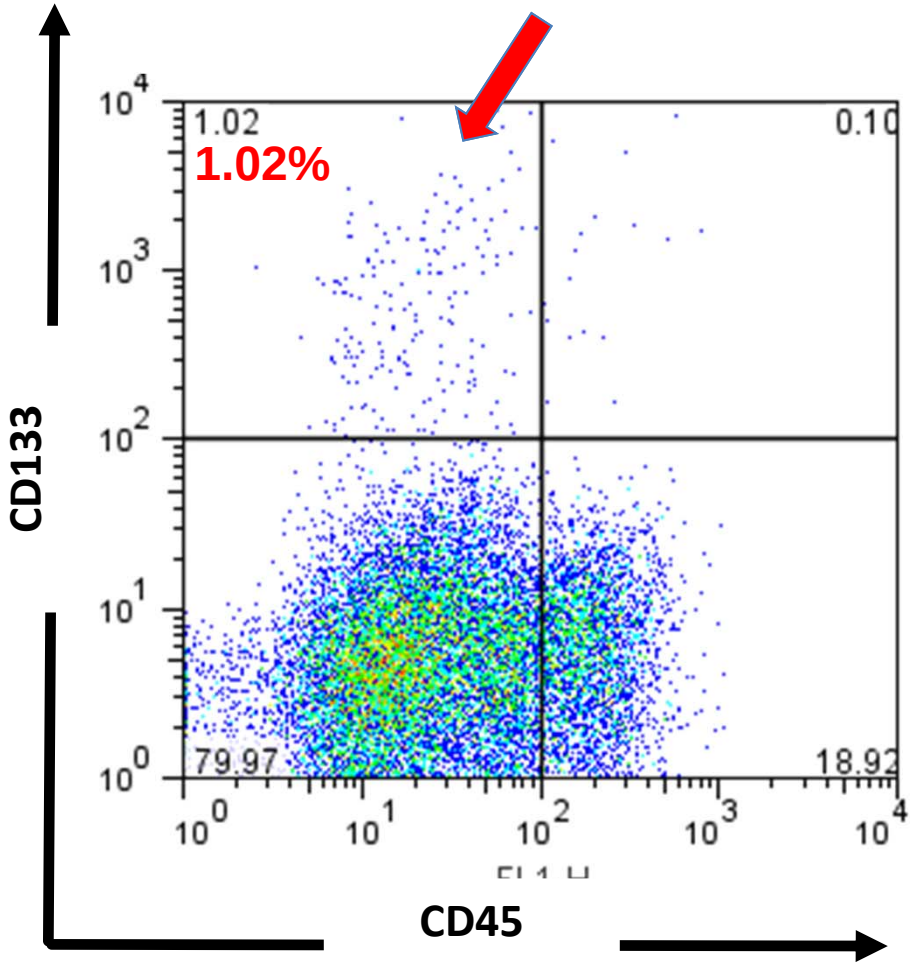


CD31 (pecam)
CD133 (promin1)
Dapi

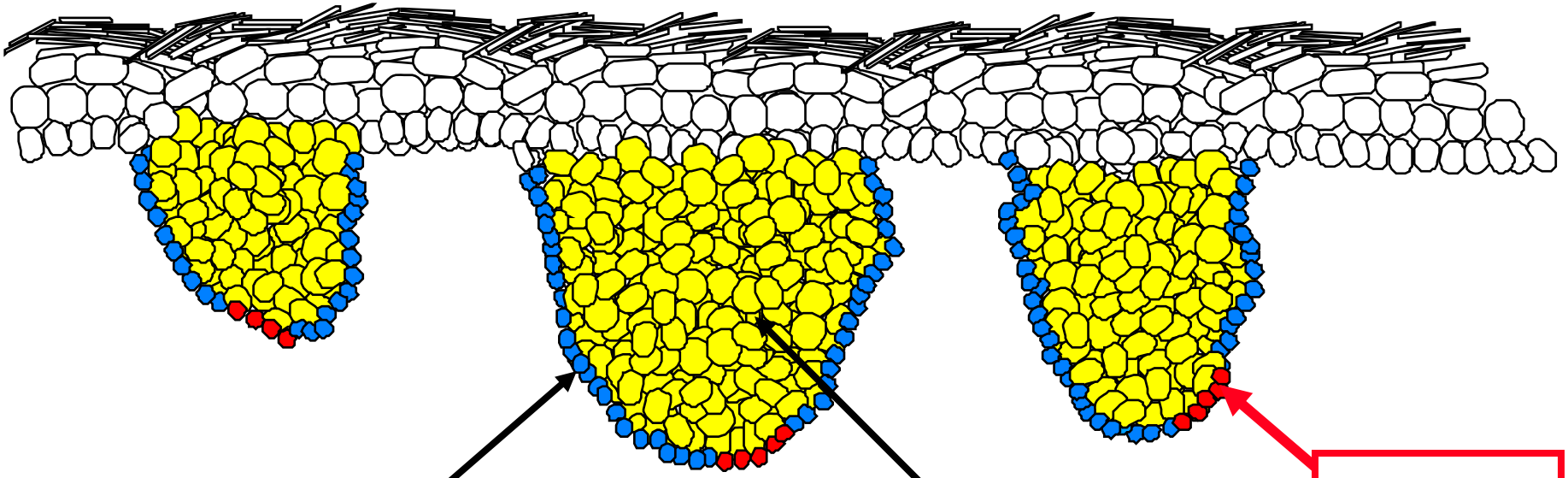
CD133+ cells represent a rare subset of human SCC cells

Mean 0.81% +/-0.86% n=31





Summary of Cell Surface Markers in SCC



CD133-Hi

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

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

CD200+ cells are not present in SCC and CD44+ cells were CD45+

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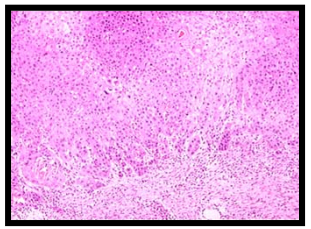
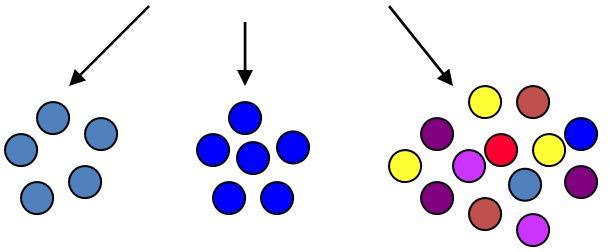
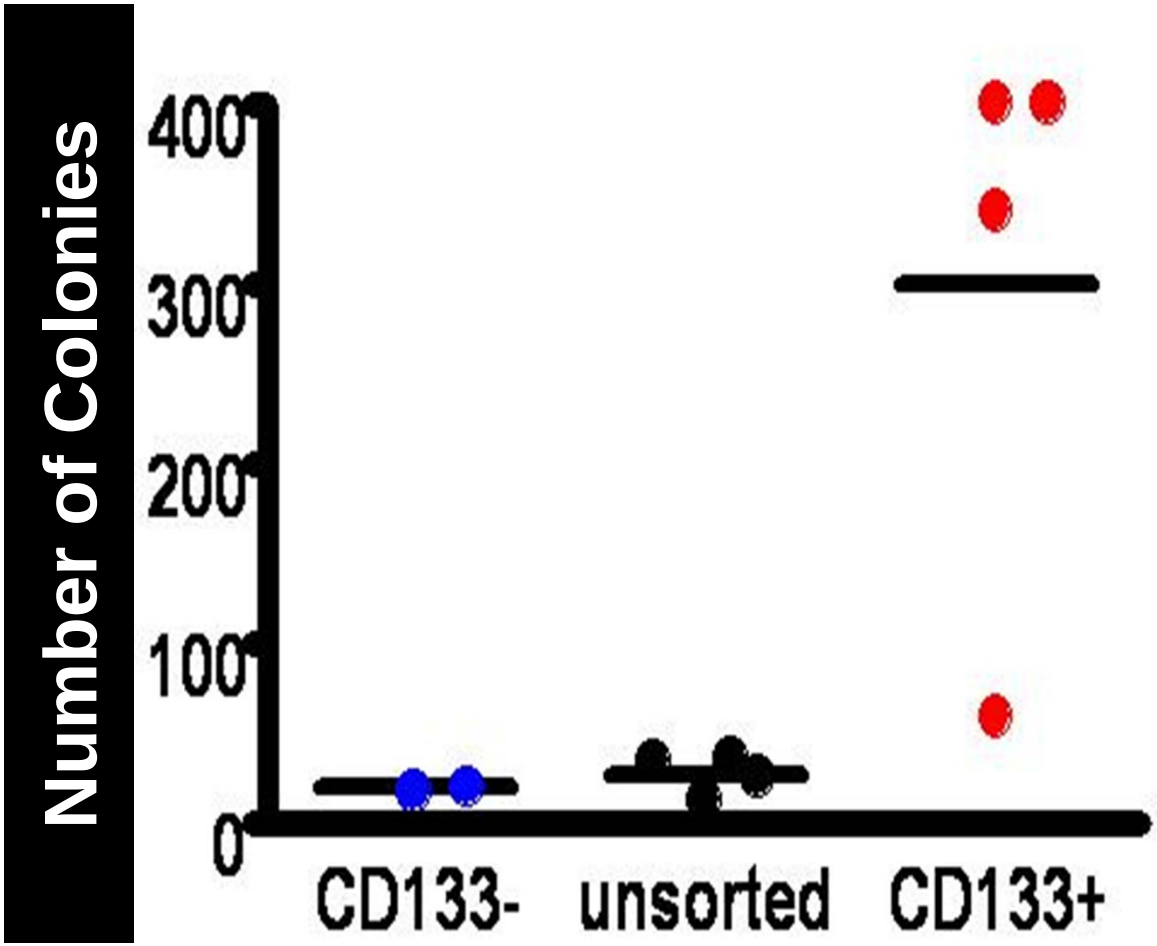
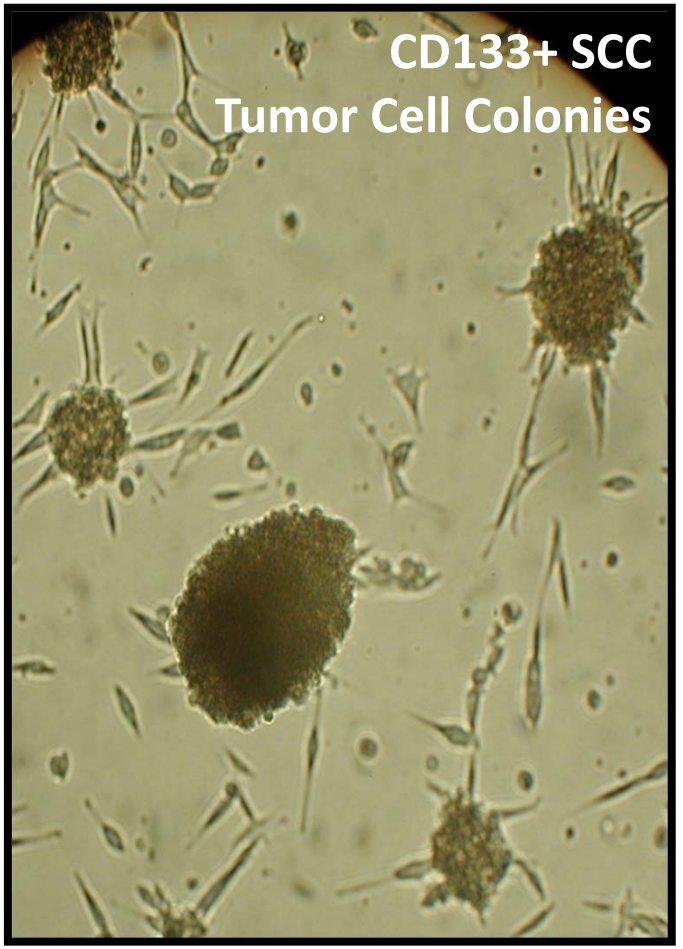




CD133+ cells isolated from SCC are enriched for spheroid colony formation





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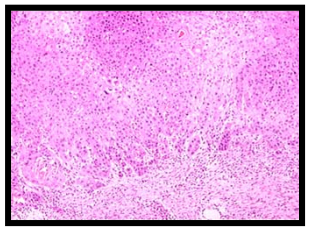
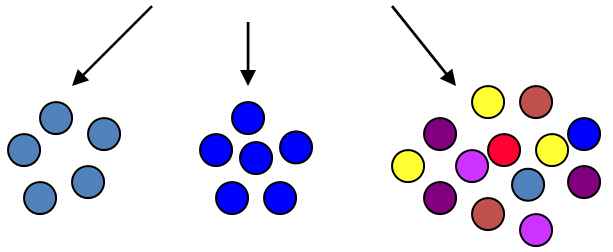
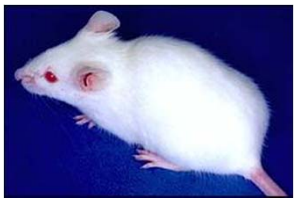
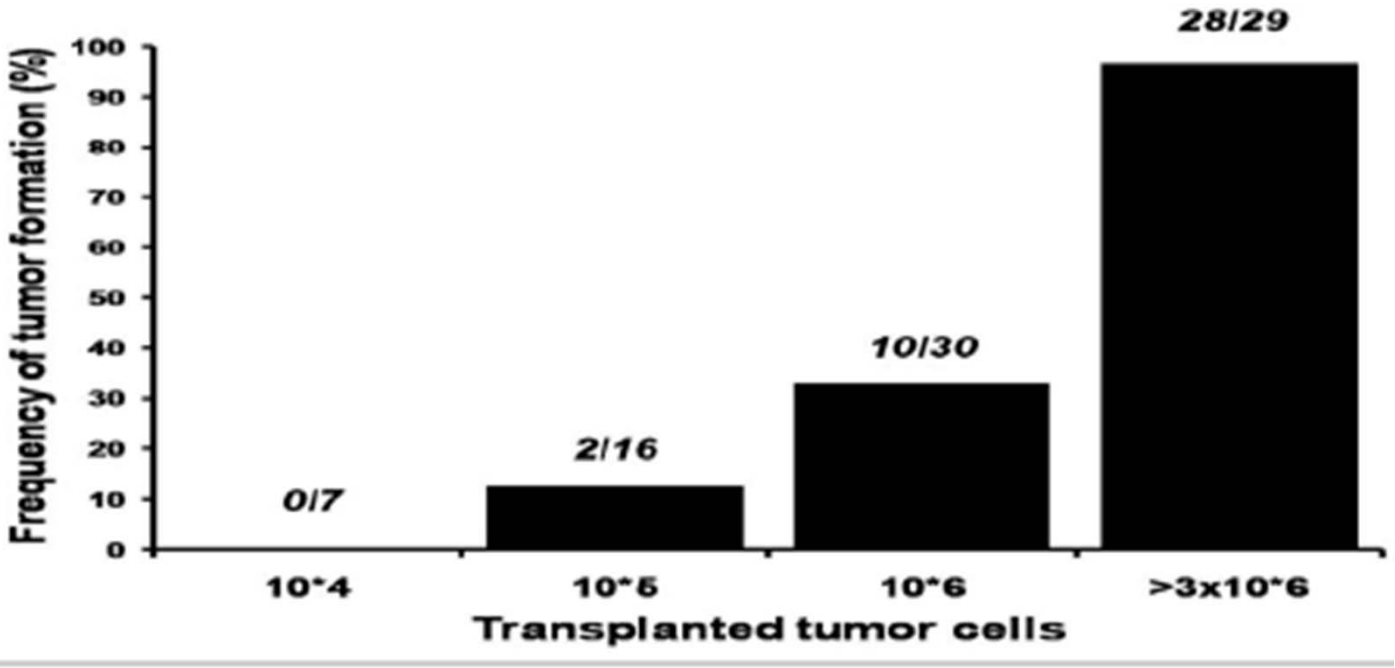






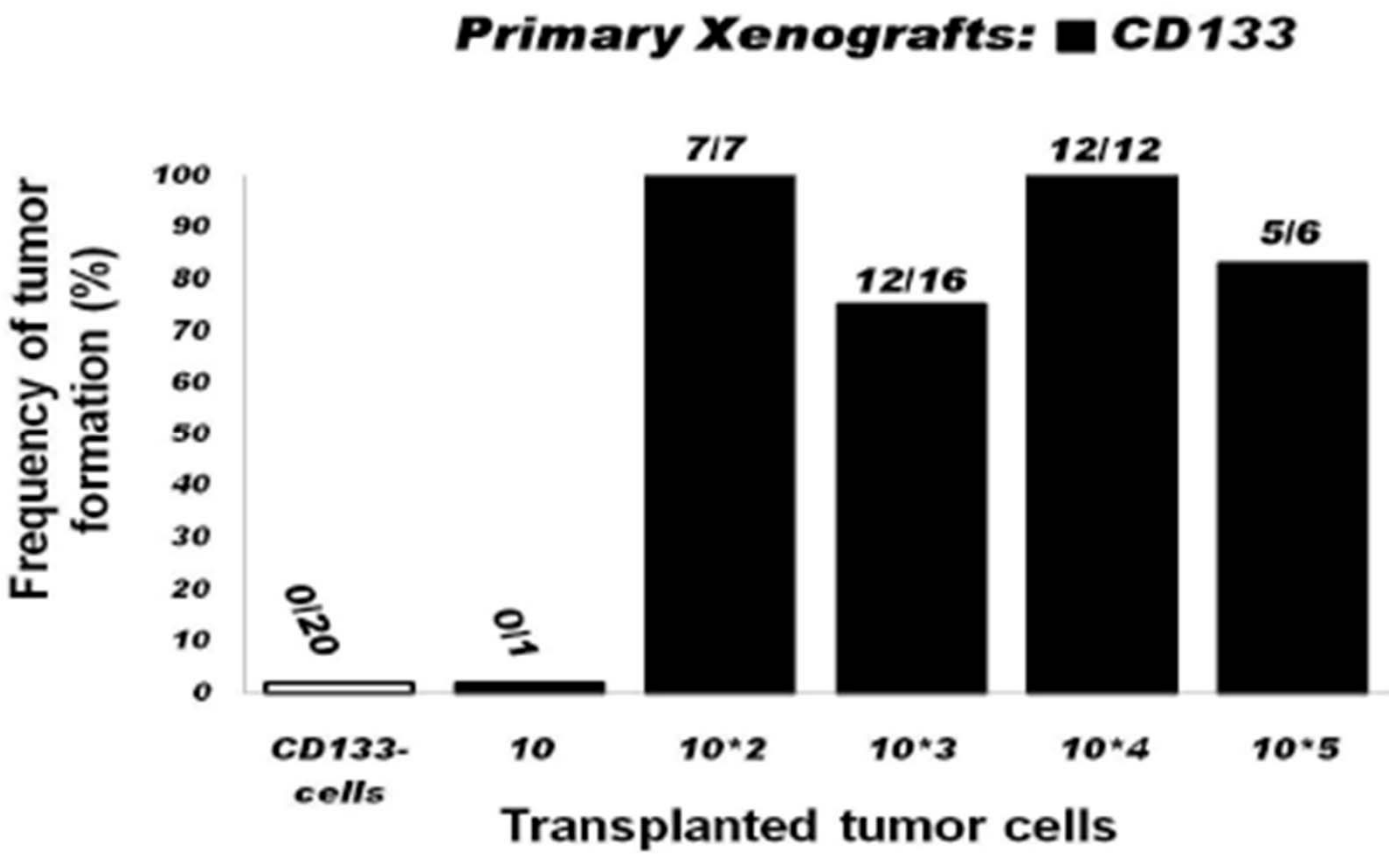
Tumor growth was dependent on the number of unsorted human SCC cells xenotransplanted



82 Total Xenographs into Nude Mice

TIC frequency = 1 / 1,400,000 Total SCC cells

Xenotransplanted CD133+ SCC cells are highly enriched for TIC

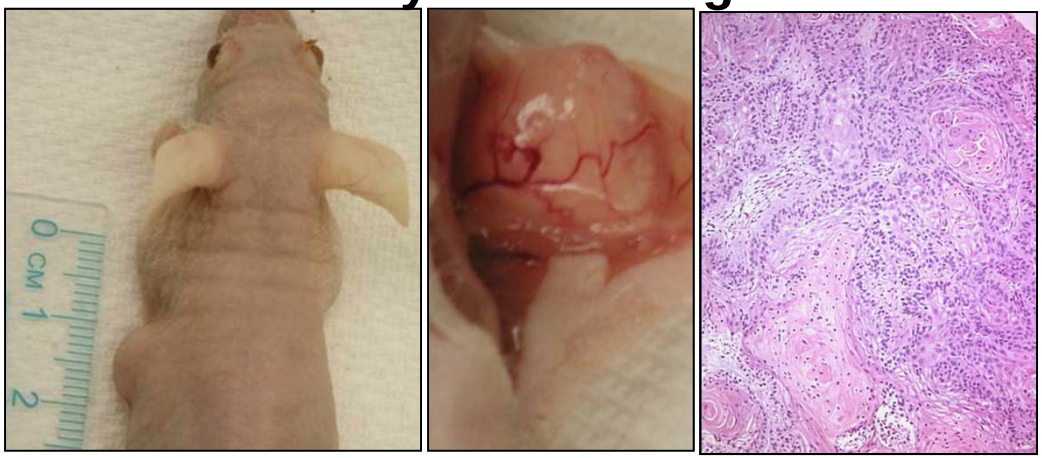


42 total xenografts from 28 different human SCC specimens

TIC frequency = 1 / 483 CD133+ cells

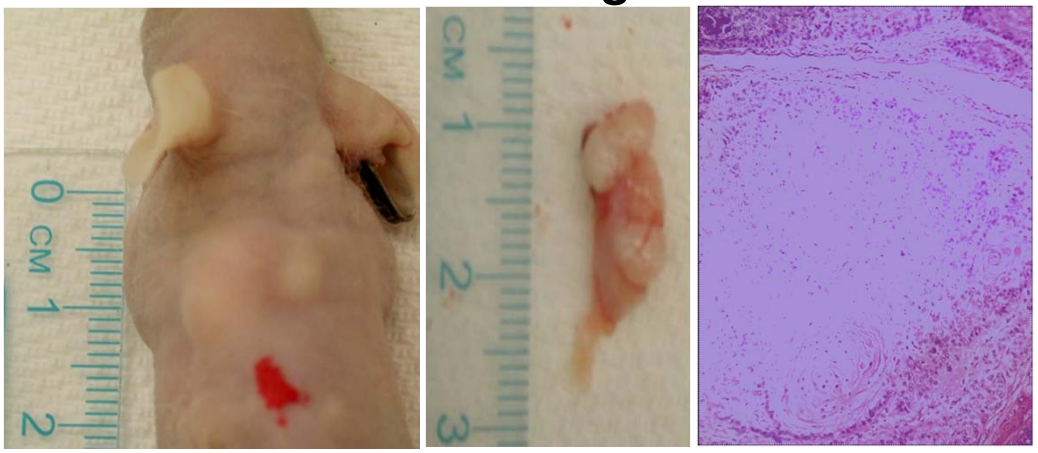
CD133+ SCC can be serially transplanted - demonstrating the stem cell properties of self-renewal and tumor reconstitution

Primary SCC Xenografts



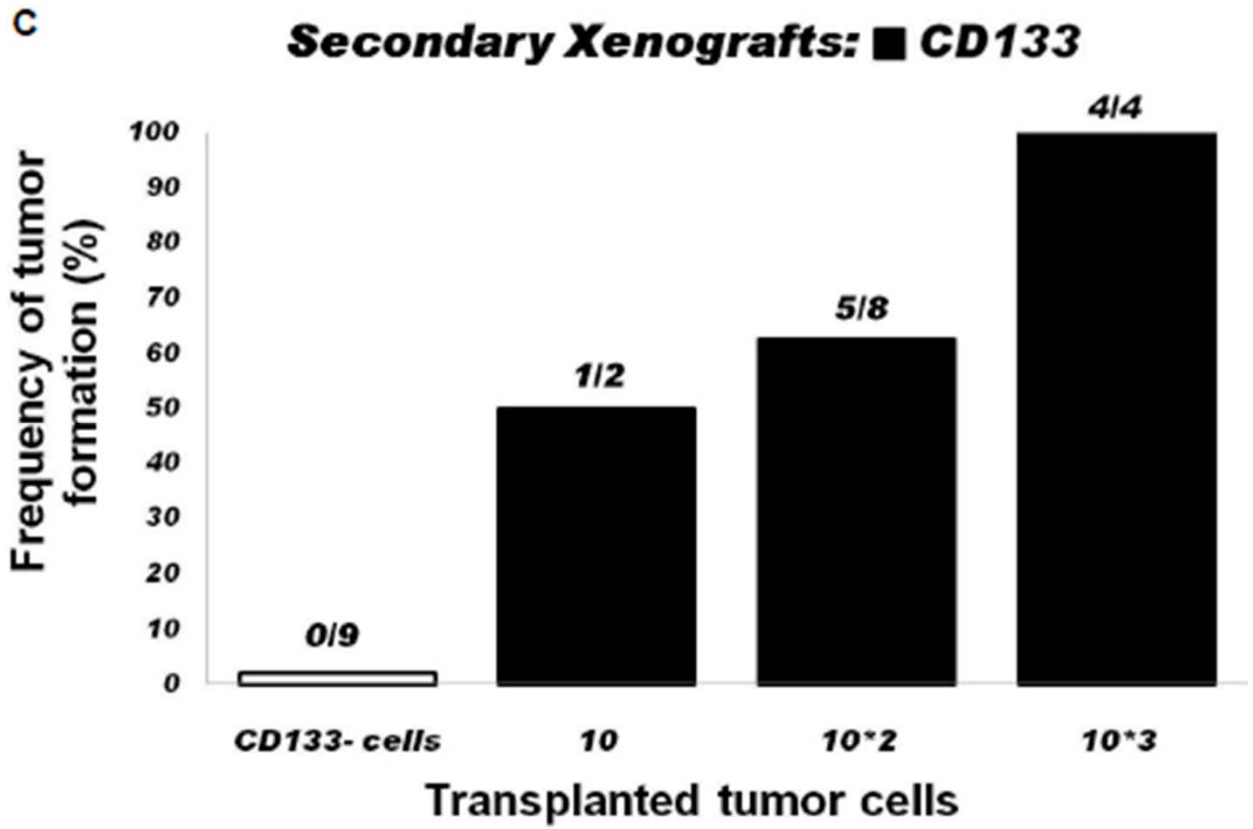
FACS analysis of CD133+ / CD45- cells in primary SCC xenograft = 0.7% **(n=11)**

Serial transplants of CD133+ from 1' xenografts



Secondary SCC Xenografts

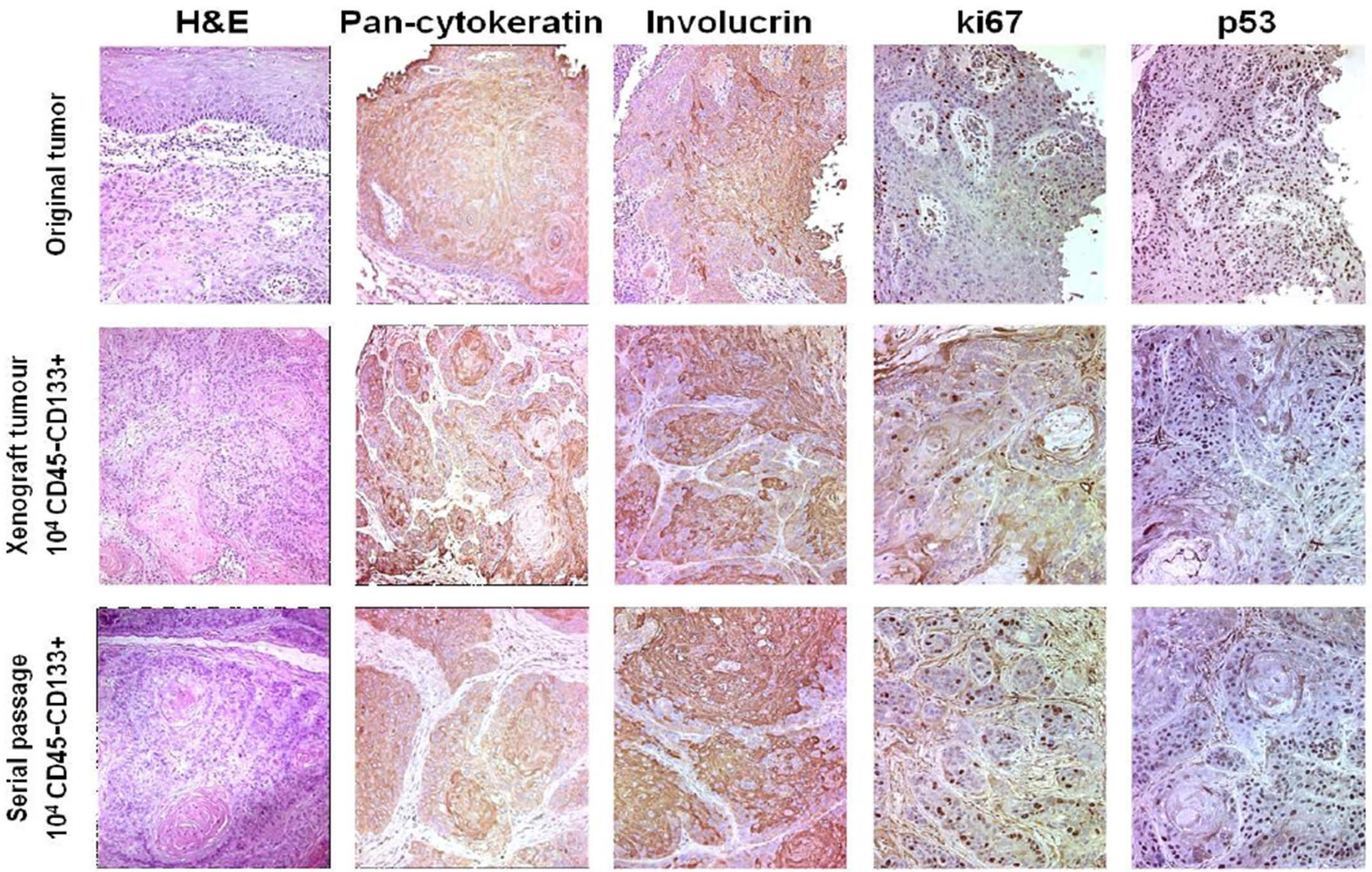
Human CD133+ SCC cells are enriched for TIC when serially transplanted into mice



14 total serial xenografts from 8 different human SCC specimens

TIC frequency = 1 / 863

Xenotransplanted CD133+ SCC cells recreates the original tumor morphology



Conclusions

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

The ability to isolate and characterize enriched TIC subpopulations, such as CD133+ cells in human SCC, will be important for understanding how normal tissue developmental programs have been altered in cancer. TIC can be analyzed for:

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

In vivo animal models will need to be optimized to more closely mimic the human microenvironment.

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

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NCAB Questions

1. What criterion should be applied to the development of cancer stem cell (CSC) lines use for therapeutic screening purposes?

2. What is the best approach to analyze the diagnostic and/or prognostic value of CSC markers in human cancer?

3. What are the minimum requirements for the xenograft in vivo models to convincingly demonstrate the presence of cancer stem cells in sub-populations of cancer cells?

4. Once enriched populations of CSC are isolated, can we leverage these discoveries into therapeutics before their biology is completely understood? If so, by what methods?