



# Mammary Stem Cell Biology

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My laboratory is interested in identifying and characterising the cells that make up the normal mammary epithelium. We are particularly interested in studying mammary stem and progenitor cells since we hypothesise that these cells are the initial targets for malignant transformation and that they may function as cancer stem cells which propagate tumour growth. We are also interested in identifying the cellular mechanisms that regulate the growth of normal mammary stem and progenitor cells since cancer stem cells may be dependent on the same mechanisms, and these mechanisms represent potential therapeutic targets.

My laboratory currently has four main research themes:

### **1. Characterization of normal mammary epithelial stem and progenitor cells.**

The mammary epithelium is composed of two general lineages of epithelial cells: luminal cells and basal-positioned myoepithelial cells. A self-renewing population of mammary stem cells, in conjunction with more differentiated progenitor cells, maintains the mammary epithelium. Mammary stem and progenitor cells are perceived to be the cell of origin of breast tumours since only these cells have the replicative

capacity that allow the multiple mutations required for tumour progression to accumulate. My previous research demonstrated that mammary stem and progenitor cells can be detected via the use of functional assays and can be prospectively isolated using fluorescence-activated cell sorting (Figure 1) (Stingl et al., *Nature* 2006; 439:993). We are now extending these studies by developing strategies to purify the cells to a level that allows us to obtain an accurate gene expression profile and to identify genes important in stem cell function. During this process we have also identified two novel types of progenitor cells within the mammary epithelium and are currently determining their properties and their developmental fates.

### **2. Determining the cell of origin in basal breast cancer.**

Breast cancer is a heterogeneous disease with approximately five molecular subtypes and 18 histological subtypes identified. Our laboratory is interested in elucidating the mechanisms that account for this heterogeneity. One possible mechanism is that different types of breast cancers initiate in, and are propagated by, different types of mammary cells. To test this directly, we are conducting experiments in which we are reverse engineering human breast tumours onto different cellular backgrounds. We will also be examining the influence of a variety of tumour suppressor genes on mammary stem and progenitor cell function in the course of these experiments. Studies in the hematopoietic field have demonstrated that loss of common tumour suppressor genes can impart some properties of stem cells to committed progenitor cells (Akala et al., *Nature* 2008; 453:228). We are currently conducting similar experiments to determine if a similar phenomenon occurs in breast cancer. The tumour suppressor genes that we are focusing on are those associated with basal-like breast tumours, which is a very aggressive type of breast cancer.

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\*Joined during 2008

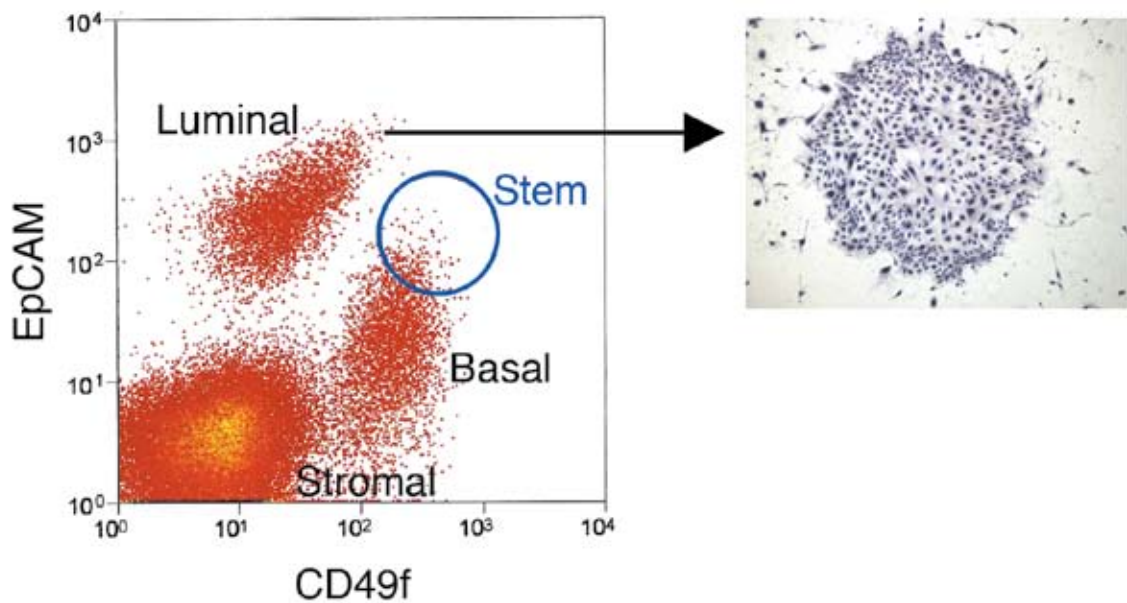


Figure 1. Flow cytometry dot plot demonstrating the distribution of epithelial cell adhesion molecule (EpCAM) and  $\alpha_5$ -integrin (CD49f) among freshly dissociated mammary epithelial cells. The stromal, luminal and basal cell populations are indicated. Luminal progenitors can be detected by their ability to generate pure luminal cell colonies in vitro. Mammary stem cells are localised at a high frequency within the basal cell population in the region indicated by the blue circle.

### 3. Identification of the molecular mechanisms that regulate stem cell self-renewal.

Self-renewal is perceived to be a defining property of stem cells. Cellular pathways that regulate stem cell self-renewal are considered to be good targets for therapeutic intervention since tumours should eventually exhaust their proliferative capacity in the absence of these pathways. Since we have developed strategies that enable us to isolate stem cells to very high purities, our approach to identify these pathways is to compare the gene expression patterns of mammary stem cells isolated from different developmental states (e.g., states of stem cell expansion vs. non-expansion).

### 4. Characterization of human breast tumour stem cells (collaborative project with Carlos Caldas, CRI).

Previous reports in the literature have demonstrated that human breast tumours contain a cancer stem cell component and that these cells can be prospectively isolated on the basis of the expression of certain cell surface markers and intracellular enzymes (reviewed in Stingl and Caldas, *Nat. Rev. Cancer* 2007; 7:791). It is not known if these properties are universal for all breast cancer stem cells or if different types of breast tumours have stem cells with unique properties. To address this issue, we have initiated experiments in which the breast cancer stem cell component of human breast tumours is identified and characterized using flow cytometry in combination with functional assays. We will be able to understand the cellular context of these cancer

stem cells by using the markers that we have previously determined to characterise different subsets of normal mammary epithelial cells. Long-term experiments include tracking the evolution of these cancer stem cells over time and examining their functional heterogeneity, both of which have large implications for response to therapy.

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