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Differential alteration of stem and other cell populations in ducts and lobules of TGFα and c-Myc transgenic mouse mammary epithelium

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Abstract

Genes associated with proliferation are active in stem and progenitor cells, and their over-expression can promote cancer. Two such genes, c-Myc and TGFα, promote morphologically dissimilar mammary tumors in transgenic mice. We investigated whether their over-expression affects population size and cell cycle activity in stem and other cell populations in non-neoplastic mammary epithelia. Results indicated that both cell population and cell cycle regulation are cell type- and microenvironment-specific. To create a tool for identifying and categorizing the five cellular phenotypes by light microscopy, we adapted previously established ultrastructural criteria. Using nulliparous MMTV-*c-myc* or MT-*tgfα* mice, we determined and compared the relative sizes the putative stem, progenitor and differentiated cell populations. PCNA staining was used to compare the portion of each cell population in the cell cycle. Cell population sizes were analyzed relative to: (1) their location in ducts versus lobules (microenvironment), (2) genotype, and (3) cell type. Population sizes differed significantly by genotype, depending on microenvironment ($p = 0.0008$), by genotype, depending on cell type ($p < 0.0001$), and by microenvironment, depending on cell type ($p = 0.03$). The number of cycling cells was also affected by all three factors, confirming that the interplay of cell type, gene expression and three-dimensional organization are very important in tissue morphogenesis and function. We describe a structure in mammary epithelium consistent with that of a stem cell niche, and show that it is altered in MMTV-*c-myc* and likely altered in MT TGFα transgenic epithelia.
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1. Introduction

It is now accepted that most, if not all, adult tissue harbors a population of tissue-specific stem cells that maintain and repair the tissue. There is also increasing evidence that it is members of the stem and early progenitor cell populations (Cozzio et al., 2003), rather than the differentiated cells that propagate the genetic alterations that initiate, and ultimately cause cancer in a tissue (Al Hajj et al., 2003). This new evidence has promoted the understanding that cancer, as a genetic disease, hijacks the mechanisms of tissue repair and renewal, specifically in the stem cell populations, disrupting their programs and creating chaotic growth dysregulation. This appreciation of the nature of cancer has clarified the role that stem cell regulation and biology will now play in cancer research, from prevention to cure and management. For this reason it is imperative that we quickly phenotype the stem, progenitor, differentiated and aging cell populations in tissues and learn how they are regulated.

It is now clear that stem cells occupy highly specialized spaces in all tissues investigated (Spradling et al., 2001). These include brain (Palmer et al., 2000), hair follicle (Kishimoto et al., 2000), muscle (LaBarge and Blau, 2002), intestine (Nishimura et al., 2003; Kayahara et al., 2003), tooth (Harada et al., 1999) and gonads (Xie and Spradling, 2000; Tulina and Matunis, 2001). These small, highly regulated microenvironments, first described in the hematopoietic

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system (Nilsson et al., 2001) and *Drosophila* ovary (Xie and Spradling, 2000), are called stem cell niches; stem cell niches have recently drawn focused interest in mammalian brain (Palmer et al., 2000), gut (Yang et al., 2001), testes (Shinohara et al., 2001) and mammary gland (Chepko and Dickson, 2003). A stem cell niche is comprised of specialized cells that surround the stem cell. Similar to an ecological or social niche, it acts to: (1) shield the stem cell from the burden of incoming hormone and growth factor signals present in the surrounding tissue and larger environment and (2) supply, interpret and regulate specific signals to modulate tissue growth by directing stem cell asymmetric cell division. Although the regulation of these spaces is a new field of study, it has been shown that the disruption of the same signaling pathways that are frequently disrupted in cancer are those that also effect regulation of stem cell division (Tulina and Matunis, 2001). There is now a growing literature that supports the hypothesis that genetic alteration in a propagating cell is not the sole causal factor in tumorigenesis, but that tumorigenesis also depends on signaling from the extracellular matrix and the neighboring cells. Concisely, the microenvironment also contributes to the development and/or the progression of cancer.

Recent ultrastructural and FACS data have opened the field of mammary epithelial stem cells. Chepko and Smith (1997) have described the size and ultrastructural features of a combined putative stem and progenitor cell population in rat and mouse mammary gland, which in the rat comprises 3% of the mammary epithelium. A third, more differentiated progenitor population, comprises 5% of the rat mammary epithelium (Chepko and Smith, 1997). However, since stem cell-specific antibodies provide less than optimum in situ immunohistochemical results at present, mammary epithelial stem cells have yet to be firmly identified in situ. FACS analysis based on Sca-1 positivity and Hoechst 33342 exclusion, a marker for a small side population (SP) of rare cells in blood (Goodell et al., 1996), was used by Welm et al. (2002) to isolate a progenitor cell-enriched compartment in mouse mammary gland that represents 3% of the epithelial cells. A forward-side-scatter plot performed on this population (Welm et al., 2002) also confirmed the ultrastructural analysis (Chepko and Smith, 1997) that putative mammary stem cells are small, with scant cytoplasmic complexity. However, Alvi et al. (2002), using only Hoechst 33342 exclusion with FACS analysis, demonstrated that the SP population, in both mouse ($0.45 \pm 0.22\%$) and human mammary epithelium is much smaller ($0.18 \pm 0.23\%$, with the deviation related to number of pregnancies, contraceptive status, and day of menstrual cycle), and similar in size to that of mouse bone marrow (Goodell et al., 1996). This was consistent with the prediction from ultrastructural evidence, that the putative stem cell population is actually a combined population of stem and primary progenitors (Chepko and Smith, 1997).

Recently, cells with the molecular markers CD44+, $CD24^{-/low}$, $lineage^{neg}$ were found in human breast cancers (Al Hajj et al., 2003). These cells are seen as "cancer stem cells", because like normal mammary epithelial stem cells (Al Hajj et al., 2003), they can be serially transplanted to repeatedly replicate the tumor (Welm et al., 2002). The stem-like nature of these "cancer stem cells" was demonstrated when as few as 100 of them were capable of producing new tumors which contained cells with the same complement of molecular phenotypes as the cells of the original tumor, including both cancer-initiating cells and differentiated cancer cells without self renewal capacity. This shows that cancer population dynamics are based on some manner of hierarchical cellular organization, comparable to that of normal tissues which contain both self- and non-renewing cells.

Additional evidence that stem cells in normal tissues are at risk for cancer-initiating events is the effectiveness of the stem cell receptor inhibitor Gleevec against the population of c-kit-expressing cells in hematopoietic and lung cancers (Druker et al., 2001). However, the ultimate cancer stem cell was shown to mutate (Mauro and Druker, 2001; La Rosee et al., 2002) in order to escape cell death caused by this inhibitor, thus conserving a small population of cancer stem cells. Using differential cell counts, based on ultrastructural characteristics, Chepko and Smith (1997) demonstrated that there are at least six discrete populations of cells in rat mammary epithelium. These populations are represented by five structural phenotypes that, due to the unavailability of specific antibodies, were identified according to their staining and morphological characteristics. The cell with the most primitive morphology, the small light cell (SLC), is believed to comprise two division competent cell populations: a primary stem cell compartment and a transit amplifying compartment. However, the undifferentiated large light cells (ULLC; Chepko and Smith, 1997) are a somewhat more differentiated, mitotically competent population that may represent a slightly more restricted progenitor cell population. The differentiated large light cells (DLLC) are nearly differentiated and may possess organelles characteristic of either the LDC (luminal) or the MYO (myoepithelial) functional types (Chepko and Smith, 1997). These latter three morphotypes appear to be mitotically inactive and represent populations of either differentiated or nearly differentiated cells. All five morphotypes are present in mammary epithelia of mouse, human (Ferguson, 1985b), sheep (Ellis, 1995) and cow (Ellis and Capuco, 2002), and at least the ULLC is mitotically active in human (Ferguson, 1988).

Transplantation experiments of primary cultures of mammary epithelial cells have demonstrated that a stem cell hierarchy, similar to that known for blood, may exist in mammary epithelium (Smith, 1996; Kordon and Smith, 1998). Chepko and Smith proposed that the five morphotypes in mammary epithelium represent such a hierarchy that sustains and renews the epithelium during the life of the animal (Chepko and Smith, 1997). Ellis and Capuco (2002) have confirmed that cells morphologically similar to the mitotically active cells in rodent are the primary proliferating compartment in heifer mammary gland. Recently, the ultrastructural characteristics of a putative stem cell niche in rat mammary

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