Commentary

Mammary cancer and epithelial stem cells: a problem or a solution?

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Abstract

The existing paradigms for stem cells in adult tissues include the integument, the alimentary canal, the lung, the liver, skeletal muscle and bone marrow. The mammary gland, by contrast, is the 'new kid on the block'. What little is known about stem cells in the mammary gland indicates that they possess a prodigious capacity for self-renewal. More importantly, in rodents, they persist with undiminished reproductive vigor throughout the organism's lifetime without regard to age or reproductive history. Do these stem cells represent primary targets for mammary neoplasia? If so, what are the implications for prevention/therapy?

Keywords: aging, mammary, neoplasia, stem cell, transplantation

Introduction

Evidence for mammary epithelial stem cells derives from studies of the glands from rodents, canines and humans. In the human, genetic analysis [1] of contiguous portions of individual human mammary ducts within the same breast signifies their clonal derivation and forecasts the existence of multipotent mammary epithelial cells in the human breast [2–4]. This work was confirmed and extended by the demonstration of a common loss of heterozygosity in normal cells in individual lobules within breast tissue from patients with carcinoma, in duct-lobular units from paraffin-embedded mammary tissue, and from normal luminal and myoepithelial cell clones derived from reduction mammoplasty patients [5–7].

Cell lines from canine mammary spindle-cell tumors exhibited mixed phenotypes on transplantation but possessed identical allelic patterns in microsatellite analysis, suggesting that canine mammary tumors arise from pluripotent stem cells [8].

Rat mammary glands contain a subpopulation of clonogenic epithelial cells that, when transplanted, give rise either to alveolar or ductal structures [9]. In quantitative rat mammary cell transplantation studies, both alveolar and ductal colonies were developed that support the conclusion that both colony types are derived from a single clonogenic mammary cell [10,11].

In the mouse, it was recently shown that the epithelial population of a fully developed lactating mammary outgrowth in mice could consist of the progeny from a single cell [12]. Serial transplantation of epithelial fragments from the clonally derived gland demonstrated that the subsequently generated outgrowths are also comprised of progeny from the original antecedent. All epithelial cell types were found to be present within these clonal normal populations including luminal, myoepithelial, ductal and lobule-committed epithelial progenitors and fully competent mammary epithelial stem cells. These observations demonstrate the presence of multipotent

LacZ = β-galactosidase (*Escherichia coli*); TGF-β1 = transforming growth factor-beta 1; WAP = whey acidic protein.

tissue-specific epithelial stem cells among the parenchyma of the mouse mammary gland.

The prevailing view regarding stem cells is that they are cells with the capacity for prolonged, if not unlimited, self-renewal and that they can produce at least one type of fully differentiated descendant. Between the stem cell and its differentiated progeny, there may often be an intermediate population of committed progenitors with a restricted differentiation potential and a limited capacity for self-renewal. It has become increasingly clear that stem cells do not exist in organs as independent units; rather, their behavior and maintenance is dependent on signals emanating from neighboring somatic cells [13,14]. In mammalian epidermis, Notch/Delta signaling [15] and c-*myc* activation [16] control important aspects of epidermal stem cell behavior. Likewise, in the mammary gland, activation of Notch brings about severe limitation of stem cell function by preventing the development of the secretory epithelial cell lineage [17].

An important key to understanding stem cell activity and persistence in adult organs is the determination of the physical microenvironment and molecular milieu within which the stem cell resides (stem cell niche), and the determination of the conditions that allow for stem cell expansion or directed differentiation of stem cell progeny.

Stem cell aging in clonal mammary populations

In the author's laboratory, clonal mammary populations derived from outgrowths of individual mammary fragments from old, multiparous donors were created and carried through six transplant generations to growth senescence. The purpose of the experiment was to establish the 'repertoire' of the original stem cell antecedent by evaluating its clonogenic (stem cell) progeny through successive transplantation generations toward reproductive senescence.

A calculation of the total number of new stem cells required during repopulation of each individual outgrowth indicates that 10^{12}–10^{13} multipotent progenitors will be generated anew from the original antecedent before growth senescence is reached [12]. This number is reached because all parts of each newly generated mammary gland are capable of recapitulating glandular regeneration on subsequent transplantation (reviewed in [18]).

Using this approach, several new characteristics of mammary epithelial stem cells have been discovered. For example, local stem cells within individual outgrowths display different patterns of growth senescence when propagated in impregnated hosts. In this scenario, a fully competent implant produces a gland with full secretory lobule development filling the fat pad. Senescing outgrowths may fail to produce full secretory lobular development but generate a complete system of branching ducts or, alternatively, may yield only secretory lobule development in the absence of ductal branching morphogenesis. This illustrates that, during growth senescence, multipotent stem cells independently lose their capacity to originate the progeny necessary for lobular and ductal morphogenesis. Consequently, the property of producing progeny committed to either ductal or lobular morphogenesis is not only intrinsic to the mammary stem cell, but is also subject to independent regulatory control (Smith GH, Boulanger C, Strickland P, Daniel C, manuscript submitted). Growth senescence of these cells may be reached only after serial transplantation through multiple generations [19–21].

A prediction ensues that, in growth senescent populations, there will be an absence of stem cells. Previously, epithelial cells with a unique ultrastructural morphology were described among the mammary epithelium of rats and mice [22,23], and were shown to be proliferation competent. These distinctive cells are omnipresent at all stages of mammary development and differentiation. It was proposed that these ultrastructurally distinct epithelial cells represent mammary stem cells. Careful examination of fully senescent mammary epithelial populations reveals the absence of these ultrastructurally distinct cells, supporting the conclusion that they are indeed mammary epithelial stem cells (Smith GH, Boulanger C, Strickland P, Daniel C, manuscript submitted).

Mammary stem cell control of ductal patterning

Our observations in the mouse mammary gland suggest that multipotent progenitors can strictly limit the behavior of their progeny within a given environment and instruct, quite specifically, the extent to which these daughters can react to the local surroundings. This capability is demonstrated in experiments where it is shown that progenitors from the same clonal population can produce, within the same host, vastly different mammary ductal structures. Subsequent transplantation indicates that the multipotent progeny found in these disparate mammary ductal populations retains the specific capacity to reproduce their specific ductal pattern. Local mammary stem cells therefore accommodate the capacity not only to control the specific patterns of the branching ducts they produce, but also to control the ability to convey this dominion to their stem cell progeny (Smith GH, Boulanger C, Strickland P, Daniel C, manuscript submitted).

Premalignant populations from regional mammary stem cells

The mammary stem cells (clonogens) produce the cellular and tissue diversity of the gland. As already discussed, much of this configuration may be cell autonomous (i.e. programmed by its progenitor, the stem cell). This concept

becomes more fundamental when applied to the appearance of mammary hyperplasia and other parenchymal irregularities. The local tissue derangement is often demonstrably monoclonal in humans and rodents [5–7,24]. The present author hypothesizes that these cellular lesions exhibit cell autonomous characteristics conferred on them by the transformed local stem cell. In support of this speculation, local regions of serially transplanted epithelial clones occasionally manifested focal regions of hyperplastic lobular development. These lesions proved to be clonal in origin and repeatedly produced hyperplastic lobular mammary outgrowths on transplantation. One normal fragment, a fourth-generation transplant, generated an aggressive mammary neoplasm *in situ*. This growth was also composed of cells derived from the original clonogen and spawned several metastases to the lung of the tumor host. The lung lesions proved, as well, to be composed of cells descended from the primary founder. It therefore appears that premalignant and malignant clones may constitute a lineage potential of aging mammary epithelial stem cells (Smith GH, Boulanger C, Strickland P, Daniel C, manuscript submitted).

Proliferative capacity of an individual mammary stem cell

The capacity of a single multipotent mammary antecedent to reproduce mammary gland growth through multiple transplant generations is unprecedented and represents direct evidence of the prodigious reproductive potential of a single mammary stem cell in the gland of an aged multiparous mouse. The presence of such cells in the mammary gland must represent an enormous risk to subsequent mammary cancer development. A means to reduce mammary cancer risk may thus be to limit the reproductive capability of individual stem cells *in situ*. Boulanger and Smith demonstrated that stem cell self-renewal is curtailed in mice expressing transforming growth factor-beta 1 (TGF-β1) from the whey acidic protein (WAP) promoter in the differentiating progeny of the mammary stem cell, resulting in early growth senescence [25]. As a consequence, the subsequent risk for mammary tumorigenesis in these glands is startlingly reduced, as demonstrated by challenging TGF-β1 transgenic and wild-type sisters with mouse mammary tumor virus and tabulating the total mammary tumor incidence over 80 weeks. This observation provides a 'proof of principle' that stem cell proliferative power (self-renewal) and tumor risk are inversely related.

A new study [26] utilizing WAP-driven Cre and Rosa 26-fl-stop-fl-LacZ mammary glands shows great promise for further clarification of a possible stem cell niche in the mammary gland. Cells whose reporter gene has been activated by expression of WAP-Cre appear in late pregnancy. A portion of this parity-induced epithelium survives postlactation involution (~5–10% in primiparous females) and accumulates on successive pregnancies, representing >60% of the total epithelium in multiparous females.

A similar accumulation does not occur in nulliparous females, although activated cells appear transiently during the estrus cycle. The new parity-induced population contributes extensively to secretory epithelial development on successive pregnancies. Transplantation of fragments or dispersed cellular populations containing the activated cells indicates that they may contribute extensively to the resulting outgrowth in which they appear at regular intervals throughout the branching ducts [26]. An intriguing possibility is that these sites represent newly formed stem cell niches. The LacZ reporter gene enables enrichment of these cells by fluorescence-activated cell sorting, opening the possibility of a more detailed examination of their biochemistry and molecular biology. In addition, the behavior of the parity-induced epithelium in WAP-TGF-β1 mice may enlighten further the relationship between WAP-expressing somatic cells and the behavior of mammary stem cells.

Conclusions

The presented data and those of other workers indicate that proliferative capacity resides in a small number of cells (clonogens) among the mammary epithelium. The evidence also supports the conclusion that transiently amplifying, lineage-limited cells are produced from omnipotent epithelial stem cells. Of interest is the observation that genes expressed in the differentiating somatic cells may modulate the proliferative lifespan of mammary stem cells. The discovery of a new proliferation-competent epithelium, tagged by WAP expression in the mammary glands of parous females, creates an experimental model for examining the effects of somatic cell signaling on stem cell behavior. Modulation of stem cell behavior holds exceptional promise for a new prophylactic approach for controlling mammary cancer risk. An important step towards the achievement of this control will be the characterization of the stem cell niche in the rodent mammary gland and, ultimately, in humans.

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