

Adult stem cells and stem cell niche play important roles in primary mammary tumors

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Abstract

Cancer biology and molecular biology of mammary tumors have been studied so far. New cell biology concept about stem cells and stem cell niches provides the important knowledge that mammary stem cells differentiate to myoepithelial and luminal epithelial cell lineages. Early detection and tumor differentiation are the targets of therapeutic plan. In general, the terminal end bud (TEB) composes of cap cell layer that surrounds the body cells. As mammary stem cell reservoirs, cap cells give rise to myoepithelial and/or luminal epithelial cells. The novel p53 homologue, p63, provides an informative data that relate to primary canine mammary tumors derived from myoepithelial lineage. A combination of two monoclonal antibodies, AE1 and AE3, relates to luminal epithelial lineage. Vimentin, a member of intermediate filaments, is immunoreactive to myoepithelial cell and mesenchymal tissues. In summary, mammary stem cells have the potential to differentiate into two categories of epithelial cells, myoepithelial and luminal epithelial lineages in canine mammary tumors. AE1/AE3 and Vimentin may be used as specific biomarkers to distinguish the mammary stem cell origin.

Keywords: mammary tumor, stem cells, stem cell niche, myoepithelial cells, luminal epithelial cells

สเต็มเซลล์และสเต็มเซลล์นิชมิมบทบาทสำคัญในเนื้ออกเต้านม ชนิดปฐมภูมิ

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บทคัดย่อ

การศึกษาทางชีววิทยาและทางชีวโมเลกุลของเนื้ออกเต้านมดำเนินการมาเป็นเวลานาน จากข้อมูลสำคัญและข้อสังเกตใหม่ๆ ทางชีววิทยาของเนื้ออกเกี่ยวกับสเต็มเซลล์และสเต็มเซลล์นิชมิมสามารถจำแนกสเต็มเซลล์ในเนื้อเยื่อเต้านมออกเป็นเซลล์กล้ามเนื้อของเยื่อหุ้มท่อ และเซลล์เยื่อหุ้มท่อ การวินิจฉัยโรคโดยเร็วและการจำแนกประเภทของเนื้ออกเป็นเป้าหมายหลักของการวางแผนการรักษา จากการศึกษาในปัจจุบันพบว่าTerminal end bud (TEB) ประกอบด้วยเซลล์ 2 ชนิด ได้แก่ cap cells และ body cells โดย cap cells ทำหน้าเป็นแหล่งสำรองของสเต็มเซลล์ของเต้านม ซึ่งสามารถพัฒนาไปเป็นเซลล์กล้ามเนื้อของเยื่อหุ้มท่อและ/หรือเซลล์เยื่อหุ้มท่อก็ได้ และได้มีการค้นพบ p63 ซึ่งมีลักษณะใกล้เคียงกับ p53 ทำให้ได้ข้อมูลเพิ่มเติมเกี่ยวกับเซลล์กล้ามเนื้อของเยื่อหุ้มท่อซึ่งเป็นต้นกำเนิดของเนื้ออกเต้านมชนิดปฐมภูมิในสุนัข การผสมผสานระหว่าง AE1 และ AE3 antibody ก็สามารถใช้ข้อมูลเกี่ยวกับเซลล์ต้นกำเนิดที่เป็นเซลล์เยื่อหุ้มท่อได้ดียิ่งขึ้น นอกจากนั้นแอนติบอดีต่อVimentin ซึ่งเป็น Intermediate filament ประเภทหนึ่งก็ให้ผลตอบสนองต่อเซลล์กล้ามเนื้อของเยื่อหุ้มท่อ และ Mesenchymal tissues กล่าวโดยสรุปสเต็มเซลล์ในเนื้อเยื่อเต้านมมีความสามารถในการพัฒนาไปเป็นเนื้ออกเต้านมในสุนัขที่มีต้นกำเนิดมาจากเซลล์กล้ามเนื้อของเยื่อหุ้มท่อและ/หรือเซลล์เยื่อหุ้มท่อได้ การวินิจฉัยเนื้ออกเต้านมโดยใช้ AE1/AE3 และ Vimentin antibody อาจเป็นไปไอมาร์คเกอร์ที่จะช่วยในการจำแนกประเภทของเนื้ออกที่มีต้นกำเนิดมาจากสเต็มเซลล์ในเต้านมได้

คำสำคัญ: เนื้ออกเต้านม สเต็มเซลล์ สเต็มเซลล์นิชมิม เซลล์กล้ามเนื้อของเยื่อหุ้มท่อ เซลล์เยื่อหุ้มท่อ

Mammary gland development

Mammary gland composes of parenchymal tissues which invade the mammary fat pad. In mouse, there are 5 pairs of mammary fat pads located beneath skin. The fat pads extend from thoracic to inguinal area. The mouse mammary gland is continually undergoing changes in structures and functions.

Embryonic development

Embryonically, the mammary gland invades the underlying stroma and subsequently to form rudimentary ductal system. This ductal system embedded in the mammary fat pads that extend along the mammary line. The penetration of the epithelial bud begins at day 10-11 when the mammary streak appears as a line extending from the anterior to the posterior limb bud. After day 12, the mammary epithelium consists of several layers. Then the mammary rudiment becomes bulb-shaped by day 14 and elongates beginning at day 16. At day 17, a mammary sprout invades the underlying mammary fat pad precursor tissue and continues to grow until birth to form a rudimentary ductal tree with several branching ducts (Richert et al., 2000). After embryonic developmental time, the mouse mammary gland remains inactive and the gland undergoes most of its development after birth.

Postnatal development

At birth, the mouse rudimentary and small ductal trees undergo complexity. Each mammary gland composes of the epithelial cords and stroma. The small ductal tree consists of a single layer of epithelial cells surrounding a central lumen and a small amount of connective tissues, fibroblasts and adipose tissues. The cells bordering the lumen refer as luminal epithelium which underline by myoepithelium. The myoepithelial cells are contractile and responsible for milk let down. At the early stages of development, luminal epithelial and myoepithelial cells control their interactions with the basement membrane by remodeling the immediate environment through secretion of protease and protease

inhibitors (Talhouk, Bissell and Werb, 1992). The basement membrane composes of network of proteins and proteoglycans which secreted by the myoepithelial cells. Normally, the ductal structure maintains as the intact membrane, however, it may influences by reproductive hormones.

Puberty and Postpuberty

Until puberty, the mammary glands grow symmetrically with the rest of the body. In rodents the mammary glands do not regress during prepuberty but maintain a small ductal tree which initiates rapid growth at the onset of puberty. The development of the ductal system is dramatically preceded under regulation of ovarian hormones. The ductal system penetrates the mammary fat pads by dichotomous branching until they reach the edge of the fat pad by week 10-12. At the tip of the ducts, the club-shaped structure called Terminal end buds (TEBs), contain highly proliferative cells. The TEBs could response to the specific hormonal system. During estrous cycle, they increase their complexity of the branching system by addition of side branches and further branching during pregnancy. Like the structure of the small ductal tree, the secondary and tertiary ductal structures compose of a single layer of cuboidal epithelium surrounding a central lumen. The luminal epithelium surrounds by a layer of myoepithelium and a thin layer of dense stroma.

Pregnancy and Lactation

The massive proliferation of ductal branches and formation of alveolar bud-like observed during postpuberty. The mouse mammary differentiation peak around 19-20 days of pregnancy which formation of alveoli and fully lactating glands at parturition. During the second half of pregnancy, the alveolar buds gradually cleavage and differentiate into individual alveoli. Then the alveolar becomes milk-secreting lobules during lactation. By late pregnancy, the alveoli

grew the majority of the fat pad and the alveolar epithelial cells produced milk protein and lipid. The luminal epithelial cells changed from cuboidal to flatten. In this stage, the myoepithelial cells still surround the alveoli but discontinuity (Richert et al., 2000).

Involution

After weaning, the mouse mammary glands remodel but it is reversible and lactation can be

reinitiated upon suckling (Furth, 1999). During involution, the secretory epithelial cells undergo apoptosis and the epithelial structures appear organized. However, the epithelial structures appear disorganized as much as the alveolar epithelial cells die and collapse. After involution, all of the alveoli collapsed then luminal epithelial and myoepithelial cells are being rearranged. The remodeled mammary gland appears more differentiated than a virgin mouse (Richert et al., 2000).

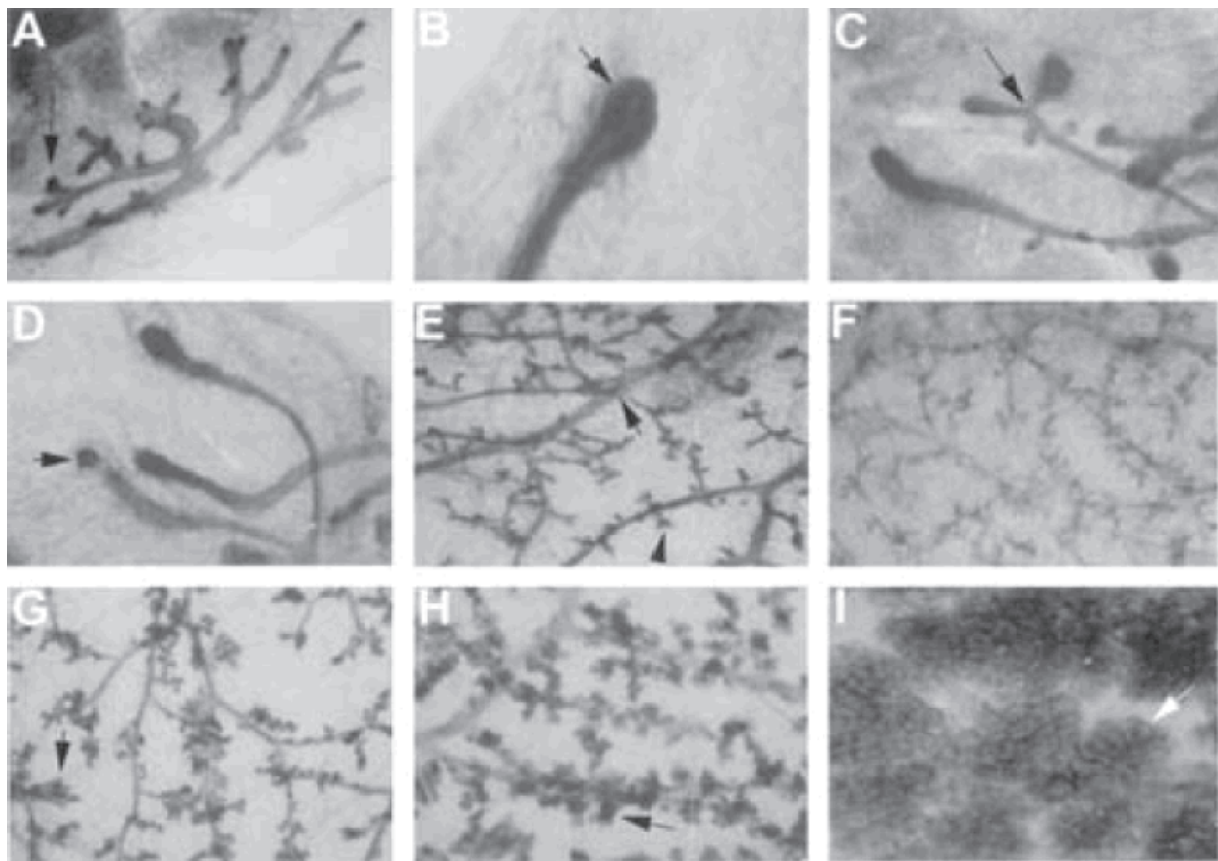


Figure 1: Whole-mount analysis of mammary glands from FVB mice. (A) Epithelial structures from a 3-week virgin. TEBs are indicated by the arrow. (B) A TEB (arrow) from a 5-week virgin. (C) Epithelial structures from a 5-week virgin. Bifurcation of the growing duct is indicated by the arrow. (D) Epithelial structures from a 10-week virgin showing a regressing end bud (arrow). (E) Ducts from a 10-week virgin showing both lateral branches (arrow) and alveolar buds (arrowheads). (F) Epithelial structures from a mammary gland at day 21 of involution. (G) Alveolar structures from a mammary gland at day 8 of pregnancy. The alveolar lobules are beginning to develop (arrow). (H) Alveolar structures from a mammary gland at day 12 of pregnancy. The alveoli are continuing to develop (arrow). (I) Alveolar structures from a mammary gland at day 18 of pregnancy. The arrow indicates the alveoli that have filled the majority of the fat pad (Richert et al., 2000).

Note: All panels were photographed at 45x magnification. Panel A-D had been cropped and enlarged to show the structure of interest, so magnification is not exact.

Recently, the tissue recombination technique revealed that the female reproductive hormones act sequentially during mammary gland development.

Estrogen, progesterone, and prolactin act through their respective receptors in the mammary epithelium. (Richert et al., 2000; Brisken and Duss, 2007).

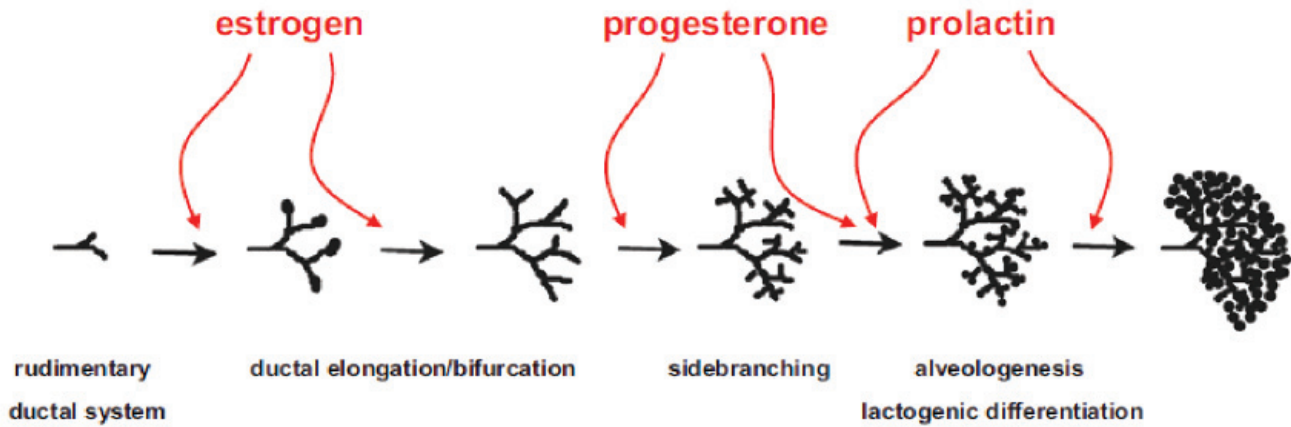


Figure 2: Schematic representation of mammary gland development (black) and hormonal control (red) of different morphogenetic steps (Brisken and Duss, 2007).

Stem Cells and Stem Cell Niche

Stem cells are highly capability cells to reproduce and/or differentiate, including self-renewing and growing into various cell types that are needed. They are responsible for the growth, homeostasis and repair of many tissues such as hematopoietic system, skeletal muscle and mammary gland. The maintenance and survival of stem cells is regulated by inputs from their local microenvironment, often referred to as the stem cell niche. The stem cell niche is quite complex and a dynamic group of cells. It could transmit and receive signals through various mediators. Jones and Wagers (2008) purposed the schematic which hypothesized the niche composite. They summarized the known components of previously described mammalian and non-mammalian niches: the

stem cell, stromal cells, soluble factors, extracellular matrix, neural inputs, vascular network, and cell adhesion components. As an unusual group of cells, the niche may conserve all of the components listed or it may compose of a selection component that relevant to its particular functions. The niche may provide structural support, trophic support, topographical information and/or physiological cues (Jones and Wagers, 2008).

Stem cell niches provide the answer how the local microenvironment can protect stem cells and influence their behavior. The niches cells normally regulate the proliferation of stem cells nearby which losing the signals from the niches may permit over-proliferation of stem cells and could be predisposing cause of cell transformation.

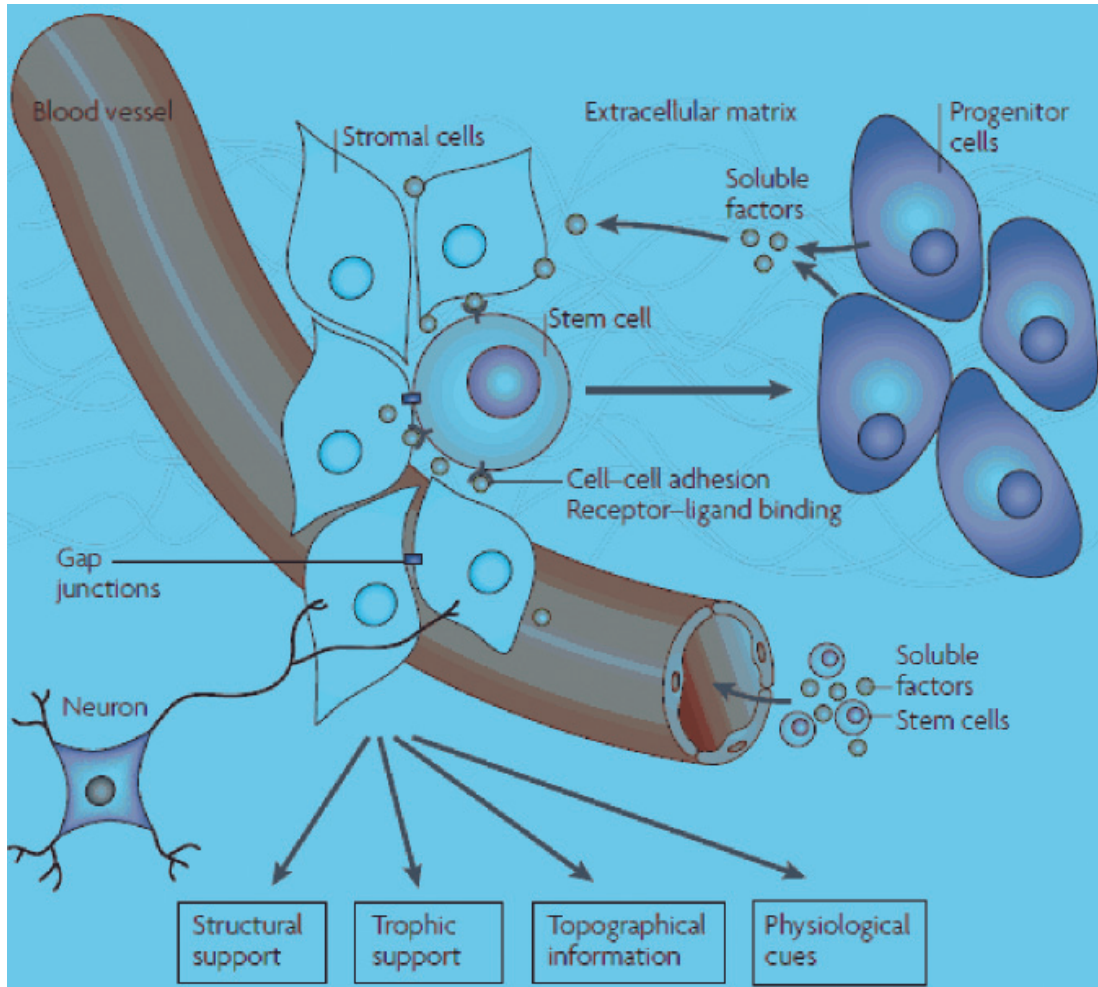


Figure 3: Components and functions of stem cell niches (Jones and Wagers, 2008).

Mammary stem cells

Mammary gland is a complex tissue, which is organized into a tree-like structure, and continually undergoing changes in its structure and function. It is composed of hollow branches and fruit-like terminal end buds. There is an inner layer of luminal epithelial cells that face the lumen and are surrounded by an outer layer of myoepithelial cells that secrete the basal lamina separating the mammary parenchyma from the stroma. The terminal end bud (TEB) appears at the onset of puberty, then undergoes rapid growth and differentiation under hormonal regulation. The TEB

composes of two major populations; 1) cap cell layer which surrounds the second most population 2) the body cells.

The cap cells may undergo either a myoepithelial lineage or a luminal epithelial lineage and therefore are thought to be multipotent stem cells. Differentiated myoepithelial and luminal epithelial cells line the neck of the TEB and the subtending duct. The ducts are surrounded by a basal layer of overlapping myoepithelial cells, whereas the alveoli cells are surrounded by a basket-like layer of myoepithelial cells (Richert et al., 2000).

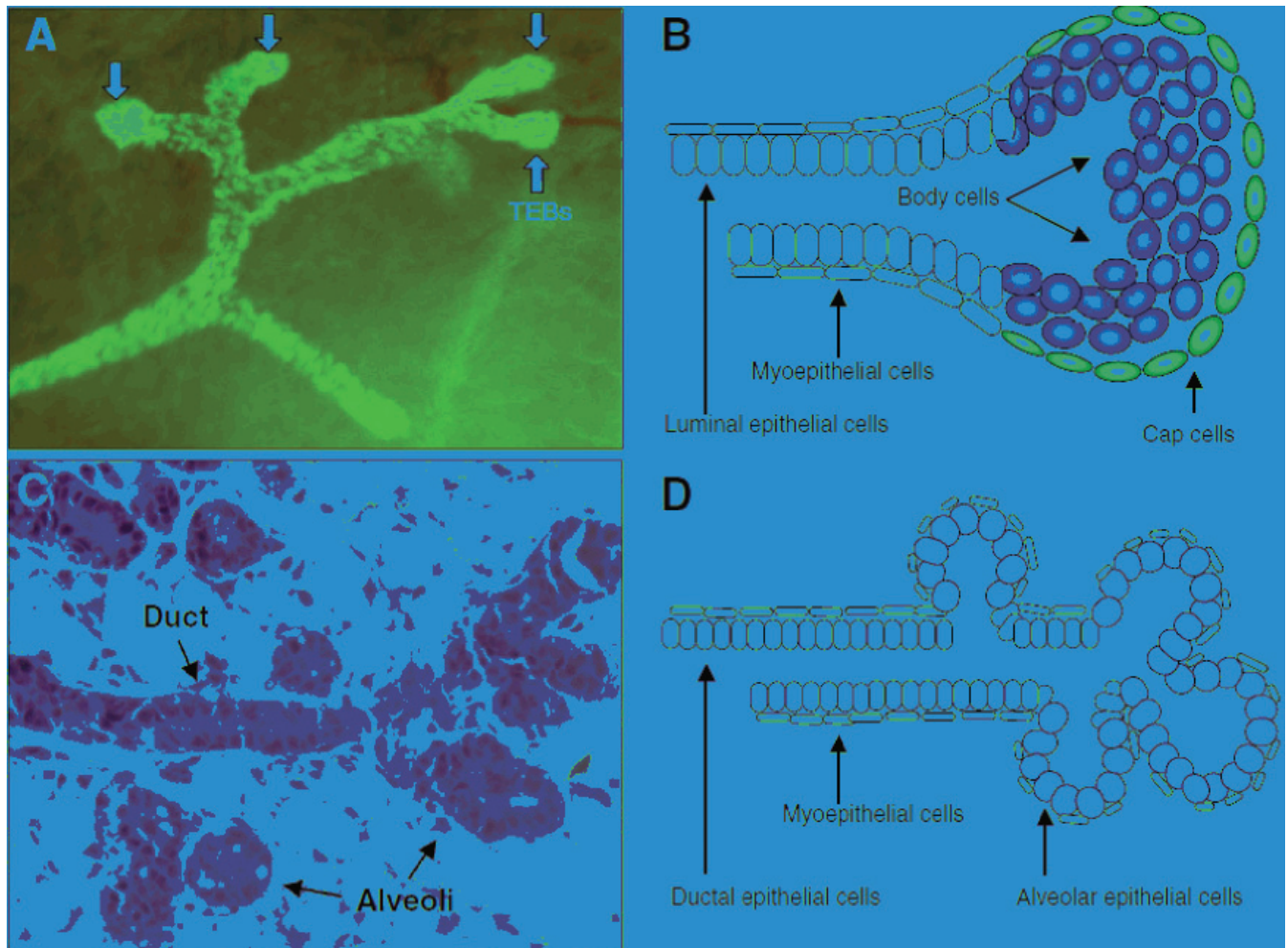


Figure 4: The terminal end bud (TEB). (A) Expression of Sca1 is enriched in TEBs (arrow) and ducts of six-week-old in Sca1-GFP knock-in mice. (B) Schematic view of the TEB. (C) H&E section of a midpregnant mammary gland from C57BL/6 mice indicating the locations of the ductal and alveolar cells. (D) Schematic view of the ductal and alveolar cells during midpregnancy (Woodward et al., 2005).

Mammary gland is one of a unique organ in reproductive system that undergoes most of its development after birth under the control of systemic hormones. At the moment of time, during puberty, estrus cycle, and/or pregnancy, mammary cells are response to specific hormones and generate new tree-like structures. Briskin and Duss (2007) suggested that mammary epithelial cells taken from any area of the mammary gland are capable to fully reconstruction a ductal tree when grafted to cleared fat pads. That indicates stem cells and their niches are distributed at regular intervals throughout the mammary gland.

Mammary gland stem cells are a quiescent and self-renewing population within the mammary gland that are capable of giving rise to the differentiated ductal, alveolar and myoepithelial cells (Woodward et al., 2005) Adult stem cells are long-lived, generally quiescent cells that generate new stem cells, and thereby maintain the stem cell pool, as well as more committed progeny, which populate the organ through proliferation (Molofsky et al., 2004; Reya et al., 2001).

However, the degree of stem cell properties potentially decreases from top to bottom: as the cell becomes more committed, the cell gradually loses its

properties. Many previous studies suggest that stem cells are able to self-renew and proliferate within the niche. The stem cells could maintain in their un-differentiated state by cell-matrix and cell-cell interactions with the niche cells, involving integrins and cadherins, respectively.

These stem cells can be distinguished by their long-term label-retaining cell (LT-LRC) properties, which are thought to reflect a state of quiescence. Stem cells may exit the quiescent state by becoming short-term (ST)-LRCs when exposed to the particular stimuli. As they become further committed, they become the transit-amplifying progenitors (TAs), comprising the side

population (SP) that are able to efflux the Hoechst dye. The SP/TA cells eventually give rise to more committed progenitors that are Sca1⁺. The Sca1⁺ population differentiates into luminal and myoepithelial cells. Stem cells are thought to possess many of the features that constitute the tumor phenotype, including self-renewal and unlimited replicative potential. Tumorigenic mutations are presumably sustained in expanding SP/TA population. These cells give rise to tumorigenic progenitor cells. CD44⁺ CD24⁻ may be markers that distinguish tumorigenic progenitor cells from normal progenitor cells (Woodward et al., 2005).

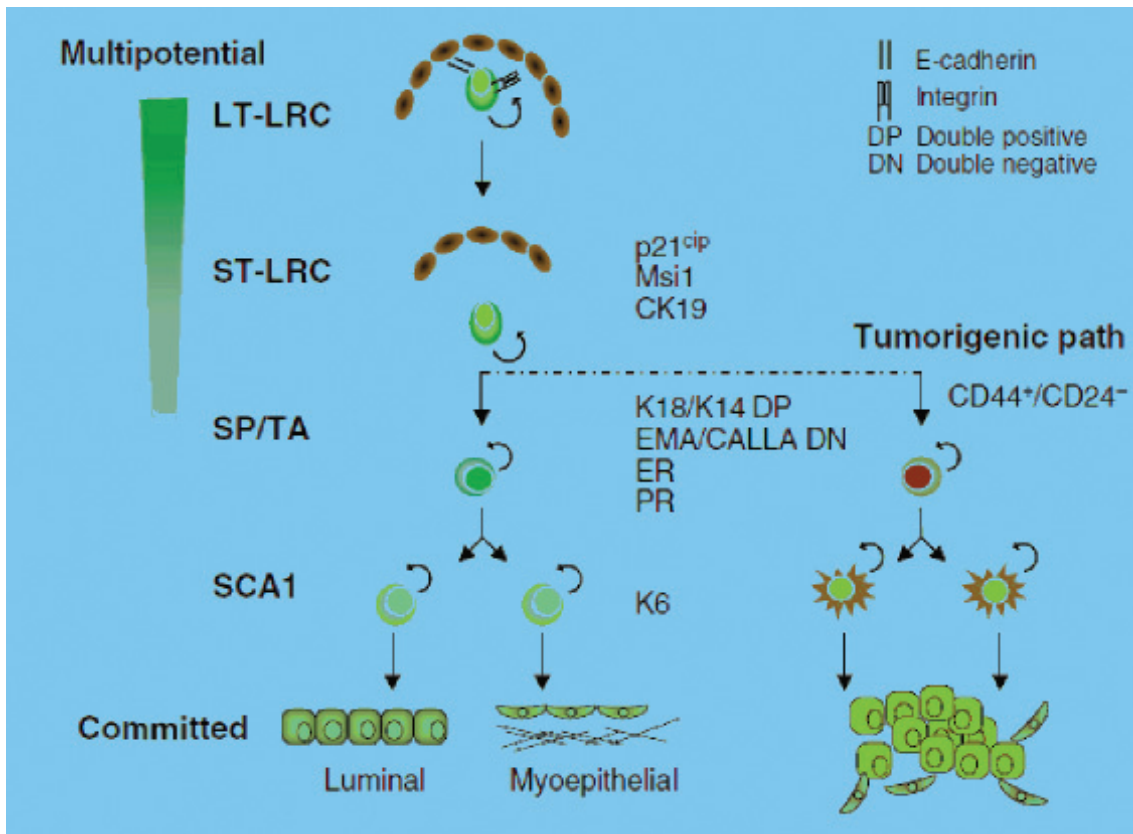


Figure 5: Mammary gland stem/progenitor-cell fate (Woodward et al., 2005).

Cancer biology

Secretory organ, including mammary gland, composes of 2 epithelial cell types. There is an inner luminal cell layer and an incomplete outer myoepithelial cell layer. Since the myoepithelium is incomplete, some luminal cells reach the basement membrane. The myoepithelial cells attached to the basement membrane by hemidesmosomes and to adjacent luminal epithelial cells by desmosomes. Although the myoepithelial cell involves to mammary tumors, however, it cell is the Cinderella of mammary biology when compares to the luminal epithelial cell (Lakhani and O'Hare, 2000). So far, the luminal epithelial cell is an ugly sister because they believe that it related to most human breast cancer.

The position of myoepithelial cells is an ideal situation to control many aspects of luminal epithelial cells functions. They could regulate polarity, electrolyte and fluid flow, filter and process signals of endocrine and paracrine nature. They perhaps act as an intermediary in such signaling processes by passing information both inwards and outwards in a paracrine fashion or via intra-epithelial gap-junctions (Locke et al., 2000).

The interaction between stroma, extracellular matrix (ECM) and epithelium may drive mammary gland development and function. There are 2 assumptions in chemical carcinogenesis of the mammary gland; 1) carcinogens induce neoplasia by causing mutations in the epithelial cells DNA and 2) the alterations of tissue architecture observed in neoplasm are consequence of the primary mutational event.

Maffini et al. (2004) applied N-nitrosomethylurea (NMU) to Wistar-Furth rat mammary tissue recombination model. They aimed to determine whether the primary target of the carcinogen is the epithelium, the stroma or both tissue compartments. In their experiment, they observed that neoplastic transformation of the mammary epithelial cells occurred only when the stroma was exposed to NMU. It was regardless of whether or not the epithelial cells were exposed to the carcinogen.

The previous studied published an experiment that the crucial obligatory target in chemical carcinogenesis of the rat mammary gland was not the mammary epithelial cell but the mammary stromal cell (Maffini et al., 2004). There is an opposite experiment of Medina and Kittrell (2005) who could not demonstrate a tumorigenic effect of carcinogen-treated stroma in the mouse model. They purposed that stroma is not a major target in 7, 12 dimethylbenzanthracene (DMBA)-mediated tumorigenesis of mouse mammary pre-neoplasia.

It may conclude that carcinogen-induced alterations in stroma can influence tumorigenesis in some, but not all. The evidence belongs to Medina and Kittrell (2005) provides the information of the events that promote or progress initiated cells. They also found that mammary epithelial cells are targets for chemical carcinogens and that effect on the stroma alone are not the keyfactors in some mammary tumor models. However, Maffini et al., (2005) used a tissue recombination model and observed that the stroma plays a crucial role in mammary gland carcinogenesis. As susceptibility to carcinogenesis decreases, the ability of the stroma to reprogram neoplastic epithelial cells increases.

Cancer biomarkers

The AE1/AE3 recognized a wide spectrum of cytokeratins that are expressed during epithelial cell differentiation. Compared with other epithelial markers, such as the epithelial membrane antigen (EMA), AE1/AE3 is more specific and has been widely used for clinical determination of the epithelial origin of malignant cells (Bussolati et al., 1986; Bonnie, 2002). Vimentin is a member of intermediate filaments which are parts of cytoskeleton in cytoplasmic matrix world. Normal canine mammary tissues, mixed tumors or complex adenoma are immunopositive to vimentin in every type of myoepithelial cells and mesenchymal tissues (Destexhe et al., 1993; Rabanal and Else, 1994). Therefore, vimentin can be used as a myoepithelial cells and mesenchymal cells markers.

The function of p53, tumor suppressor, is a principal mediator of growth arrest, senescence and apoptosis in response to a broad array of cellular damage (Levine, 1997; Wu and Levine, 1997). Overexpression of p53 protein has been reported in feline and canine mammary gland tumors and squamous cell carcinomas (Murakami et al., 2000; Lee, 2004), and in human malignant epithelial tumors of the breast (Baccouche et al., 2003), stomach (Kasper et al., 1999), kidney (Haitel et al., 2000), and liver (Endo et al., 2000). p53 gene mutations and protein overexpression using the PAb240 anti-p53 antibody were useful predictors of increased malignant potential and poor prognosis in canine mammary tumors (Lee et al., 2004). p63, a recently characterized p53 homologue nuclear transcription factor, is necessary to maintain an epithelial stem cell population (Ribeiro-Silva et al., 2003). It is consistently expressed in basal cells of several types of multilayered epithelial organs (Gama et al., 2003). p63 is a sensitive and specific myoepithelial markers in canine mammary tumors (Batistatou et al., 2003). Reis-Filho et al., (2002) reported that p63 positive myoepithelial cells observed more frequently in ductal carcinoma in situ (DCIS) cases. However, they concluded these presences could not be used as a criterion to rule out invasion in breast fine needle aspiration biopsy (FNABs) because they are present in up to 60% of invasive cases.

In summary

Prognosis and measurement of therapeutic responses of cancer is a major topic of interest in current cancer research. However, early detection and tumor differentiation are still important. Understanding the cancer biology and molecular biology of cancer may facilitate the linkage between diagnosis, prognosis, therapy, and survival time. For example, AE1/AE3 and Vimentin may be used as specific biomarkers to distinguish the mammary stem cell origin. Another application is inhibition of estrogen receptor at the selective binding site. Consequently, these selective drugs inhibit estrogen-

stimulated breast cancer cell growth. In addition, they may interrupt cell cycle and induce apoptosis regulatory gene expression.

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