Introduction

The fertilized oocyte is a totipotent stem cell, capable of giving rise to all the cell types of the embryo and the tropheoblast (1,2). Even after the first few divisions, embryonic cells give rise to totipotent stem cells, those capable of recreating an entire organism (3). Twinning is often a result of early embryo splitting and subsequent totipotent development (4,5). Embryonic germ cells and embryonic/carcinoma cells are each types of pluripotent stem cells that can be isolated from embryonic or fetal tissue or germ cell tumors. These pluripotent stem cells can be grown in culture to some extent, using feeder layers and growth factors to maintain differentiation capacity. Pluripotent stem cells have a restricted differentiation capacity as compared with totipotent stem cells. There are a plethora of recent reports of both totipotent and pluripotent mammalian stem cells growing in culture: Some of these have been used for mammalian cloning experiments (6). Each tissue, as it differentiates, gives rise to the multipotent stem cells of the body (7). For example, the hematopoietic stem cell is capable of giving rise to all of the cells in the blood (8,9). All stem cells have the property of giving rise to additional stem cells when they divide. This property is self-renewal (10). As self-renewal occurs, cells confront a decision point. At this point, cells commit to differentiate and eventually stop dividing, undergoing senescence or apoptosis, or continue dividing (9,11). When the decision point results in self-renewal, it permits a nearly immortal lifespan for the stem cell (12). The mechanism underlying the self-renewal decision point is a subject of active investigation (13,14). Some stem cells, such as hematopoietic stem cells and breast cancer, where are we? A concise review of literature

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SUMMARY: Stem cells and breast cancer, where are we? A concise review of literature.

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There is an analogy between embryogenesis and cancer and the attention is on increasing the rate of cell division and on a small percentage of perennial cells. The key to understanding is to be found in the properties of these cells developed in the form of perennial totipotency, multipotency and unipotency. The normal life cycle involves epigenetic mechanisms that are deregulated in cancer cells; these tumor cells appear to belong to deregulation since its progeny. Here is a review of the literature on embryogenesis of the breast, endocrine system interactions and the proper development and functioning of the various cell lines and to the importance of cancer stem cells.

KEY WORDS: Breast - Embryogenesis - Anatomy of the gland - Stem cells - Cancer.

Mammella - Embriogenesi - Anatomia della ghiandola - Cellule staminali - Cancro.
Breast anatomy

In 1840 Astley Paston Cooper published The Anatomy of the Breast (27). The striking plates in this classical text are based on the author’s studies of the breasts of seven previously lactating cadavers through dyed paraffin injection. The illustrations identified numerous structures in the vascular and ductile system and clarified, for the first time, the gross anatomy of the drainage network. The staining techniques were apparently very advanced for that era but crude when compared to modern imaging methods; the wax may have changed and displaced some of the delicate ductile structures of the breast. Moreover, studying a non-lactating breast from a cadaver does not reveal its normal, hormone-mediated growth and development. The gross anatomy of the human breast shows it to be one of the only organs not fully developed at birth. The breast changes in size, shape, and function through puberty, pregnancy, and during and after lactation (28,29). Breast growth and development involve two distinguishable processes: organogenesis (ductile and lobular growth) and lactogenesis. In the developing mammary gland, three cell lineages have been described: myoepithelial cells that form a basal cell layer, ductal epithelial cells, and milk producing alveolar cells (30-32). Although transplantation studies in mice have demonstrated that most mammary cells have a limited capacity for self-renewal, clonal populations that can recapitulate the entire functional repertoire of the gland have been identified (33,34). In an elegant study, human mammary epithelial cells derived from reduction mammoplasties were used to generate non-differentiated spheroids (designated mammospheres) in cell culture and demonstrate the presence of three mammary cell lineages. More importantly, the cells in the mammospheres were clonally derived, providing evidence for a single pluripotent stem cell (35). These same approaches are being used to isolate and characterize breast cancer stem cells (35).

Breast embryology

From the fifth to seventh week of pregnancy, a human fetus develops a mammary ridge, which rises from the axilla to the inguinal region (36). By the sixth gestational week, this ridge depresses into the pectoral region, forming primary breast buds (37). At birth the main lactiferous ducts are present as well as the nipples and areola. After puberty, estrogen secretion at each menstrual cycle stimulates proliferation and active growth of breast tissue. Breast development proceeds with growth of the ductile system and the formation of ductile buds. Surrounding fat pads also develop, giving the breast size and shape unrelated to functional capacity (38-40). Estrogen is a potent mammary mitogen that has numerous salutary systemic effects (41): estradiol, the most active form, decreases risk of coronary artery disease in women between puberty and menopause, a decrease in risk that is not observed in postmenopausal women (42). Experimental studies have showed exogenous estrogen can preserve endothelium critical for coronary artery dilation, reduce infant size, decrease the occurrence of ventricular arrhythmias, and protect against ischemia reperfusion injury. Estradiol is also a neuroprotective and neurotrophic factor: it has a positive influence on memory and cognition and may decrease the risk of Alzheimer disease and stroke (44,44bis). Finally, estrogen receptor immune staining has enabled observation of hormonal effects on osteoblasts on the medullary bone surface. Such studies show that estrogen receptors are present in osteogenic cells and suggest that estrogen directly acts on medullary bone ostogenesis (45). In spite of all these positive activities, exogenous estrogens bring a risk of neoplasia in responsive tissues, probably because of their potent activity as mitogens (47-49). Obesity has been associated with breast cancer risk (50). Because adipose tissue se-
cretes estrogens, the mechanism through which it acts may be by accumulation of excess estrogen (51). Breast tissue is exquisitely sensitive to the hormonal changes of early pregnancy (52). Many women report breast tenderness as a first sign of pregnancy. The human breast is capable of lactation from 16 weeks post-fertilization, with differing rates of growth and breast development before and after parturition (53). During the first trimester of pregnancy, mammary epithelial cells proliferate and duct cells branch in response to estrogen. The breast duct epithelium proliferates into the breast fat pads where end buds develop into secretory alveoli in response to human placental lactogen, human chorionic gonadotropin, and prolactin (54-56). While progesterone stimulates an increase in the size of the lobes and lobules, somatotropin and ACTH interact with prolactin and progesterone fostering maimorphogenesis. During the second trimester, there is further enlargement of the duct system and additional growth of the lobules. At approximately 12 weeks, a secretory substance that is similar to colostrum becomes visible in the acini. Subsequent prolactin production from the anterior pituitary together with placental lactogen triggers mammary alveolar differentiation, followed by the glandular secretion of colostrum. The alveoli then become distended with colostrum (57). The dozen or so lactiferous sinuses radiate from the areola, draining into the nipple.

Soil and seed

In 1889, the English physician Stephen Paget introduced the “soil and seed” hypothesis of metastasis to English-speaking medicine, by crediting the idea to Fuchs (58). In Paget’s study of 735 fatal cases of breast cancer, he concludes that the distribution of metastases cannot be due to chance alone and that different tissues provide optimal conditions for the growth of specific cancers. He noticed that patients with primary breast cancers had secondary tumors that developed preferentially in regional lymph nodes, bone marrow, lung, and liver (59). In the “soil and seed” metaphor, the “soil” refers to the secondary site of tumor development, and perhaps the chemical signals produced in the microenvironment at the potential site of metastasis (60,61). The “seed” is the ostensible stem cell or tumor initiating cell from the primary tumor (62). Genetic variations that affect signaling molecules in the metastatic microenvironment can impact the “soil” (63,64). Over expression of cellular migration factors could encourage a faster movement or more rapid growth of tumor cells and could challenge the capacity of immune surveillance to keep a tumor in check. Up regulation of cell surface receptors on tumor cells could provide a propitious key to unlock a fertile new “soil” for them. Mutations that affect the autocrine and paracrine signaling, in for example chemokine receptors and their effector molecules, could play an important role on tumor growth exacerbation or inhibition. Relief of immune inhibition is known to play an important role in immune surveillance and could be responsible for a significant amount of tumor escape. Variations that augment inhibitory factors could have a protective effect by decreasing the rate of tumorigenesis. In a variation of this idea, called the “homing” hypothesis, a secondary signal secreted by cells at the future metastatic sites “calls” the tumor cells and permits them to proliferate there (65,66). In this hypothesis, the “seed” produces cell surface receptors able to recognize the site demarcated by the “soil”. Although the mechanisms of tissue specificity remain obscure, researchers have focused on small messenger molecules as attractants and larger cell surface receptors guiding the tumor-initiating cells or “seeds”. Muller (66) and Murphy (67) have each focused on chemokines and chemokine receptors as viable candidates for “soil and seed” signaling. Murphy specifically proposes a “spatial and temporal code” made up of specific combinations of such molecules, and others being responsible for neo vascularization, metastasis, and immune surveillance avoidance. Chemokines and their receptors have been implicated in three distinct stages of neoplasia: transformation, tumor development, and metastasis. Expression of specific receptors on KSHV-infected B-lymphocytes and the expression of specific receptors in HIV patients, such as CCR5 or CXCR4, are sufficient to dictate the future course of their respective diseases. Other cancers may involve specific chemokine receptor expression (68).

Importance of the stroma

Luminal epithelial cells interact with a surrounding microenvironment. In part, these interactions direct normal mammary gland development. Altering luminal epithelial cell interaction with the extracellular matrix and local microenvironment might induce abnormal intracellular signaling pathways that affect the development and progression of breast tumors. A central signal pathway for mammary gland development and breast cancer progression involves the expression of estrogen receptors (69-70). In a study using cultured nonmalignant mammary epithelial cells, the basement membrane molecules, laminin-1 and collagen-IV, were found to be involved in maintenance of estrogen receptor alpha expression (71). This response could be interfered through the disruption of cell–extracellular matrix adhesion. Phenotypically normal mammary epithelial cells have been used to dissect the promoter region of the ER alpha receptor involved in response to the basement membrane. A malignant cell line sharing a common lineage with nor-
Stem cells and breast cancer: where are we? A concise review of literature

Breast cancer stem cells

A University of Michigan group recently identified a small population of cancer stem cells in breast tumors that has changed the way many scientists view cancer (73,74). These cancer stem cells represent only 1% of the tumor and were the only cells in the tumor capable of transplanting the tumor into nude mice. This suggests that the terms cancer stem cells and tumor-initiating cells are functionally synonymous. Additional studies have presented data that long-established cell lines, even HeLa cells, contain a minor population of cells with some of the same tumor-initiating properties as stem cells (75,76). Many researchers now suspect that all cancers are composed of a mixture of stem cells and proliferative cells with a limited life span. The implications of this concept are far reaching. The rapid growth of many cancers following chemotherapy could result from the survival of cancer stem cells. This is paralleled in the body with the regrowth of hair due to the survival of hair follicles and the recovery of blood cells due to the survival of hematopoietic stem cells. Can these results be extrapolated to most or all solid tumors? Are there therapeutic approaches targeting these cancer stem cells with application to a wide array of cancers? These are critical questions remaining to be addressed in the cancer stem cell field. Researchers have known for decades that there exist a proportion of cells in a tumor capable of surviving radiation treatment and cytotoxic drug exposure (77). These cells are capable of DNA repair and can survive and reproduce under hypoxic conditions (78). Stem cells must also survive many genetic insults in the life of the individual and express drug transporters and DNA repair systems. Stem cells are necessarily refractory to programmed cell death and can be quiescent for long periods of time, all properties that would allow a cancer cell to resist standard therapeutic approaches (79-81).

Stem cells activated and cancer

Most stem cells in the body remain in a dormant state. These cells are surrounded by other, differentiated cells that are functionally synonymous. Additional studies have presented data that long-established cell lines, even HeLa cells, contain a minor population of cells with some of the same tumor-initiating properties as stem cells. Many researchers now suspect that all cancers are composed of a mixture of stem cells and proliferative cells with a limited life span. The implications of this concept are far reaching. The rapid growth of many cancers following chemotherapy could result from the survival of cancer stem cells. This is paralleled in the body with the regrowth of hair due to the survival of hair follicles and the recovery of blood cells due to the survival of hematopoietic stem cells. Can these results be extrapolated to most or all solid tumors? Are there therapeutic approaches targeting these cancer stem cells with application to a wide array of cancers? These are critical questions remaining to be addressed in the cancer stem cell field. Researchers have known for decades that there exist a proportion of cells in a tumor capable of surviving radiation treatment and cytotoxic drug exposure (77). These cells are capable of DNA repair and can survive and reproduce under hypoxic conditions (78). Stem cells must also survive many genetic insults in the life of the individual and express drug transporters and DNA repair systems. Stem cells are necessarily refractory to programmed cell death and can be quiescent for long periods of time, all properties that would allow a cancer cell to resist standard therapeutic approaches (79-81).

Stem cell activated and specific cancers

While a model of a small population of self-renewing cells as the key to all cancers is an attractive idea, can the model be extended to the wide variety of tumor types and specific agents implicated in causing these tumors? Three distinct types of cancers have been described: embryonic, conditional growth, and renewal (81,82). Embryonic cancers derive from rapidly dividing embryonic tissue and therefore contain a population of actively dividing stem cells. The prototype embryonic cancer is retinoblastoma, but Wilm's tumor, Ewing's sarcoma,
childhood bone and brain cancers each fall under this rubric. Retinoblastoma arises in embryonic cells in the developing eye, known as retinoblasts. These cells are highly proliferative and are naturally activated stem cells. The mutation or loss of the RB1 gene transforms these embryonic stem cells into cancer stem cells. These cells would be expected to have lost the response to growth regulatory signals shutting down the stem cell, once the development of the eye was complete. Other childhood cancers could involve multipotent stem cells in other tissues suffering genetic damage during development. These cancers require the fewest number of genetic events, because the target cell is a fully activated stem cell. Stem cells can also be activated during the normal process of expansion of certain tissues due to the action of hormones, particularly during puberty (conditional growth tissues). Examples would be the breast and prostate, which undergo dramatic expansion and growth during puberty under the control of estrogen, testosterone, and other hormones (83). Activated stem cells in the breast would be the target cell for breast cancer. Inactivation of specific tumor suppressor genes, like TP53, would transform the breast tissue stem cells into unregulated cells, initially resulting in pre-malignant lesions. There is good evidence that p53 haplo insufficiency accelerates cancer onset, perhaps by diminishing DNA repair, thereby facilitating mutation of activated stem cells. These uncontrolled stem cells would be the target for additional events leading to the progression of the pre-malignant lesion into a fully malignant tumor. Consistent with this model, the major risk factors for breast cancer involve hormonal and reproductive variables (84). Women with an early onset of puberty have a higher rate of breast and ovarian cancers than those with later menarche. Premenarche, especially those starting at a relatively younger age, decrease cancer risk. These factors influence neither the number nor activation of breast stem cells. Several drugs able to decrease cancer risk and/or cancer reoccurrence have been developed. These include agents reducing the production of estrogen or blocking its action on cells. Similarly, the removal of the ovaries reduces cancer risk in those with an extensive family history of breast and ovarian cancer (85). Mutations in the BRCA1 and BRCA2 genes dramatically increase the risk of breast cancer. However, unlike many other tumor suppressor genes, BRCA1 or BRCA2 mutations are not commonly found in sporadic breast tumors. The BRCA1 and BRCA2 proteins play a role in the DNA repair process. These mutations can be thought of as increasing the probability of genetic events associated with tumor progression. Since these genes are not in the main pathway leading to the breast cancer, they are not frequently mutated in sporadic tumors, but do increase an individual’s risk of disease, when mutated.

Current paradigms envision a small stem cell compartment possessing cells with the capacity for perpetual self-renewal existing alongside a much larger proliferative compartment whose cells have a finite ability to proliferate before presumably arresting and/or undergoing apoptosis. These paradigms can explain the low cloning efficiency of most cell lines, their inefficiency at colony formation in soft agar, and their limited tumorigenicity. But none can explain how the stem cells remain a constant fraction of the total population, if indeed they do. Any proposal will require stem cells to divide slowly and must recognize that in a cell line derived from a solid tumor the number of cells undergoing apoptosis is relatively small. One possibility is that there is an interchange of cells between a proliferative compartment and the stem cell pool. That such an interchange might occur is not improbable since the cell line almost certainly originated from a stem cell with a proliferative advantage.

Cancer therapy “causes” cancers

If most solid tumors are composed of a minor population of self-renewing (stem) cells and a large fraction of non-renewing cells, cancer therapy failure following radiation and chemotherapy treatments is not the result of a rare cell evolving from within the tumor, but the regrowth of the cancer stem cells. Of course, tumor stem cells could accumulate genetic changes rendering them even more drug resistant, radiation resistant, or aneuploid. Because cures are achieved for many types of cancer, the cancer stem cells must be eliminated by a given therapeutic strategy. Mature, committed stroma in the tumor microenvironment are likely to play a role in supporting or stimulating the stem cells, forming a “tumor niche”. The rapid regression of the tumor could lead to disruption of the tumor niche and the elimination of the cancer stem cells. Immune surveillance is clearly important in many cancers (86), and reducing the mass of the tumor may allow the immune system to efficiently recognize the remaining cells. Targeted therapies directly suppressing or killing tumor stem cells may synergize with established therapies to provide increased efficacy. Angiogenesis likely to be critical to provide blood supply to the tumor stem cells, and strategies to inhibit the development of blood vessels are likely to be effective (87). One of the protective mechanisms of stem cells against toxins is the expression of one or more ATP-binding cassette (ABC) efflux transporters. These pumps protect stem cells from xenobiotic toxins (88). The ABCG2 and ABCB1/MDR1 genes are expressed in the majority of stem cells and in most tumor stem cells (89,90). These transporters can efflux fluorescent dyes such as rhodamine and Hoechst 33342, and this property allows stem cells to be separated from non stem cells on a cell sorter (91). The combined use of chemotherapy drugs and
ABC transporter inhibitors could be used to specifically target cancer stem cells (92). There are highly specific inhibitors of ABCB1 in clinical use and ABCG2 inhibitors in development (93). Transporter inhibition therapies are likely to have toxic effects on the patient’s normal stem cells, and both ABCG2 and ABCB1 play a role in the blood–brain barrier. Therefore, this approach would have to be carefully adjusted to avoid excessive toxicity.

How the stem cells can suggest new approaches

Another approach to inhibiting cancer stem cells is to target the proteins essential for the growth and maintenance of stem cells. Because of the fundamental research in Drosophila, mice, C. elegans, zebrafish, and other developmental systems, a tremendous amount is known about the growth regulatory pathways functioning in embryonic cells (94). One pathway, controlled by the Hedgehog (HH) and WNT signaling molecules, contains several genes functioning as either tumor suppressor genes or oncogenes (95). For example Patched (PTCH) is the receptor for HH molecules and PTCH is mutated in patients with nevoid basal cell carcinoma syndrome (96). The PTCH gene is also mutated in virtually all sporadic basal cell carcinomas and in some medulloblastomas, rhabdomyomas, and rhabdomyosarcomas (97). The mammalian HH genes (IHH, SHH, DHH) are overexpressed in a large number of cancers including small cell lung, pancreas, gastric, breast, and prostate (98–101). HH ligand overexpression and PTCH mutation both have the effect of constitutive expression of smoothened (SMO), a G-protein coupled receptor family protein, a key signaling protein in the pathway. Constitutive HH expression could be an important component to the stem cell activation in many cancers and therefore represents an attractive target for cancer therapy. Cyclopamine is a compound discovered in the Corn Lily (Veratrum californicum), a plant teratogenic to sheep (102). Cyclopamine binds to and inhibits the SMO protein and suppresses the growth of cells and tumors with activated HH signaling (103). Human prostate tumor cell lines grown as xenografts in mice were eliminated following 21 days of treatment with cyclopamine (104), and UV-induced basal cell carcinomas were suppressed in mice given low levels of cyclopamine in their drinking water (105). Recently it has been demonstrated that vitamin D3 is a critical signaling molecule between PTCH and SMO. PTCH normally secretes vitamin D3 and this molecule inhibits SMO on that cell as well as adjacent cells (106). HHs inhibit this secretion and cause a release from repression. Cyclopamine competes for the binding of vitamin D3 on SMO and so appears to act in a similar manner. It is likely that vitamin D3 and/or other steroidal analogues could have a similar effect and be candidate anticancer compound. Other pathways critical to embryonic development and potentially important in cancer have also been described and include the WNT and NOTCH pathways. A number of experimental inhibitors of these pathways have been developed. These pathways are also the subject of drug development for a number of conditions and one example is the drug MK0752, which is in clinical trials for the treatment of acute T-cell lymphoblastic leukemia, myelodysplastic leukemia, chronic lymphocytic leukemia, and myelodysplastic syndrome. Gamma-secretase is required for the maturation of the NOTCH protein, and g-secretase inhibitors have been developed for a number of pathological conditions. In a recent study, one such gamma secretase inhibitor was effective in the inhibition of stem-like cells in embryonal brain tumors (107).

Future research

The identification of cancer stem cells in solid tumors has important implications for basic cancer research. Most analyses of tumors such as gene expression, microarray, proteomic, and many phenotypic assays have been performed on whole tumors and have not revealed data on the small fraction of tumor stem cells. In addition, screens for cancer cytotoxic drugs have involved cell cultures treated over short time periods (108). Drugs specifically targeting cancer stem cells may display modest activity in short-term proliferation assays and be rejected for further follow-up study in animals or humans. Several important questions remain from the current data. Are current markers for cancer stem cells adequate? Do the side population cells isolated from cell lines (109) bear a relationship to cancer stem cells? In principle in any permanent cell line there must be self-renewing cell population. If the characterization of the SP cells in cell lines could be applied to cancer stem cells, this could advance understanding rapidly. One property of cancer cells is the ability, like stem cells, to grow in soft agar cultures (110). It has been found that only a fraction of cells in a tumor cell culture can form a colony in soft agar. Are the cells forming soft agar colonies cancer stem cells? This would be a logical conclusion from the information at hand. It is known that the clonogenicity varies substantially between different tumor cell lines. If clonogenicity is related to self renewing cells in the culture then assays based on colony formation may be useful for screening for stem-cell targeting therapies. Such assays would be more time consuming and have a lower throughput, but might in the end prove more informative.
Conclusions

The identification of cancer stem cells in certain solid tumors has created considerable excitement in the field and generated new research possibilities. If these results can be extended to most or all cancer cell types, a considerable advancement in understanding will be achieved. Separating the cancer process into a stem cell activation phase and a tumor progression phase allows an understanding of how the myriad cancer causing agents can have their effect on specific tissues. Research efforts directed to understand the growth requirements of tumor stem cells as well identify tumor stem cell antigens could lead to new targeted approaches. The isolation and characterization of cancer stem cells from other tissues will be a great aid in cancer diagnostics, cancer prevention, and therapeutics. Normal stem cell-based approaches are being intensively developed as an aid in replacing damaged cells and tissues in the body. The insights from the growth and characterization of normal stem cells will aid in the understanding of cancer stem cells and in new therapeutic approaches.

References