G Chir Vol. 32 - n. 10 - pp. 438-446
October 2011

mini-review

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

A. SANGUINETTI[1], G. BISTONI[2], N. AVENIA[1]

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

A. SANGUINETTI, G. BISTONI, N. AVENIA

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 unipotent. 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 Delna the proper development and functioning of the various cell lines and to the importance of cancer stem cells.

RIASSUNTO: **Cancro della mammella e cellule staminali, dove siamo? Concisa revisione della letteratura.**

A. SANGUINETTI, G. BISTONI, N. AVENIA

Esiste una analogia tra embriogenesi e cancro e l'attenzione ricade sull'aumento della velocità di divisione cellulare e su di una piccola percentuale di cellule perenni. La chiave di lettura è da ricercare nelle proprietà di queste cellule perenni: totipotenza, multipotenza ed unipotenza. Il normale ciclo biologico comporta meccanismi epigenetici che risultano deregolati nelle cellule neoplastiche; queste deregolazioni sembrano appartenere alla cellula neoplastica sin dalla sua progenie. Riportiamo una revisione della letteratura in merito all'embriogenesi della mammella, alle possibili interazioni deln sistema endocrino sul corretto sviluppo e funzionamento delle varie linee cellulari ed alla importanza delle cellule staminali tumorali.

KEY WORDS: Breast - Embryogenesis - Anatomy of the gland - Stem cells - Cancer.
Mammella - Embriogenesi - Anatomia della ghiandola - Cellule staminali - Cancro.

Introduction

The fertilized oocyte is a totipotent stem cell, capable of giving rise to all the cell types of the embryo and the trophoblast (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 cel-

[1] University of Perugia, Italy
Endocrine Surgery Unit
[2] "Sapienza" University of Rome, Italy
Plastic and Reconstructive Surgery Department

© Copyright 2011, CIC Edizioni Internazionali, Roma

© CIC Edizioni Internazionali

ls, maintain relatively rapid division, to produce the large numbers of lymphocytes and red blood cells required by the body (15). This is true also of the rapidly cycling cells of the broad band at the middle of the intestinal crypt (16). Other stem cells, such as those in the skin and colon, maintain a slow and constant growth, replenishing the tissue (17,18), and yet other stem cells in the brain and most other tissues remain quiescent and are only activated when stimulated by tissue damage or hormonal exposure (19). When a cell is sufficiently differentiated that it still proliferates, but gives rise to only one cell type, one can speak of it as determined (20), canalized (21), or unipotent (22). An example would be a megakaryocyte that can give rise only to platelets. The only subsequent steps in multicellular organism development are "terminal differentiation" and senescence. Whether or not de-differentiation or trans-differentiation occurs, once cells have progressed through their developmental path, remains a matter of controversy (21,22). Stem cell migration is a normal part of mammalian development and is a particular characteristic of the early genital ridge (**1**). The regulation of stem cell division in the adult is controlled through epigenetic mechanisms, such as DNA methylation and histone acetylation (22). Orderly "replenishment" of tissues requires cell division and the capability of repairing some damaged tissues, as in liver regeneration (23). It is generally acknowledged that the latter requires a certain amount of cellular "reprogramming". A central feature of the involvement of stem cells in cancer is the dysregulation of the epigenetic control of stem cell proliferation (24-26).

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-adherent 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 (30). 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 infarct 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 osteogenesis (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-

© CIC Edizioni Internazionali

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 mammogenesis. 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-

© CIC Edizioni Internazionali

mal mammary cells provide the insight that over expression of ER alpha accompanied unresponsiveness to normal basement membrane regulation found in those malignant cells. One interpretation of these data is that crosstalk between different signaling pathways is a requirement in the constitution or proper functional tissue organization and when this cell-cell interaction goes awry, the malignant phenotype may result. Normal tissue homeostasis is maintained by dynamic interactions between epithelial cells and their microenvironment. As tissue becomes cancerous, there are reciprocal interactions between neoplastic cells, adjacent normal cells such as stroma and endothelium, and their microenvironments. The current dominant paradigm wherein multiple genetic lesions provide both the impetus for, and the Achilles heel of, cancer might be inadequate to understand cancer as a disease process.

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 regrowth 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 within the tissue microenvironment often described as a "niche". The cells of the niche regulate the stem cells via cell–cell contacts, interactions with the extracellular matrix, and secretion of inhibitory factors. The disruption of the niche microenvironment, through infection, inflammation, tissue damage, or chemical assault, can activate the division of the stem cells The activated stem cell gives rise to additional stem cells as well as cells committed to differentiate. These new cells repair the damaged area of tissue, and the stem cells return to their quiescent state. Virtually all of the agents described to confer a risk for cancer also result in tissue alteration (and therefore activation of stem cells) including radiation, wounding, chemical damage, infectious agents, and inflammation. Cancer can be thought of as a disease resulting from the abnormal growth of stem cells, resulting from chronic activation of stem cells (caused by disruption of the niche) and leading to the long-term proliferation of the stem cells. Upon tissue damage they divide and repair the damage. However, chronic tissue damage leads to continually divided (activated) stem cells that are the target for later mutagenic events that create a cancer stem cell and a tumor resulting in genetic damage to the cell (mutation of tumor suppressor genes and activation of oncogenes). This disruption of the niche and subsequent stem cell activation could occur by hormonal stimulation, tissue damage caused by inflammation, radiation, chemicals, or infections, or inactivation of certain tumor suppressor genes. The abnormally dividing stem cell could be subject to additional genetic events leading to autonomous growth, the loss of cell cycle regulation, and resistance to apoptosis all well understood properties of cancer cells (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,

© CIC Edizioni Internazionali

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. Pregnancies, especially those starting at a relatively younger age, decrease cancer risk. These factors influence either the number or 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. Angiogenesisis 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). TheABCG2 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 Hoecht 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

© CIC Edizioni Internazionali

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, myelogenous 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.

© CIC Edizioni Internazionali

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 insight from the growth and characterization of normal stem cells will aid in the understanding of cancer stem cells and in new therapeutic approaches.

References

1. Gilbert SF, Singer SR, Tyler MS, Kozlowski R N. (2006). 'Developmental biology.' Sinauer Associates, Sunderland, MA. Goodell, M. A., Brose, K., Paradis, G., Conner, A. S., and Mulligan, R. C. (1996). Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 183, 1797–806.
2. Kimball JW. Kimball's biology pages. John W. Kimball [Andover, MA], 2003.
3. Seydoux G, Braun R E. Pathway to totipotency: lessons from germ cells. Cel 2006; 127, 891–904.
4. Hall J G. Twinning. Lancet 2003;362, 735–43.
5. Stevenson, R E, Hall J G. Human malformations and related anomalies.' Oxford University Press, Oxford, 2006.
6. Jaenisch R, Hochedlinger K, Blelloch R, Yamada Y, Baldwin K, Eggan K. Nuclear cloning, epigenetic reprogramming, and cellular differentiation. Cold Spring Harb Symp Quant Biol 2994;69, 19–27.
7. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 2000;6, 1229–34.
8. Alberts B. (2008). "Molecular biology of the cell. Reference edition." Garland Science, New York. Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J., and Clarke, M. F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100, 3983–8.
9. Schlessinger, D, and Van Zant G. Does functional depletion of stem cells drive aging? Mech Ageing Dev 2001;122, 1537–53.
10. McKenzie JL, Gan OI, Doedens M, Wang JC, Dick JE. Individual stem cells with highly variable proliferation and self-renewal propertiescomprise the human hematopoietic stem cell compartment. Nat Immunol 2006;7,1225–33.
11. Morrison SJ, Shah NM, Anderson D. Regulatory mechanisms in stem cell biology. Cell 1997;88, 287–98.
12. Huang X, Cho S, Spangrude GJ. Hematopoietic stem cells: generation and self-renewal. Cell Death Differ 2007;14, 1851–9.
13. Potten CS. Regeneration in epithelial proliferative units as exemplified by small intestinal crypts. CIBA Found Symp 1991;160, 54–71; discussion 71–6.
14. Alonso L, Fuchs E. Stem cells in the skin: waste not, Wnt not. Genes Dev 2003;17, 118 200.
15. Bjerknes M. Expansion of mutant stem cell populations in the human colon. J Theor Biol 1996;178, 381–5.
16. Schatton T, Frank MH. Cancer stem cells and human malignant melanoma. Pigment Cell Melanoma Res 2008; 21, 39–55.
17. Spemann H. U¨ ber die Determination der ersten Organanlagen des Amphibienembryonen. Zool Jahr Suppl 1918;15, 1–48.
18. Waddington CH. Principles of development and differentiation. Macmillan, London, 1966.
19. Blanpain C, Horsley V, Fuchs E. Epithelial stem cells: turning over new leaves. Cell 2007;128, 445–58.
20. Leri A, Kajstura J, Anversa P. Cardiac stem cells and mechanisms of myocardial regeneration. Physiol Rev 2005;85, 1373–416.
21. Raff M. Adult stem cell plasticity: fact or artifact? Annu Rev Cell Dev Biol 2003;19, 1–22.
22. Do JT, Han DW, Scholer HR. Reprogramming somatic gene activity by fusion with pluripotent cells. Stem Cell Rev 2006;2, 257–64.
23. Michalopoulos GK. Liver regeneration. J Cell Physiol 2007;213, 286–300.
24. Bapat SA. Evolution of cancer stem cells. Semin Cancer Biol 2007;17, 204–13.
25. Bengochea A, de Souza M M, Lefrancois L, Le Roux E, Galy O, Chemin I, Kim M, Wands JR, Trepo C, Hainaut P, Scoazec JY, Vitvitski L, Merle P. Common dysregulation of Wnt/Frizzled receptor elements in human hepatocellular carcinoma. Br J Cancer 2008;99, 143–50.
26. Karpinets TV, Foy BD. Tumorigenesis: the adaptation of mammalian cells to sustained stress environment by epigenetic alterations and succeeding matched mutations. Carcinogenesis 2005;26, 1323–34.
27. Cooper A. On the anatomy of the breast. Longman, Orme, Green, Brown, and Longmans, London, 1840.
28. Lawrence RA, Lawrence R A. Breastfeeding, a guide for the medical profession. Mosby, St. Louis 1985.
29. Creasy RK, Resnik R, Iams JD. Maternal–fetal medicine: principles and practice. Saunders, Philadelphia, PA, 2004.
30. Dontu G, Al-Hajj M, Abdallah WM, Clarke MF, Wicha MS. Stem cells in normal breast development and breast cancer. Cell Prolif 2003;36 Suppl 1, 59–72.
31. Dean M, Annilo T. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. Annu Rev Genom Hum Genet 2005; 6, 123–42.
32. Deugnier MA, Faraldo, MM, Janji B, Rousselle P, Thiery JP, Glukhova MA. EGF controls the in vivo developmental potential of a mammary epithelial cell line possessing progenitor properties. J Cell Biol 2002;159, 453–63.
33. Smith G H (2006). Mammary stem cells come of age, prospectively. Trends Mol Med 12, 287–9.
34. Smith G H, Boulanger CA. Mammary stem cell repertoire: new insights in aging epithelial populations. Mech Ageing Dev 2002;123, 1505–19.
35. Xiao Y, Ye Y, Yearsley K, Jones S, Barsky SH. The lymphovascular embolus of inflammatory breast cancer expresses a stem cell-like

© Edizioni Internazionali

phenotype. Am J Pathol 2008;173, 561–74.
36. Ameryckx L, Leunen M, Wylock P, Amy JJ. Breast problems in children and adolescents. Eur Clin Obstet Gynaecol 2005;1, 151–63.
37. Moore KL, Persaud TVN, Moore KL, Moore K L (1993). Study guide and review manual of human embryology. Saunders, Philadelphia, 1993.
38. Black RF, Jarman L, Simpson J. Lactation specialist self-study series. Jones and Bartlett, Sudbury, MA, 1998.
39. Clarke RB. Steroid receptors and proliferation in the human breast. Steroids 2003;68, 789–94.
40. Riordan J.Breastfeeding and human lactation. Jones and Bartlett, Sudbury, MA, 2005.
41. Ho K J, Liao JK. Nonnuclear actions of estrogen. Arterioscler Thromb Vasc Biol 2002;22, 1952–61.
42. Rossouw JE. Debate: The potential role of estrogen in the prevention of heart disease in women after menopause. Curr Control Trials Cardiovasc 2000;Med 1, 135–8.
43. McCullough LD, Alkayed NJ, Traystman RJ, Williams MJ, Hurn PD. Post ischemic estrogen reduces hypoperfusion and secondary ischemia after experimental stroke. Stroke 2001;32, 796–802.
44. Zhai P, Eurell TE, Cooke PS, Lubahn DB, Gross DR. Myocardial ischemia-reperfusion injury in estrogen receptor-alpha knockout and wild-type mice. Am J Physiol Heart Circ Physiol 2000a;278, H1640–7.
44bis. Zhai P, Eurell TE, Cotthaus R, Jeffery EH, Bahr JM, Gross, DR. Effect of estrogen on global myocardial ischemia-reperfusion injury in female rats. Am J Physiol Heart Circ Physiol 2000b. 279, H2766–75.
45. Jilka RL. Cytokines, bone remodeling, and estrogen deficiency: a 1998 update. Bone 1998; 23, 75–81.
46. Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC. Ovarian surface epithelium: biology, endocrinology, and pathology. Endocr Rev 2001;22, 255–88.
47. Jordan VC, Morrow M. Tamoxifen, raloxifene, and the prevention of breast cancer. Endocr Rev 1999;20, 253–78.
48. McLachlan, JA. Environmental signaling: what embryos and evolution teach us about endocrine disrupting chemicals. Endocr Rev 2001;22, 319–41.
49. Vona-Davis, L, Rose DP. Adipokines as endocrine, paracrine, and autocrine factors in breast cancer risk and progression. Endocr Relat Cancer 2007; 14, 189–206. Breast Cancer Stem Cells 191.
50. Chlebowski RT. Obesity and early-stage breast cancer. J Clin Oncol 2005;23, 1345–7.
51. Deroo BJ, Korach KS. Estrogen receptors and human disease. J Clin Invest 2006;116 561–70.
52. Leslie K K, Lange C A. Breast cancer and pregnancy. Obstet Gynecol Clin North Am 2005;32, 547–58.
53. Harrison RF, Biswas S. Maternal plasma, human placental lactogen, alphafetoprotein, prolactin and growth hormone in early pregnancy. Int J Gynaecol Obstet 1980;17, 471–6.
54. Parmar H, Cunha GR. Epithelial–stromal interactions in the mouse and human mammary gland in vivo. Endocr Relat Cancer 2004;11, 437–58.
55. Riordan J. Breastfeeding and human lactation. Jones and Bartlett, Sudbury, MA, 2005.
56. Buhimschi CS. Endocrinology of lactation. Obstet Gynecol Clin North Am 2004;31, 963–79, xii.
57. Fuchs E. Das Sarkom des Uvealtractus. Graefe's Arch Ophthalmol XII, 233, 1882.
58. Paget S. The distribution of secondary growths in cancer of the breast. Lancet 1889;133, 571–3.
59. Langley RR, Fidler IJ. Tumor cell-organ microenvironment interactions in the pathogenesis of cancer metastasis. Endocr Rev 2007;28, 297–321.
60. Strieter RM. Chemokines: not just leukocyte chemo attractants in the promotion of cancer. Nat Immunol 2001;2, 285–6.
61. Chung LW, Baseman A, Assikis V, Zhau, HE. Molecular insights into prostate cancer progression: the missing link of tumor microenvironment. J Urol 2005;173, 10–20.
62. Crawford NP, Hunter KW. New perspectives on hereditary influences in metastatic progression. Trends Genet 2006;22, 555–61.
63. Eccles SA, Welch DR. Metastasis: recent discoveries and novel treatment strategies. Lancet 2007; 369, 1742–57.
64. Hewitt RE, McMarlin A, Kleiner D, Wersto R, Martin P, Tsokos M, Stamp GW, Stetler-Stevenson W G. Validation of a model of colon cancer progression. J Pathol 2000;192, 446–54.
65. Stetler-Stevenson WG. The role of matrix metallo-proteinases in tumor invasion, metastasis, and angiogenesis. Surg Oncol Clin North Am 2001;10, 383–92, x.
66. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME. McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001; 410, 50–6.
67. Murphy PM.Chemokines and the molecular basis of cancer metastasis. N Engl J Med 2001;345, 833–5.
68. Strieter RM. Chemokines: not just leukocyte chemoattractants in the promotion of cancer. Nat Immunol 2001;2, 285–6.
69. Sternlicht MD. Key stages in mammary gland development: the cues that regulate ductal branching morphogenesis. Breast Cancer Res 2006;8, 201.
70. Gupta G P, Massague J. Cancer metastasis: building a framework. Cell 2006;127, 679–95.
71. Novaro V, Roskelley C D, Bissell MJ. Collagen-IV and laminin-1 regulate estrogen receptor alpha expression and function in mouse mammary epithelial cells. J Cell Sci 2003;116, 2975–86.
72. Novaro V, Radisky DC, Ramos Castro NE, Weisz A, Bissell MJ. Malignant mammary cells acquire independence from extracellular context for regulation of estrogen receptor alpha. Clin Cancer Res 2004;10, 402S–9S.
73. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci 2003;USA 100, 3983–8.
74. Hemmati HD, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind, DH, Bronner-Fraser M, Kornblum HI. Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci USA 2003; 100, 15178–83.
75. Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA, Brenner MK. A distinct "side population" of cells with high drug efflux capacity in human tumor cells. Proc Natl Acad Sci 2004;USA 101, 14228–33.
76. Kondo T, Setoguchi T, Taga T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proc Natl Acad Sci USA 2004;101, 781–6.
77. Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. Br J Cancer 1955;9, 539–49.
78. Teicher BA. Hypoxia and drug resistance. Cancer Metastasis Rev 1994;13, 139–68.
79. Kim M, Turnquist H, Jackson J, Sgagias M, Yan Y, Gong M, Dean M, Sharp JG, Cowan K. The multidrug resistance transporter ABCG2 (breast cancer resistance protein 1) effluxes Hoechst 33342 and is overexpressed in hematopoietic stem cells. Clin Cancer Res 2002;8, 22–8.
80. Zhou, S., Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa,M, Nakauchi H, Sorrentino BP.The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 2001; 7, 1028–34.

© CIC Edizioni Internazionali

81. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. Nature 2004;432, 324–31.
82. Knudson AG. Antioncogenes and human cancer. Proc Natl Acad Sci USA 1993;90,10914–21.
83. Trichopoulos, D, Lagiou, P, Adami HO. Towards an integrated model for breast cancer etiology: the crucial role of the number of mammary tissue-specific stem cells. Breast Cancer Res 2005; 7, 13–7.
84. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, Mulvihill JJ. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst 1989;81, 1879–86.
85. Kauff ND, Satagopan JM, Robson ME, Scheuer L, Hensley M, Hudis C A, Ellis NA, Boyd, J, Borgen PI, Barakat RR, Norton L, Castiel M, Nafa K, Offit K. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2002;346, 1609–15.
86. Nakachi K, Hayashi T, Imai K, Kusunoki Y. Perspectives on cancer immuno-epidemiology. Cancer Sci 2004;95, 921–9.
87. Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol 2002;29, 15–8.
88. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2002;2, 48–58.
89. Kim M, Turnquist H, Jackson J, Sgagias M, Yan Y, Gong M, Dean M, Sharp JG, Cowa K. The multidrug resistance transporter ABCG2 (breast cancer resistance protein 1) effluxes Hoechst 33342 and is overexpressed in hematopoietic stem cells. Clin Cancer Res 2002;8, 22–8.
90. Scharenberg C W, Harkey MA, Torok-Storb, B. The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors. Blood 2002;99, 507–12.
91. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 1996;183, 1797–806.
92. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005; 5, 275–84.
93. Henrich CJ, Bokesch HR. Dean M, Bates SE, Robey RW, Goncharova EI, Wilson JA, McMahon JB. A high-throughput cell-based assay for inhibitors of ABCG2 activity. J Biomol Screen 2006;11, 176–83.
94. Nusslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in Drosophila. Nature 1980;287, 795–801.
95. Dean M. Towards a unified model of tumor suppression: lessons learned from the human patched gene. Biochim Biophys Acta 1997;1332, M43–52.
96. Chidambaram A, Goldstein AM, Gailani MR, Gerrard B, Bale SJ, Di Giovanna JJ, Bale AE, Dean M. Mutations in the human homolog of the Drosophila patched gene in Caucasian and African American nevoid basal cell carcinoma syndrome patients. Cancer Res 1996; 56, 4599–601.
97. Tostar, U., Malm, C.J., Meis-Kindblom, J.M., Kindblom, L. G., Toftgard, R., and Unden, A. B. (2006). Deregulation of the hedgehog signalling pathway: a possible role for the PTCH and SUFU genes in human rhabdomyoma and rhabdomyosarcoma development. J Pathol 208, 17–25.
98. Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, Parker AR, Shimada Y, Eshleman JR, Watkins DN, Beachy PA. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. Nature 2003;425, 846–51.
99. Karhadkar SS, Bova GS, Abdallah N, Dhara S, Gardner D, Maitra A, Isaacs J T, Berman DM, Beachy P A. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature 2004;431, 707–12.
100. Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers,GY, Qi, YP, Gysin S, Fernandez-del Castillo C, Yajnik V, Antoniu B, McMahon M, Warshaw AL, Hebrok M. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 2003;425, 851–6.
101. Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 2003;422, 313 7.
102. James LF, Panter KE, Gaffield W, Molyneux RJ. Biomedical applications of poisonous plant research. J Agric Food Chem 2004;52, 3211–30.
103. Chen JK, Taipale J, Young KE, Maiti T, Beachy PA. Small molecule modulation of Smoothened activity. Proc Natl Acad Sci USA 2002;99, 14071–6.
104. Karhadkar SS, Bova GS. Abdallah N, Dhara S, Gardner D, Maitra A, Isaacs JT, Berman DM, Beachy, PA. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature 2004;431, 707–12.
105. Athar M, Li C, Tang X, Chi S, Zhang X, Kim A L, Tyring, SK, Kopelovich L, Hebert J, Epstein E.H, Jr, Bickers DR, Xie J. Inhibition of smoothened signaling prevents ultraviolet B-induced basal cell carcinomas through regulation of Fas expression and apoptosis. Cancer Res 2004;64, 7545–52.
106. Bijlsma MF, Spek CA, Zivkovic D, van de Water, S, Rezaee F, Peppelenbosch MP. Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion. PLoS Biol 2006;4, e232.
107. Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, Eberhart CG. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. Cancer Res 2006;66, 7445–52.
108. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 1988; 48, 589–601.
109. Kondo T, Setoguchi T, Taga T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proc Natl Acad Sci USA 2004;101, 781–6.
110. Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA, Brenner MK. A distinct "side population" of cells with high drug efflux capacity in human tumor cells. Proc Natl Acad Sci USA 2004;101, 14228–33.
111. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. Science 1977;197, 461–3.

© Copyright Edizioni Internazionali