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The Stem Cell Hypothesis in Head and Neck Cancer

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Abstract Cancer stem cells (CSCs) are tumoral cells which have stem features such as self-renewal, high migration capacity, drug resistance, high proliferation abilities. In the last 10 years the pathological meaning and the existence of CSCs have been matter of discussion and a large number of articles have been published about the role that these cells play in the development and maintenance of the tumors. Head and neck squamous-cell carcinoma (HNSCC) is the sixth most common cancer worldwide: early diagnosis of high-risk premalignant lesions are high priorities for reducing deaths due to head and neck cancer. In the last years the CSCs hypothesis has been faced also for head and neck cancer, with the aim of a better comprehension of the tumor biology and an early diagnosis. The evidence that the development of a tumor comes from a small number of cells with stem-like characteristic, could bring too to the identification of therapies against these cellular target, fundamental for maintenance and progression of the lesion. Here, a literature review has been reported about the detection of supposed CSCs in head and neck cancer. J. Cell. Biochem. 103: 408–412, 2008.

Key words: stem cell; cancer

The word "stem" comes from an ancient Greeks term that means "girder, shore." The word has been used by biologists and physicians to explain the properties of an undifferentiated cell, progenitor of all the other cells. The hallmarks of the stem cells are self-renewing capacity and the possibility to extensively proliferate, giving rise to daughter cells or progenitor cells (transit cells), committed to differentiate towards different cytotypes giving origin to tissue and organs. Maintenance of stem lines is ensured by symmetric division, according to different signals in the microenvironment or niche [Calvi et al., 2003] where the cell lays. The stem cells can be divided into two main categories: embryonic stem cells and adult stem cells. The latter's role is to substitute old cells and repair tissue injuries. Adult stem cells can be founded in most of the tissues, where they remain in a quiescent status, embedded into their niches, for several years, till an activation signal wake them up from their sleep. Cancer stem cells (CSCs) are tumoral cells which have stem features such as self-renewal, high migration capacity, drug resistance, high proliferation abilities. In the last 12 years the pathological meaning and the existence of CSCs have been matter of discussion. A large number of articles have been published about the role that these cells play in the development and maintenance of the tumors.

It is well known that solid tumors are built by heterogeneous cell populations, where it has been possible to observe cells with stem properties: high proliferation, expansion and growth in tissue environments different from the original one [Reya et al., 2001]. Tumor can be seen as a whole organ and the different cells that build it have different goals and roles in the tumor economy. As in a beehive, there are worker cells whose aim is to build the tumor mass and queen cells, able to move, invade and

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colonize new tissues. Within the latter group there is the nest of CSCs, the only able to reproduce the tumor in a new and different environment.

The existence of CSCs has been supported by numerous research performed on acute myeloid leukemia, where it has been demonstrated that only a specific cellular subset, that express antigenic markers similar to hematopoietic stem cells, has clonogenic activity in immunocompromised mice [Lapidot et al., 1994; Bonnet and Dick, 1997]. Similar researches, conducted on tumors of epithelial origin such as breast cancer [Dontu et al., 2005] evidenced the presence of stem-like cells within the cancer lesions: only CD44+CD24− cells [Ponti et al., 2005], isolated from breast cancer, are able to generate tumors in immunocompromised mice. Moreover, it has been reported that cancer cells with stem markers show self-maintaining, extensive proliferation, and differentiation ability into all other cell lineages present within the tumors. The evidence that the development of a tumor comes from a small number of cells with stem-like characteristic, could bring to the identification of therapies against these cellular target, fundamental for maintenance and progression of the lesion.

Head and neck squamous-cell carcinoma (HNSCC) is the sixth most common cancer worldwide. Moreover, HNSCC has a severe impact on the quality of life of patients and survivors, and the significant morbidity subsequent to treatment often mandates long-term multidisciplinary care. Therefore, early diagnosis of high-risk premalignant lesions are high priorities for reducing deaths due to head and neck cancer [Hunter et al., 2005]. In the last years the CSCs hypothesis has been faced also for head and neck cancer [Zhang et al., 2006], with the aim of a better comprehension of the tumor biology and an early diagnosis.

PATHOGENETIC HYPOTHESES

Numerous hypotheses exist about the pathological activation of a stem cell. The first hypothesis considers the signals that can reach the stem cells within their niches. A pathological condition can change the balance between the signals that come from the niche and the ones that come from surrounding environment, generating a signal that makes the cells exit out the niche without a clear differentiation fate, helping the formation of highly proliferative population [Sell, 2004]. Oct-4, Wnt, Notch, Shh are genes involved in self-renewal and determination of differentiation fate. A deregulation of these signals can be often related with development and progression of a CSC [Ezeh et al., 2005; Suo et al., 2005].

The second hypothesis considers an extremely active process: cellular fusion that brings to transdifferentiation. Cellular fusion is characteristic of immature cells such as the stem and tumor cells, whose genome is instable. In the eukaryotic organisms cellular fusion is a physiologic phenomena, finely tuned during development and differentiation. The importance of the cellular fusion during development and diseases is evidenced by numerous biological processes: fecundation, with the fusion between oocyte and spermatozoi; during muscle development mononuclear myoblasts fusion for the formation of multinucleated muscle fibers. During bone formation osteoclasts genesis from the fusion of monocytes; in the placenta the fusion between trophoblasts and sincitiumtrophoblasts [He et al., 2005]. Fusion between a stem and a cancer cell, with reassembling of genotype, may be the first step of neoplastic transformation [Aractingi et al., 2005]; Houghton et al. [2004] described selective migration of hematopoietic stem cell into the gastric epithelia during Helicobacter felix infection, observing that the potentiality to develop a gastric carcinoma is owned only by this bone marrow population. These observations consider the possibility that fusion between a migrated stem cells and an adult mutated cell within the tissue may lead to carcinoma formation.

Actually it is not yet clear if the cancer cells with stem properties isolated within the tumors are born as mutated stem cells with tumoringenic activity or if the latter is the result of the recruiting of stem cells by the cancer [Sell and Pierce, 1994; Wicha et al., 2006], reprogramming the stem cell fate through factors released by cancer cells [Christel et al., 2005].

THE NICHE OF CARCINOMA STEM CELLS

Over 90% of all human neoplasias are derived from epithelia. In normal epithelial tissues, stem cells, usually located in the basal layers, divide to produce cells, termed transient amplifying cells, which undergo a few rounds of

more rapid division before they terminally differentiate. A key feature of normal stem cells is their asymmetrical division, the mechanism that allows stem cell self-renewal while producing hierarchies of amplifying and differentiating cells that form the bulk of the tissue. Normal tissue renewal of epithelia comes from stem cells or their differentiating progeny. The degree of differentiation of a carcinoma depends on the proportion of undifferentiated tumor stem cells, the stage of maturation arrest of the majority of cells in the tumor, and on the ability of some cells to escape arrest and to differentiate [Mackenzie, 2006].

Locke et al. [2005] showed that the maintenance of a subpopulation of stem cells during passage of carcinoma cell lines indicates that the key stem cell property of asymmetrical division persists but is shifted towards enhanced stem cell self-renewal. The presence of malignant epithelial stem cells in vivo has been shown by serial transplantation of primary cancer cells and the present observations indicate that stem cell patterns are robust and persist even in cell lines. There is increasing evidence that the growth and spread of cancers is driven by a small subpopulation of CSCs—the only cells that are capable of long-term self-renewal and generation of the phenotypically diverse tumor cell population. Failure of current cancer therapies may be due to inefficacy on potentially quiescent CSCs that remain vital and retain their full capacity to repopulate the tumor. Treatment strategies for the elimination of cancer therefore need to consider the consequences of the presence of CSCs. However, the development of new CSC-targeted strategies is currently hindered by the lack of reliable markers for the identification of CSCs and the poor understanding of their behavior and fate determinants. Recent studies of cell lines derived from oral squamous cell carcinoma (OSCC) indicate the presence of subpopulations of cells with phenotypic and behavioral characteristics corresponding to both normal epithelial stem cells and to cells capable of initiating tumors in vivo [Costea et al., 2006]. The cancer-derived differentiated cells are benign if not normal leading to the conclusion that attempts to direct normal differentiation of malignant stem cells might serve as an alternative to cytotoxic therapy [Locke et al., 2005].

The epithelial stem cells lack form clusters in association with a specialized mesenchymal environment (the "niche"), where they are well protected and feeded [Miller et al., 2005]. Stem cells separated from their niche lose their stemness, although such a loss may be reversible, becoming "transit-amplifying cells" that are rapidly proliferating but have a more limited proliferative potential, and can give rise to terminally differentiated cells. The identification of the stem cell subpopulation in a normal epithelium leads to a better understanding of many previously enigmatic properties of an epithelium including the preferential sites of carcinoma formation, as exemplified by the almost exclusive association of corneal epithelial carcinoma with the limbus, the corneal epithelial stem cell zone [Sun and Lavker, 2004]. Being long-term residents in an epithelium, stem cells are uniquely susceptible to the accumulation of multiple, oncogenic changes giving rise to tumors. The application of the stem cell concept can explain many important carcinoma features including the clonal origin and heterogeneity of tumors, the occasional formation of tumors from the transit amplifying cells or progenitor cells, the formation of precancerous "patches" and "fields," the mesenchymal influence on carcinoma formation and behavior, and the plasticity of tumor cells. While the concept of CSCs is extremely useful and it is generally assumed that such cells are derived from normal stem cells, more work is needed to identify and characterize epithelial CSCs [Locke et al., 2005].

The cells can escape the niche through high levels of matrix metalloproteinases [Ikebe et al., 2004]; (MMP)-9 was found present in the tissue of squamous cell carcinoma (SCC) [Rosenthal et al., 2005]. When tumor cells and fibroblasts were isolated from the tissue and cultured separately, significant levels of MMP-9 were lost in the culture media of tumor cells as well as fibroblasts. Laminin and tenascin can form a complex to escape the niche and invade the tissues [Franz et al., 2006].

Inflammation, induced by microbial agents, radiation, endogenous or exogenous chemicals, has been associated including cancer. Since carcinogenesis has been characterized as consisting of the "initiation," "promotion," and "progression" phases, the inflammatory process could affect any or all three phases and pull the stem cell out the niche [Castellsague et al., 2004; Trosko and Tai, 2006].

ANTIGENS

It has been demonstrated in experimental models of human tumors that tumor lesions are built up by heterogeneous population of cancer cells and the presence of stem antigens can be evidenced by phenotypical analysis. Cytofluorimetric analysis can play an important role in this phase of identifying and recognize CSCs. The most used markers have been not only CD34, but also CD133 and CD24, as markers of non-differentiated cells, often coupled with migration molecules as CD44, CD29, CD31 and other integrins. The different cell surface phenotypes prospectively identify tumor-initiating subpopulations in solid tumors and even cell lines derived from tumors retain hierarchical stem cell patterns demonstrable as differing clonogenic abilities related to cellular properties such as size, adhesiveness, dye exclusion, and patterns of gene expression [Mackenzie, 2006]. Barth et al. [2004] investigated tumor-free mucosa and 39 SCCs of the oral cavity, the pharynx, and larynx with respect to the presence of stromal CD34+ fibrocytes and stromal expression of CD117, the receptor of stem cell factor (SCF). CD117 expression was absent from the tumor-free stroma. Of 39 SCCs, 33 were free of stromal CD34+ fibrocytes, and CD117-positive stromal spindle cells were found in 25 carcinomas. The SCF is a soluble factor that can be isolated from the plasma [Tan et al., 2006] and from the epithelium [Sheu et al., 2005] of patients affected by nasopharyngeal and in esophageal and gastric mucosa; in the latter case within the epithelium can be found its receptor too, CD117 or c-kit [Sheu et al., 2005]. Kojc et al. [2005] analyzed the expression of CD34, a stem cell marker, and transforming growth factor beta1 (TGFbeta1), a protein involved in cell differentiation, in squamous intraepithelial lesions (SILs) and SCC of the larynx and hypopharynx. The stroma in normal laryngeal mucosa and SILs contained scattered CD34-positive cells, while the stroma of SCC did not contain CD34-positive cells. The same pattern of stromal reaction was also observed in the peritumoral zone, defined as a band of host tissue between the invasive tumor front and adjacent normal tissue. In adjacent normal tissue, there were CD34-positive stromal cells and no myofibroblasts. This study put the accent on the disappearance of the stem marker CD34 and the transformation of laryngeal SILs to SCC, perhaps the proof that a loss of stem cells, following particular stimuli, can alter the tissue homeostasis. On the other hand Prince et al. [2007], in an immunodeficient mouse model, showed that CD44(+) head and neck SCC cells, usually $<10\%$, but not the CD44(−) cancer cells, can give origin to new tumors in vivo. The tumors derivated from purified CD44(+) cells reproduced the original tumor and could be serially passaged, confirming the ability of CD44(+) to self-renew and to differentiate. Furthermore, the CD44(+) cells in the tumor express high levels of nuclear BMI1, that has been demonstrated to play a fundamental role in self-renewal and to be involved in tumorigenesis. In this study, the data suggest that the CD44 presence, a mesenchymal progenitor marker in this case, identity a population of human HNSCC that possess properties of CSCs; this experiment seems to confirm the hypothesis that a "wrong" or "exaggerated" exit of the stem cells from their niche, can alter their cycle and tissue cellular turnover, leading to cancer.

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