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Review article

Cancer stem cells: the theory and perspectives in cancer therapy

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Abstract. The cancer stem cell theory elucidates not only the issue of tumour initiation and development, tumour's ability to metastasise and reoccur, but also the ineffectiveness of conventional cancer therapy. This review examines stem cell properties, such as self-renewal, heterogeneity, and resistance to apoptosis. The 'niche' hypothesis is presented, and mechanisms of division, differentiation, self-renewal and signalling pathway regulation are explained. Epigenetic alterations and mutations of genes responsible for signal transmission may promote the formation of cancer stem cells. We also present the history of development of the cancer stem cell theory and discuss the experiments that led to the discovery and confirmation of the existence of cancer stem cells. Potential clinical applications are also considered, including therapeutic models aimed at selective elimination of cancer stem cells or induction of their proper differentiation.

Keywords: cancer, cancer stem cells, cancer stem cell theory, stem cells, therapeutic model.

Introduction to the clonal evolution model and the cancer stem cell model

Neoplasms are defined as tissue consisting of a heterogeneous population of cells that differ in biological characteristics and potential for self-renewal (Reya et al. 2001). According to the model of clonal evolution of tumour cells, cancer is formed through the accumulation of genetic changes in cells and gradual selection of clones (Figure 1a). The majority of therapeutic approaches (conventional therapies) that aim at eliminating tumour cells are based on this theory (Clarke and Becker 2006). The limited effects of these therapies (poor prognosis for patients in advanced stages of cancer, particularly with solid tumours) suggested that tumour cells include a population of cells responsible for the initiation of tumour development, growth, and tumour's ability to metastasise and reoccur. Because of some similarities between these cells and stem cells (SCs), the former have been named cancer stem cells (CSCs) (Figure 1b). The CSC model assumes that CSCs have the following characteristics: (1) self-renewal; (2) heterogeneity, i.e. potential for multidirectional differentiation; and (3) resistance to apoptosis. It is believed that these properties decrease the effectiveness of conventional therapies that act mainly on the differentiated or differentiating tumour cells. The population of undifferentiated CSCs, forming a minor ('silent') fraction of tumour mass, remains spared (Ponti et al. 2005; Costa et al. 2006; Kucia et al. 2006).

The concept of CSCs assumes that they arise from SCs or progenitor cells (precursor cells, partly differentiated, with a limited proliferation potential) (Costa et al. 2006). According to the pretumour progression hypothesis, the development of tumour results from the clonal evolution of the CSC population (Calabrese et al. 2004). The transformation of a normal SC into a CSC is due to the accumulation of genetic modifications (mutations in oncogenes, suppressor genes and miss-match repair genes) and epigenetic alterations (abnormal methylation, histone modification) (Costa et al. 2006).

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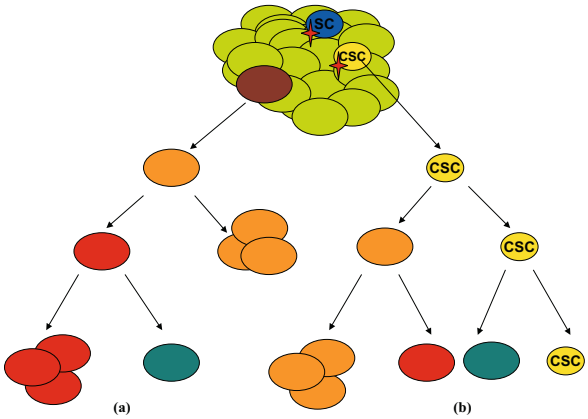

Figure 1. Models of tumour development: (a) clonal evolution model; (b) cancer stem cell model. Green = niche cells; blue = stem cell (SC); yellow = cancer stem cells (CSCs); red star = adhesive molecules; brown, orange, red, dark turquoise = cells accumulating genetic alterations.

Characteristics of stem cells and cancer stem cells

SCs are resistant to apoptosis and have the ability to self-renew, differentiate into a variety of cells, and to generate numerous daughter cells. A characteristic feature of self-renewing cells is an increase in telomerase activity, due to which the length of telomeres remains constant after cell division. This means that the cells are not subject to the aging effect and apparently have an infinite replication potential (Huntly and Gilliland 2005).

In respect to differentiation potential, SCs can be divided into the following groups:
(a) totipotent – such as a fertilized egg cell and early blastomeres, capable of giving rise to any cell type of an organ or placenta;
(b) pluripotent – embryonic cells, capable of giving rise to any cell type of an organ, but not placenta;
(c) multipotent – cells of the ectoderm, endoderm and mesoderm;
(d) unipotent – cells capable of giving rise to only one cell type of a tissue.

A special microenvironment (natural tissue niche) is necessary to regulate the function of SCs, where they are surrounded by a special type of cells, such as tissue stromal cells in the bone marrow. Crypts in the gut, stomach, and hippocampus in the brain may act as niches for SCs. With few exceptions, SCs always remain inside their niche ('silent' state) and sometimes are attached to it by adhesive molecules (Figure 2a). The number of SCs in a given tissue as well as SC self-renewal and differentiation processes are controlled by niche regulatory systems (Spradling et al. 2001).

Environmental stimulation may induce SCs to generate progenitor cells by entering the accelerated division phase. Self-renewal ensures constant replacement of mature cells of a given tissue and its regeneration in case of injury. After a symmetric division of the cell, which is driven by needs of the organism, the daughter cells either remain undifferentiated (retaining SC properties), or form 2 progenitor cells and begin to differentiate (Figure 2b,d). An asymmetric division generates 2 daughter cells, one of which remains in the niche (a cell identical to the SC) (Figure 2c). The other cell is removed from the niche (normally with some of the neighbouring 'nursing' cells) and it turns into a precursor/progenitor cell (Clarke and Becker 2006). The progenitor cells proliferate intensively, differentiating at the same time (specialization), ensuing the removal of the daughter cell from the microenvironment of the niche. As the cells differentiate and give rise to mature cells of a given tissue or organ, the progenitor cells lose their ability to self-renew. The decrease in the number of cell divisions probably results from loss of telomerase activity (Clarke and Fuller 2006). The self-renewal process may be disturbed by alterations of asymmetric division control. It has been shown in studies on SCs in *Drosophila melanogaster* that aberrations in asymmetric cell division, caused by mutations in genes controlling polarity (*aps, mira, numb, pros*), increase the frequency of self-renewal and cause the malignant conversion of neuroblasts to forms similar to

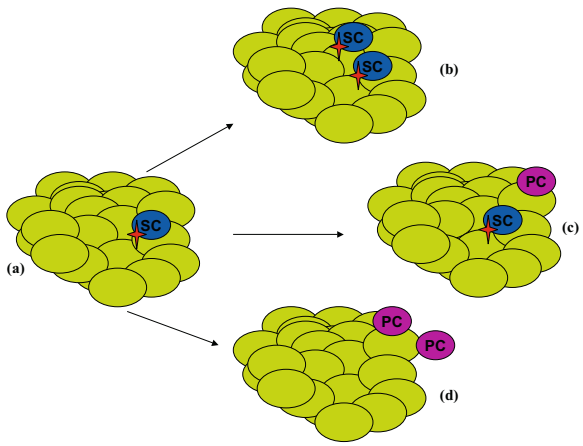

Figure 2. Model of stem cell division: (a) stem cell (SC) in the niche, before division; (b) symmetric division generates 2 SCs; (c) asymmetric division generates a SC and a progenitor cell (PC); (d) symmetric division generates 2 PCs. Green = niche cells; blue = SCs; purple = PCs; red star = adhesive molecules.

neuroblastoma. Consequently it has been suggested that the suppressor gene *LKB1*, which also takes part in controlling polarity and is deleted in Peutz-Jeghers syndrome (susceptibility to cancer), can play a role in mammalian carcinogenesis (Guo et al. 2006).

The process of differentiation of progenitor cells is likely to be induced by a different degree of precursor and SC sensitivity to niche signalling and outer cell environment. Normal, differentiated cells removed from their environment and cultured in vitro can acquire epigenetic changes warranted by the culturing conditions. This may cause a loss of functional differentiation. However, SCs cultured in vitro begin to proliferate rapidly and to differentiate (features encoded in these cells) and therefore must be cultured under special conditions in order to remain undifferentiated. The role of niche signalling (regulation) in keeping the SCs undifferentiated and 'silent' until they are stimulated to produce new cells, suggests that local environment signalling can also affect CSCs, hence influencing initiation and tumour growth. It has been shown that CSCs displaced into an atypical niche (lack of subsequent genetic and/or epigenetic changes) do not produce cancer, whereas normal SCs placed in a damaged tissue (by radiation, for example) can initiate tumour growth (Clarke and Fuller 2006).

The formation of CSCs outside the influence of the microenvironment (niche signalling, i.e. epigenetic factor) may also be related to alterations in signal transmission inside the cell and from cell to cell (genetic factor) (Guo et al. 2006). There are similarities between signalling pathways that govern normal SC proliferation (self-renewal control) and those promoting carcinogenesis, by initiating CSC proliferation. Deregulation (by hyperactivation, for example) of signalling pathways, such as Notch, Sonic hedgehog (Shh) Wnt/-catenin, factor Bmi-1, and *Hox* gene family products, can lead to transformation of SCs into CSCs (Bjerkvig et al 2005; Huntly and Gilliland 2005). The Bmi-1 protein also plays a crucial role in regulating the self-renewal process of SCs and CSCs. The *Bmi-1* proto-oncogene takes part in haematopoietic and neural SC self-renewal maintenance (Park et al. 2003, Molofsky et al. 2003). In normal conditions, factor Bmi-1 inhibits the transcription of the *INK4A* locus that encodes 2 cyclin-dependent kinase inhibitors: $p16^{INK4A}$ and $p14^{INK4A}$. A lack of the p16 inhibitor, accompanied by abnormal Bmi-1 function, promotes cell proliferation by increasing its self-renewal potential, whereas a lack of the p14 inhibitor hinders proapoptotic gene expression. Park et al. (2003) have shown expression of Bmi-1 in SCs in mouse foetuses, adult mice, and humans. They noticed that the number of haematopoietic SCs found in livers of Bmi-$1^{-/-}$ mouse foetuses significantly declines in postnatal life. Furthermore, they demonstrated that transplanted Bmi-$1^{-/-}$ liver and bone marrow cells are capable of transiently sustaining haematopoiesis. No evidence of self-renewal potential was found in haemato- poietic SCs of adult Bmi-$1^{-/-}$ mice. The expression of cell metabolism genes, transcription factors, and modulating cell growth genes, such as *p16* and *p14* in SCs, was altered. The expression of p16 and p14 in normal haematopoietic SCs leads to inhibition of proliferation and p53-controled cell death (Ramalho-Santos et al. 2002; Park et al. 2003).

History of hypotheses on CSC origin and experiments that confirm the existence of CSCs

A hypothesis of CSCs that have similar properties to SCs was first described by Rudolf Virchow and Julius Conheim in the 19th century (Huntly and Gilliland 2005; Kucia and Ratajczak 2006). Virchow's embryonal-rest hypothesis (cancer arises from activation of 'dormant' cells present in mature tissue, that are remainders of embryonic cells) was based on the fact that there are histological similarities between developing foetal cells (embryonal cells) and some cancer cells, e.g. their ability to proliferate and differentiate. However, Conheim postulated that the remaining embryonic cells, from which tumours form, were 'lost' during organogenesis. However, it was only the progress of molecular biology techniques that enabled the identification of CSCs in various types of tumours.

One of the first experiments confirming the existence of CSCs was preformed in the 1960s, when cells from primary sites were taken from patients with malignancies and then transplanted to other parts of their bodies. The results of this experiment showed that only a minor percentage o transplanted cells produced a tumour. Because of controversies concerning ethical aspects of that experiment, an animal model (usually a mouse line) was introduced later (Huntly and Gilliland 2005).

In 1967, Fialkow et al. showed that some leukaemic cells presented the G-6-PD protein on their surface. Those authors assumed that those

cells caused the malignancy. The introduction of flow cytometry, which enables the segregation of cells according to their surface proteins (surface markers), provided the means for further studies on SCs. In 1997, Bonnet and Dick described a subpopulation of cells that were immature and characterised by the presence of a specific surface marker CD34 ($CD34^+$) and the absence of a CD38 marker ($CD38^-$) in patients with acute myeloid leukaemia. After transplanting those cells to mice with an altered immunological system (NOD/SCID mice: non-obese/severe-combined immunodeficient), similar in histology to the donor cells, a tumour developed in some of the mice. Those authors declared that a minor subpopulation of $CD34^+/CD38^-$ cells is capable of initiating tumour development, i.e. has clonogenic properties. In acute myeloid leukaemia the frequency of this fraction is lower than 1 per 10 000 cells (Bonnet and Dick 1997). Cells with a typical leukaemic phenotype $CD34^+/CD38^+$ are not capable of initiating tumour development in NOD/SCID mice. The discovery of the $CD34^+/CD38^-$ cell subpopulation was the first proof of the existence of CSCs in haematopoietic malignancies and was the beginning of extensive research on the presence of CSCs in solid tumours (Bonnet and Dick 1997; Bjerkvig et al. 2005). Al-Hajj et al. (2003), who were the first to describe CSCs in breast cancer, found that cancer cells in this tumour are characterised by heterogeneous expression of surface proteins (markers). The identification of these markers (evaluation of cell phenotype) helped to distinguish the cells capable of initiating tumour development and the cells unable to begin such a process (diversified carcinogenic potential). Only the population of $CD44^+CD24^{-/low}Lineage^-$ cells could initiate the process of carcinogenesis in immunodeficient mice. Al-Hajj et al. (2003) found that in 8 out of 9 different types of breast cancer, a subpopulation of cells with such a phenotype exists.

The presence of a subpopulation of cells with a high proliferation potential in the tumour tissue could explain many clinical observations. For example, Al-Hajj et al. (2003) reported that in up to 30% of women with breast cancer some micro-metastases were detected in the bone marrow at the time of presentation, but only half of the women still had metastases 5 years later. According to the CSC model, the bone marrow contains dispersed tumour cells, and some of them (CSCs) have the ability to initiate carcinogenesis. Only in the case of presence of CSCs, metastases would develop.

Diagnostic tests that could identify CSCs could be a step forward in evaluating prognostic factors in people with malignancies (Al-Hajj et al. 2003). CSCs have already been identified (according to specific markers) in haematopoietic malignancies and breast, lung, ovarian, prostate, gastric, colorectal cancer and brain tumours (Costa et al. 2006). It is estimated that in these malignancies CSCs constitute <5% of all tumour cells. A recent study on the presence of CSCs in solid tumours focused on pancreatic cancer. Li et al. (2007) identified a subpopulation of cells with $CD44^+/CD24^+/ESA^+$ (epithelial-specific antigen) phenotype, which has a carcinogenic potential. They constitute 0.2–0.8% of all pancreatic cancer cells and have SC properties: self-renewal, ability to generate differentiated daughter cells, and increased expression of signalling pathway proteins (Shh). By using the animal model it has been proved that these cells have a 100-fold higher carcinogenic potential than other tumour cells (Li et al. 2007).

Although the correlations between the expression of ESA and CD24 markers and the function of CSCs have not yet been examined in other types of tumours, an association between $CD44^+$ expression and highly carcinogenic subpopulation of tumour cells with SC characteristics has been reported, for example, in breast, pancreatic and prostate cancer. Some other markers that determine the potential to generate populations of CSCs in solid tumours, such as $CD133^+$ in brain tumours, prostate and colorectal cancers, have also been described (Bao et al. 2006; Driks 2006). Studies on surface markers in tumour cells suggest that probably each type of tumour has a unique phenotype.

Recently, cancer/testis antigens (CTAs), whose expression in normal tissues is only limited to undifferentiated germ, placental and mesenchymal bone marrow cells, have also been found in various types of tumours (Costa et al. 2006). In normal, differentiated tissues, expression of these proteins is highly restricted or does not occur at all. However, in malignant tissue a high degree of CTA expression is only found in cells with SC properties. Tumour cells with high CTA expression may lose their ability to differentiate. It is this population of cells, among other tumour cells, that sustains tumour growth, proliferation, and metastasis (Costa et al. 2006). It seems that the expression of CTAs is a genuine characteristic of CSCs. Finding a therapy that would stimulate CSCs with high CTA expression

to differentiate may prove to be an effective cure for various types of tumours.

Despite numerous experimental data confirming the existence of CSCs in tumours, the background of these cells still awaits elucidation. According to one hypothesis, CSCs are derivatives of SCs residing in various organs. In these long-lived cells, mutations and epigenetic changes accumulate, which is crucial for initiation and progression of tumour growth. Transformation of SCs into CSCs initiates carcinogenesis. Somewhat more differentiated precursor cells may also transform into CSCs. Another hypothesis assumes the existence of very small embryonal SC-like cells that can be found in the blood or other tissues. If they are mobilised at a wrong time and/or displaced (exposure to damaging environmental factors), they can convert into CSCs. Mutations in other, more differentiated cells may also play a role in the development of CSCs (Kucia and Ratajczak 2006).

There is some controversy over the issue of what type of cells undergoes the transformation into CSCs. One of the models assumes that the SCs that undergo a malignant transformation, lose their property of controlling self-renewal. According to a second model, the first mutations appear in SCs, but the final stages of transformation into CSCs take place in daughter cells (differentiated cells with a less stable genome). A cell that is altered but differentiated loses its properties and regains the self-renewal potential. For example, it has been reported that both models are true for acute myeloid leukaemia (AML). The most common aberration in AML is chromosome 8 to 21 translocation, which results in producing the AML1-ETO transcript. Studies in patients with long-lasting remission showed that haematopoietic cells with the AML1-ETO transcript remain in the bone marrow. After isolating these cells it turned out that they do not have leukaemic properties and undergo proper differentiation in vitro. These results clearly confirm that the translocation in haematopoietic SCs and additional mutations in progenitor cells lead to leukaemic phenotype (Reya et al. 2001).

Perspectives in cancer therapy

The identification of CSCs brings about important therapeutic implications. Currently employed methods of treatment are usually characterised by poor selectivity, i.e. the drugs damage not only tumour cells but also normal cells (Figure 3a). This is one of the causes of ineffectiveness and serious adverse effects of such treatment. If the CSC theory proves to be true, then treatment should aim at selective elimination of CSCs from the body and not the cells that form the main mass of the tumour (Figure 3b).

The resistance of CSCs to chemotherapy may be caused by an increased expression of proteins from the BCL-2 family, which leads to an increase in expression of membrane proteins responsible for drug resistance (Al-Hajj et al. 2003). Also an increased expression of transporting proteins, such as MDR1 and ABC transporters, is an important factor in classical chemotherapy resistance (Jordan et al. 2006). Al-Hajj et al. (2003) reported that a greater expression of these proteins in breast cancer cells may make them resistant to widely applied therapies. Also the augmented expression of the *bcl-2* oncogene in haematopoietic SCs has an antiapoptotic effect and as a result the number of haematopoietic SCs increases (Reya et al. 2001). CSCs – undifferentiated and in the 'dormant' phase – are relatively resistant to cytostatic drugs, which act mainly on dividing cells. Therefore this subpopulation of CSCs is responsible for metastases and recurrence after an apparently successful treatment.

Acquiring knowledge about the biology of CSCs and discovering methods that would identify them in a heterogeneous population of tumour cells will allow for more effective treatment (Al-Hajj et al. 2003). Some hope as to finding an effective method of treatment emerged with the results of studies on malignant brain tumours, gliomas. These are tumours that have a very high death rate. Up to now they have been treated mainly by a surgical removal of the tumour mass, followed by radiotherapy that damages the cells' DNA and causes death of the cells. In most cases, it is performed only as a palliative therapy. Bao et al. (2006) reported that checkpoint proteins play a crucial role in determining the CSC resistance to radiotherapy. In response to DNA damage the checkpoint proteins are activated and their expression increases. Additionally, cells resistant to radiotherapy show expression of Prominin-1 ($CD133^+$), which also appears on the surface of neuronal and brain SCs. Cells showing expression of the $CD133^+$ marker can differentiate in many various ways, and as such they can form a tumour consisting of a heterogeneous cell population. It has been proved in vivo and in vitro that pharmacological inhibition of checkpoint proteins, e.g. Chk1 and Chk2, results in a decrease in resistance

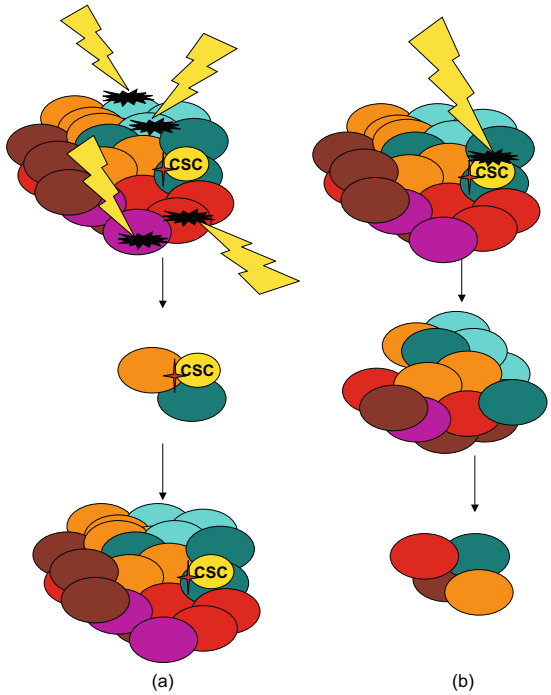

Figure 3. Model of conventional versus CSC-targeting therapy: (a) conventional cancer therapy (targeting cancer cells, but not CSCs); (b) novel cancer therapy targeting CSCs. Yellow lightnings indicate targets of anti-cancer drugs. Brown, orange, red, dark turquoise, violet, cyan = tumour cells.

of $CD133^+$ cells to ionising radiation (Bao et al. 2006). Piccirillo et al. (2006) observed a reduction of the number of CSCs initiating glioma development in culture after exposing them to morphogenetic bone proteins (BMPs). BMPs under normal conditions induce differentiation of neuron precursors into mature astrocytes. Those authors showed that BMP4 (neuronal SC regulator), which activates the BMP receptor (BMPR), had the strongest effect. In mice with transplanted human brain tumour cells, BMP4 had the effect of inhibiting tumour growth. Glioma CSCs received a signal to differentiate into non-malignant cells (Piccirillo et al. 2006). The results of these studies can point to other directions of treatment of gliomas and maybe other types of malignancies by changing the paradigm of treatment aimed not at damaging cancerous cells but at inducing CSCs to differentiate into normal cells.

Conclusions

Despite recent advances in CSC studies, the knowledge about these rare 'silent' cells, able to self-renew and sustain tumour growth and heterogeneity, is still limited. Carcinogenesis is a multi-step process related to the accumulation of genetic and epigenetic changes (Guo et al. 2006). At the molecular level, alterations in signalling pathways responsible for self-renewal of SCs are crucial in transformation of SCs into CSCs. Progress can be made only by discovering the mechanisms of control of signalling pathways. An accurate description of CSCs will strengthen our understanding of the basis of tumour development and clinical aspects, and it may lead to changing cancer classification in humans and therapeutic strategies in managing tumours. Treatment directed at eliminating those cells or inducing their proper differentiation may be an effective way to cure cancer.

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