

## Cancer stem cells – a new chance for successful treatment of cancer

### *Macierzyste komórki nowotworowe – nowa szansa na skuteczne leczenie chorób nowotworowych*

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**Słowa kluczowe:** terapia, komórki macierzyste, chemiooporność, macierzyste komórki nowotworowe, markery nowotworowe.

#### Abstract

Cancer stem cells are undifferentiated cells capable of being transformed into all types of cells comprising the tumour mass. As demonstrated by recent findings, they are responsible for the development of both haematopoietic malignancies and solid lesions. Cancer stem cells determine the unlimited growth of tumours and the variety of their morphology; they are also suspected of being the factor responsible for metastases. Their capability for renewal is the cause of disease recurrences even after long remission periods. Studies have shown that cancer stem cells may develop from normal stem cells, mature cells, and differentiated, or already transformed cells. What differentiate these cells from other cancer cells are rare mitotic divisions, which play a protective role for the cancer genome.

#### Streszczenie

Nowotworowe komórki macierzyste to niezróżnicowane komórki zdolne do przekształcania się we wszystkie typy komórek budujących masę nowotworu. Jak się okazało w ostatnich latach, odpowiedzialne są zarówno za rozwój nowotworów układu krwiotwórczego, jak i powstawanie zmian litych. To właśnie te komórki warunkują nieograniczony wzrost nowotworów, ich różnorodność w budowie morfologicznej oraz – jak się przypuszcza – są czynnikiem powodującym powstawanie przerzutów. Ich zdolność do odnowy jest przyczyną nawrotów choroby, nawet po dłuższych okresach remisji. Wyniki badań wykazały, że nowotworowe komórki macierzyste mogą się rozwijać z prawidłowych komórek macierzystych, komórek dojrziałych, zróżnicowanych oraz komórek już transformowanych nowotworowo. Cechą wyróżniającą nowotworowe komórki macierzyste, w odróżnieniu od pozostałych komórek nowotworowych, są rzadkie podziały mitotyczne, pełniące funkcję ochronną dla genomu komórek nowotworu.

#### Stem cells

Normal stem cells (NSC) are relatively non-specialised cells capable of division leading to identical offspring cells (self-renewal potential), as well as differentiation into one or more types of specialised cells in particular conditions (differentiation potential). This distinguishes these cells from other cells of the human body, which die after reaching their division limit.

Stem cell divisions can be classified into two types: symmetric division leading to two stem cells that maintain the traits of the parent cell; and asymmetric division, leading to one stem cell identical to the parent cell and another one that undergoes differentiation or, in particular conditions, apoptosis [1]. Stem

cells collected from blastocyst embryos are capable of differentiating into any type of systemic cells; these are referred to as embryonic stem cells. Stem cells are also present in mature organisms; they are known as somatic or adult stem cells. However, they are capable of differentiating only into some types of specialised cells. Although the quantity of NSCs in the human body is very low (they account for less than one per million of all body cells), ongoing studies have allowed us to isolate and multiply these cells with ever increasing efficacy while offering chances for broader therapeutic use [2].

Sources of stem cells include blastocysts formed in an *in vitro* fertilisation process, foetal germ cells following miscarriage, umbilical blood, or mature body

tissues. Collection of stem cells is a matter of much dispute related not only to scientific aspects but also primarily to ethical and legal concerns. Therefore, it seems that stem cells obtained from adult tissues have the best prospects for being used.

The classification of stem cells into conventional groups is due to their potential to differentiate into specialised cells. Cells with the highest differentiation potential are called totipotent (a.k.a. omnipotent) cells and are capable of differentiating into all embryonic cells as well as extra-embryonic cells, e.g. placental cells. Such features can be ascribed to the fertilised egg and cells formed in the two first divisions thereof. Another type of stem cells obtained from embryos and embryonic precursor germ cells are pluripotent cells capable of differentiating into cells developing from all germ layers but not into foetal membrane cells. Another group are multipotent cells isolated from individual germ layers, or organs developed therefrom. They are capable of differentiating only into cells characteristic for the germ layer or the organ from which they develop. The lowest potential for differentiation is shown by progenitor (unipotent) cells capable of differentiating into a cell type and isolated from mature body tissues [3, 4].

Stem cells are found within the tissues in regions referred to as stem cell niches. In these microenvironments stem cells maintain their readiness to perform their function. Stem cells are surrounded by escort cells that modulate the frequency of stem cell division. An important role in the development of stem cells is also played by growth factors released by adult cells and proteins present within the extracellular matrix. As the body grows, localisation of niches becomes increasingly difficult [5].

Plasticity is a very important trait of adult tissue stem cells; it consists of the cells' capability to differentiate into other cells, even those originating from other germ layers. This phenomenon may be due to transdifferentiation, cell fusion, or the presence of pluripotent stem cells in mature organisms. It is difficult to establish which of these phenomena is primarily responsible for the development of this unusual trait of stem cells. This is due to the lack of markers characteristic for stem cells of different differentiation status [6]. The theory that is most likely and best explains the observed phenomenon is the theory of pluripotent cells, preliminary confirmed by recent research results. The discovery of plasticity challenged the claim that only embryonic cells are capable of differentiating into cells of more than one tissue type. It turned out that e.g. stem cells found within the central nervous system are capable of forming blood cells [7, 8].

The factor that retains stem cells at an undifferentiated stage, detected in all stem cell types obtained from all types of sources, is the expression of the *Bcrp1* (a.k.a. *ABCG2*) gene [9]. In addition, regulation

of stem cell self-renewal is regulated by Notch, Sonic hedgehog, and WNT signalling pathways, Oct-4, BMP proteins, and Oct4, Rex1, Sox2, or *TGFB1* transcription factors [10, 11].

### Cancer stem cells

Until recently, it was believed that tumours were built of transformed monoclonal cells. This belief was disproved as cancer stem cells were identified. It turned out that tumours were complex tissues rather than homogeneous lesions.

Cancer stem cells (CSCs) are undifferentiated cells capable of being transformed into all types of cells comprising the tumour mass. They are highly similar to NSCs. As demonstrated by recent findings, they are responsible for the development of both haematopoietic malignancies and solid lesions. Cancer stem cells determine the unlimited growth of tumours and the variety of their morphology; they are also suspected of being the factor responsible for metastases. Their capability for renewal is the cause of disease recurrences even after long remission periods [12]. Studies have shown that CSCs may develop from NSCs, mature cells, and differentiated, or already transformed cells [13]. Many theories have been proposed to describe the mechanism of carcinogenesis involving stem cells.

The first of these theories suggests that CSCs are initially healthy NSCs that undergo unlimited proliferation when devoid of escort cells. Phenotypic changes in escort cells are also proposed as possible causes for unlimited divisions of stem cells. According to another hypothesis, accumulation of several mutations within the NCS genome transforms the cell into a cancer stem cell. It is also possible that the first mutation takes place within the NSC while the others occur as late as within progenitor cells.

Tumours are characteristic for their uncontrolled growth, and cancer stem cells were shown to be responsible for this process. It is assumed that cancer stem cells are mainly responsible for disease metastases and recurrences. Studies have shown that implantation of a tumour mass in another region of the body leads to the development of pathological lesion only in a small minority of cases. This suggests that tumour growth and metastasis is determined by only a small pool of cancer stem cells comprising up to several per cent of the lesion cells. What differentiate these cells from other cancer cells are rare mitotic divisions which play a protective role for the cancer genome. Therefore, cancer stem cells provide a constant pool of newly formed tumour building cells, and rare replication of their genetic material reduces the likelihood of adverse mutations. It also protects DNA from incorporation of synthetic inhibitory nucleotides used in the treatment [14]. This sheds light on the imperfections of chemotherapy and its lack of efficacy. Cytostatics

destroy rapidly dividing cells, and those are surely not the CSCs. Novel treatment methods should therefore focus primarily on elimination of the cause of the carcinogenic process, i.e. of the cancer stem cells.

A distinctive trait of cancer stem cells is increased ability to repair genomic damage caused by anticancer therapy and lowered ability to initiate apoptosis. Also atypical is the expression of antigens typical for embryonic stem cells: Oct3/4, SSEA1, CD133, and CD90 [12]. In an analogy to “healthy” stem cells, CSCs are present within special niches that provide optimum microenvironment for their development. Escort cells, identical to those in healthy tissues, regulate proliferation of CSCs and are responsible for maintaining their non-differentiated status by releasing appropriate cytokines. Strict correlations were observed between CSCs and their surrounding cells. Both types are interdependent to such degree that changes within the stem cell niche may induce or inhibit the carcinogenic process [15].

A common trait for both NSCs and CSCs, hugely important for their function, is the hypoxic condition within the stem cell niche. In physiological conditions, hypoxia is typical only for stem cells; it is believed that it is necessary for the induction of the hypoxia inducible factor-1 (HIF-1) that determines a number of stem cell functions [16]. It is suspected that HIF-1 controls about 2% of human genome. This mostly includes genes encoding enzymatic proteins for glycolysis as well as proteins that facilitate the transport of glucose into the cells, inhibit oxidative phosphorylation, or otherwise affect mitochondrial metabolism. It was shown that expression of HIF-1 reduces oxygen consumption, thus also reducing the production of reactive oxygen species that are harmful to all cells, including the CSCs [17]. Anaerobic glucose transformation pathway becomes predominant in cancer stem cells, leading to the formation of lactic acid and lowering of the pH of the environment. Acidified extracellular fluid within the region of the tumour may stimulate invasive properties of CSCs [18]. The increase in  $H^+$  ion levels is also immunosuppressive as it is believed that it contributes to inactivation of cytotoxic T cells capable of killing cancer cells [19, 20]. Another important trait of CSCs was also demonstrated, namely the capability of cell fusion with somatic cells of particular niches. This may lead to the formation of chimeric cells characterised by increased proliferation and increased resistance to treatment [21].

The above-described traits of NSCs and CSCs demonstrate significant similarities between both types of cells. However, the difference in the incidence of these cells seems to be of great importance. In contrast to normal stem cells, occurring less frequently than once per every 10,000 body cells, cancer stem cells may account for a much larger part of tumour cells. Usually they account for as little as 1–2% of the

mass of a tumour. Recently, population of CSCs that accounted for about 2.5% in colorectal cancer was described [22].

### The resistance of cancer stem cells

The resistance of stem cells to cytostatics is due to overexpression of membrane transporters responsible for multidrug resistance (MDR). Multidrug resistance, i.e. the principal mechanism via which many cancers develop resistance to chemotherapy drugs, is a major factor in the failure of many forms of chemotherapy. The best-known mechanism is often attributed to the function of drug transporter proteins in the plasma membrane, which actively remove drugs from neoplastic cells. Abnormal overexpression of these proteins is the most frequently described factor connected with resistance to cytostatics. Among cellular transporter proteins, glycoprotein P (Pgp), encoded by ABCB1 gene, plays the most important role. An increased level of this protein is considered a poor prognostic factor in many tumours [23, 24]. The clinical significance of other multidrug resistance proteins remains the subject of intensive studies. The MDR phenotype may be also characterised by the presence of other transmembrane proteins of the ABC family. These mainly include multidrug resistance protein 1 (MRP1) and breast cancer resistance protein (BCRP) [25]. The development of multidrug resistance may also be facilitated by increased activity of DNA repair enzymes, changed activity of topoisomerase II and I, reduced transport of drugs into the cell due to structural changes in the cell membrane, and the lack of activity of normal p53 protein that inhibits overexpression of Pgp in healthy cells. Another important mechanism is the development of the cells' capability to transform drugs into inactive forms [26]. Cancer stem cells have increased ability to convert the drug into a non-toxic form. An increased activity of detoxifying enzymes, such as glutathione transferase, glutathione peroxidase, and superoxide dismutase leads to neutralisation of the products formed by metabolism of drugs.

BCRP1, typical for all stem cells, was ascribed the role of an ABC family transporter that determines the occurrence of MDR in these cells. Higher expression of this protein was demonstrated in primitive resting stem cells devoid of the surface antigen CD34 (CD34<sup>-</sup>), while higher expression of Pgp and reduced expression of BCRP1 were observed in more active stem cells containing antigen CD34 [27]. In line with these findings, a hypothesis was proposed suggesting that BCRP1 maintains cells in an undifferentiated status while increased expression of Pgp has a contrary effect. Although the mechanism of this effect has not been fully explained yet, it is suspected that a change in expression of these proteins sensitises the cell to other substances, thus stimulating it to enter the differentiation stage.

BCRP1 also plays an important role in drug resistance. It was observed that cancer cells demonstrate higher drug resistance upon disease recurrence. A series of studies was therefore conducted to assess the changes in the expression of ABC transporters in the course of acute myeloid leukaemia. Expression of four MDR-related proteins was compared: BCRP1, ABCB1, MRP1, and LRP. It was shown that, compared to the post-diagnostic status, only BCRP1 had a significant increase in expression upon disease recurrence [28, 29]. This observation may be explained by the therapy being “survived” by cancer stem cells with BCRP1 proteins present within the cell wall. It is the stem cells that seem to contribute to tumour recurrence characterised by higher resistance to previous chemotherapy.

Cancer stem cells are also highly resistant to radiation therapy. This is due to the cells’ ability to rapidly activate effective mechanisms for DNA repair in response to an ionising radiation stimulus. This ability is particularly evident in CSCs while being less common in differentiated cancer cells. Its development is largely affected by kinases Chk1 and Chk2. They force the cell cycle stop, thus giving the cell time to repair its genomic material. It was shown that inhibition of these enzymes both *in vitro* and *in vivo* reduced the CSC resistance to radiation therapy [30].

The role of the Wnt/ $\beta$ -catenin pathway in the pathophysiology of CSCs has also been described.  $\beta$ -Catenin is an intracellular protein that plays an essential role in intercellular adhesion and is a key link in the signalling cascade Wingless/Wnt/ $\beta$ -catenin, which is relevant in the process of malignant transformation. This pathway is associated with the activation of cell proliferation and inhibition of apoptosis. A very clear relationship may be seen between the Wnt/ $\beta$ -catenin pathway activation and the severity of invasive lesions, the formation of metastases, and poorer clinical outcome.

The Wnt/ $\beta$ -catenin pathway has recently been implicated in radiation resistance in cells expressing CSC markers in breast cancer cell lines. Because radioresistance in CSCs may occur via concurrent but distinct mechanisms, these data regarding Wnt/ $\beta$ -catenin involvement in cell survival and self-renewal after irradiation correlate with the concept that CSCs have amplified DNA damage repair mechanisms through Chk1/2 activation. Normal stem cells activate the Wnt/ $\beta$ -catenin signalling axis during development. In non-CSCs cells this pathway promotes DNA damage tolerance. When DNA is damaged, PARP-1 is modified to prevent its interaction with Tcf-4, thus allowing Ku70 to bind in a complex with  $\beta$ -catenin to activate the Wnt pathway cellular effects. Therefore, DNA damage may enhance  $\beta$ -catenin activity. In light of this, while possibly promoting the ability of CSCs to survive extensive DNA damage until lethal damage can be repaired, the Wnt/ $\beta$ -catenin pathway

promotes genomic instability and may promote conversion of NSCs to CSCs through the destabilisation of the genome. This signalling axis could play its role by allowing radiated cells to tolerate DNA damage, while the Chk1/2 kinases cause cell cycle arrest until lethal DNA damage can be repaired. Alternatively, these pathways could both promote genomic instability while allowing tumour cells to survive after irradiation, thus accelerating the rate of genetic change in the tumour [31, 32].

### The role of stem cells in carcinogenesis

Two main models of the development of neoplastic lesions have been described to date. The first one, the stochastic model, currently only of historical value, assumed that all cancer cells were identical (structural homogeneity) and capable of disease progression. In view of recent results suggesting high heterogeneity of cancer cells in terms of their morphology, phenotype, capability to proliferate and differentiate and resistance to treatment, a second model – the hierarchic one, also known as the cancer stem cell model – has been developed. According to this model, cancer cells are organised in a hierarchical manner typical of all healthy tissues. The model distinguishes a small population of slowly dividing stem cells responsible for the formation of the lesion and a much larger group of more differentiated cells undergoing fast divisions and building up the lesion mass; these cells, however, are unable to form phenocopies of the tumour when forming metastases [33].

Another model of tumour development, known as the clonal expansion model, also exists. The model assumes that the disease develops as a result of clonal expansion of one of the altered cells characterised by the most favourable phenotypic traits due to mutations and thus capable of dominating the remaining cells. The cell was named the primary CSC. The primary CSC undergoes fast divisions, leading to pathological involvement of an increasing area. This condition lasts until the moment when mutation in one of the cells leads to an even higher acquired advantage over other cells and thus the new cell outgrows the tumour by clonal expansion. Such a cell is referred to as secondary CSC. Thus, spontaneous mutations may lead to the development of a change that would allow the altered cell to leave the niche and form a metastasis. Different resistance of cells to the applied treatment may also determine this phenomenon. The only surviving cells are those capable of adjusting to environmental changes by developing efficient protective mechanisms; it is these cells that contribute to disease recurrence [34, 35].

### Cancer stem cell markers

Current knowledge directs efficient cancer treatment strategies towards modulating the activity or

destroying CSCs. However, such treatments offer no chance of succeeding without specific marker characteristic for particular tumours. The role of cancer stem cell markers may be played by:

- cell differentiation antigens CD, expression of which is assessed at the surface of studied cells,
- cytoprotective enzymes,
- expression of ABC superfamily transporters determining the occurrence of multidrug resistance.

Currently, numerous studies are being conducted on cancer stem cell markers; unfortunately, no marker universal enough to be used in clinical practice has been identified to date. However, markers characteristic for individual tumours or tumours with primary foci originating in a particular organ have been described, but these markers are not always completely specific. This is due to the above-described theory of clonal selection and heterogeneity of cells building up the tumour. This phenomena was confirmed by the discovery of glioma CSCs showing both CD133+ and CD133– phenotypes, depending on the examined tumour part; similar features were also demonstrated in studied breast cancer stem cells. Some of those were characterised by expression of CD24–/CD44+ while others expressed CD24+/CD44– [36, 37]. Table 1 lists the better-examined markers that define CSCs.

### Novel cancer stem cell-targeting therapy

Traditional anticancer treatments in broad use today, such as chemotherapy, radiation therapy, or immunotherapy mainly target the differentiated cells that build up the tumour. They are, however, inefficient in destroying cancer stem cells most probably responsible for cancer metastases and recurrence. Therefore, novel methods allowing the neutralisation or destruction of CNCs are being investigated. Besides conventional methods, therapeutic opportunities in the course of cancer are offered by the use of healthy mesenchymal stem cells. Cytokines secreted by solid tumours have been shown to exert a positive chemotactic effect on mesenchymal stem cells. Thanks to that, these cells might be used as vectors transporting cytotoxic agents (granzymes, perforin, granulysin, cathepsins, TIA-1) that induce apoptosis in tumour cells and locally release high levels of cytokines that stimulate stronger immune responses [48, 49]. This property of mesenchymal stem cells also allows attempts to modify the cell genome in order to enhance the cellular expression of interferon  $\gamma$ , IL-12, and many other substances that accelerate cancer cell destruction. Attempts to use mesenchymal stem cells as sites for replication of deliberately introduced viral genomes containing genes that induce apoptosis of cause sensitisation of cells to cytostatics being used appear to be another interesting approach. In an analogous manner, stem cells might be sites for replication of oncolytic viruses tested in recent years

**Table 1.** Markers of tumour stem cells [38–47]

Marker CSC	Cancers that showed expression
CD24	Pancreatic cancer, breast cancer
CD44	Breast cancer, colorectal cancer, gastric cancer, hepatocellular carcinoma, ovarian cancer, pancreatic cancer, prostate cancer, head and neck cancer, ameloblastoma
CD90	Acute lymphoblastic leukaemia, hepatocellular carcinoma
CD105	Renal cancer
CD117	Ovarian cancer
CD133	Acute lymphoblastic leukaemia, colorectal cancer, endometriosis, hepatocellular carcinoma, lung cancer, primary bone tumours, ovarian cancer, pancreatic cancer, prostate cancer, brain tumours, Ewing's sarcoma
CD166	Colorectal cancer
CD200	Prostate cancer, brain tumours, breast cancer, malignant melanoma
EpCam	Colorectal cancer, pancreatic cancer, hepatocellular carcinoma
$\alpha 2\beta 1$	Breast cancer, prostate cancer
$\beta$ -Catenin	Prostate cancer, gastric cancer, breast cancer
ESA	Breast cancer, pancreatic cancer, colorectal cancer
LGR-5	Colorectal cancer
ABCG2	Breast cancer, pancreatic cancer
ALDH-1	Nasopharyngeal cancer, pancreatic cancer, breast cancer, sarcoma, colorectal cancer
Bmi1	Leukaemia

as novel anticancer drugs [50]. Oncolytic viruses themselves present with many traits that facilitate their use in anticancer therapy. They are able to replicate in cancer-transformed cells and initiate the destruction of these cells. The viral genome may be relatively easily modified by introducing fragments encoding for desired proteins active in destroying cancer cells, particularly CSCs. The superiority of viruses has higher selectivity compared to traditional methods such as chemotherapy or radiation therapy [51].

### Summary

Cancer stem cells share many characteristics with normal stem cells. With the growing evidence that

cancer stem cells exist in a wide array of tumours, it is becoming increasingly important to understand the molecular mechanisms that regulate their self-renewal and differentiation because corruption of genes involved in these pathways probably participates in tumour growth. It may also contribute to the identification of molecular targets important for future therapies. Although most questions regarding the origin and certain features of CSCs remain unanswered, their existence within tumours is widely accepted. Also, more clinical and experimental data show that curative cancer therapy is effective only when CSCs are completely eradicated. Current therapeutic approaches against cancer have been reported to control cell proliferation and tumour growth, but they are unable to completely eradicate the tumour cell mass.

Although there are many arguments for the CSC theory, some more sceptical publications have also revealed arguments against this thesis. The main problems with the CSC hypothesis include applicability of the hierarchical model, inconsistencies with xenotransplantation data, and the non-specificity of CSC markers. According to these publications, the CSC hypothesis has invigorated the research community to find novel approaches to cancer therapy. However, for many tumours, targeting a rare population of tumorigenic cells without consideration of the large bulk of proliferating cells may not change patient outcomes.

The role of CSCs in the diagnosis and therapy of cancer has recently been the subject of intense research. Therefore, improving anti-cancer treatment response requires more accurate identification of the CSCs. Some scientists believe that efforts to discover new and effective treatment methods may require much more than just targeting CSCs [52].

### Conflict of interest

The authors declare no conflict of interest.

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