REVIEW PAPER

UROLOGICAL ONCOLOGY

Cancer stem cells and their role in metastasis

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Introduction Cancer, next to cardiovascular diseases, remains the primary concern of modern medicine in developed countries. Despite the unprecedented progress in targeted therapies and personalised medicine, including immunotherapy and gene therapy, we are still unable to efficiently treat many malignancies. One of the major obstacles to treating cancer is its ability to metastasise. Hence, a better understanding of cancer biology with emphasis on the metastasis formation may hold the key to further ameliorating cancer treatment. Nowadays, there is a growing body of evidence for the common denominator of neoplasia, which seems to be universal – cancer stem cells which are being found in a growing number of cancers.

Material and methods We conducted a Web of Science and Medline database search using the terms "cancer stem cells", "carcinogenesis", and "stem cells" in conjunction with "metastasis", without setting time limits.

Results The existence of cancer stem cells was proven both in animal models and in humans. We know beyond doubt that cancer stem cells may be found in bladder cancer, breast cancer, and colon cancer, among others. The cancer stem cells in the aforementioned cancers may initiate tumour formation *ex vivo* and thus theoretically lead to tumour recurrence. Their role in the formation of metastases, however, is still under investigation.

Conclusions Although their exact role is yet to be identified, it is now obvious that cancer stem cells give rise to primary mass in solid tumours and differentiated cancer cells in leukaemias. However, the role of cancer stem cells in metastasis is still obscure.

Key Words: cancer stem cells ‹› carcinogenesis ‹› bladder cancer ‹› metastasis

INTRODUCTION

In the modern world the widespread of cancer remains a major health issue in industrialised and developing countries. In 2023, in the United States alone, over 600,000 cancer-related deaths are projected to occur [1]. Ongoing studies in the field of cancer biology are conducted to ameliorate our understanding of oncogenesis and develop novel therapeutic options for cancer patients. Over the last few decades, we achieved immense progress in the field of cancer biology thanks to discovering novel cellular biomarkers that allow precise characterisation of specific cancer cell subpopulations, which further allows the development of advanced therapeutic options, specifically targeting those cells [2]. The aforementioned advances in the molecular biology and discoveries of cellular biomarkers or clusters of differentiation, on the cellular surface as well as intracellularly, have led to the observation that the tumour mass contains a variety of cellular subpopulations including cells with stem cell-like phenotype and properties [3]. Those cells have been named cancer stem cells (CSCs) or tumour-initiating cells (TICs) and presently are thought to play a pivotal role in the cancer growth, spread, and relapse [4].

Surprisingly, the first notion of the existence of the cells that originate the tumour development comes from the mid 19th century and Julius Cohnheim's concept of "embryonic rests", i.e. embryonic cells that were not utilised during ontogenesis, as an origin of all tumours [5]. Many decades later, further research on germinal tumours has led to the discovery of embryonic stem (ES) cells in mice [6] by Stevens and Little, and as a consequence to the development of cancer stem cells theory by Kleinsmith and Pierce in 1964 [7], based on the observation that ES cells produce various differentiated tissues as well as embryonal carcinoma when transplanted in mice. That concept was confirmed by Bonnet and Dick in 1997, who observed that only a small population of acute myeloid leukaemia cells is able to initiate the acute myeloid leukemia and has vast self-renewal capacity [8]. Further studies have shown that CSCs are also encountered in solid tumours – colon and breast cancers among others [9]. The progeny of CSCs transplanted into a new host consist of cells with stem-like properties and differentiated cells without tumourigenic potential. This observation is of utmost importance because it signifies that tumours, much like healthy tissues, are hierarchically organised and there is a fraction of cells that is responsible for tumour initiation, formation, and growth – namely CSCs.

A malignant tumour's cellular architecture is highly heterogeneous. Among the tumour cells, there is a subgroup with the ability to self-renew despite cytotoxic treatment [10]. Those cells are called cancer stem cells (CSCs), a subpopulation of tumour cells characterised by low, although sustained potential for unlimited proliferation [11]. In addition to the ability to divide and increase the pool of their stem cells, CSCs are able to differentiate into non-tumourigenic cancer target types, which most likely constitute the majority of cells in the tumour mass [12]. It appears that the presence of just one CSC is sufficient to stimulate tumour growth. CSCs prefer hypoxic niches with low pH and limited availability of nutrients, making them highly resistant to challenging environmental conditions [13].

However, they are not autonomous entities but rather components of a larger ecosystem, actively influencing the tumour's microenvironment by restructuring it and receiving information from the niches they inhabit [14]. The existence of CSCs should be understood not through the prism of the hierarchy of development of normal tissues in a binary approach based on stem and non-stem elements, but based on the stem theory, understood as a series of interactions with the microenvironment, cancer cells, and other types of cells [15].

Considering the origin of CSCs, 3 crucial and well-established processes should be taken into account: spontaneous reprogramming of somatic cells, epigenetic and genetic changes such as methylation, rearrangement, demethylation in the pool of stem, progenitor and differentiated cells, and activation of the tumour microenvironment (TME) [16].

CSCs are in general characterised by 4 features: hierarchical cell organisation, hierarchy arising from the presence of self-renewing cells and those exhibiting transient proliferation, the consistent identity of CSC within the tumour structure, and the resistance of CSC to classical chemotherapy and radiotherapy [17].

RESULTS

Signal transduction pathways involved in cancer stem cell regulation

In order for CSCs to multiply and self-renew, they exploit dysfunction in stemness signalling pathways [18]. The signalling pathways that determine the specific features of CSCs mainly include the following: JAK/STAT, Wnt/β-catenin, PI3K/Akt, PTEN36, hedgehog, Notch, NF-κB, and Bcl-2 [19].

The JAK/STAT is an intracellular signalling pathway involved in cellular proliferation, differentiation, and the signal transduction [20]. This signalling pathway also mediates the process of haematopoiesis, maintaining proper immune function, tissue repair, and apoptosis [21].

In breast cancer, it was noted that constant activation of STAT3 enables CSC survival and maintenance of stemness, and interleukin-6 (IL-6) enables the transition of non-stem cancer cells into the cancer pathway, which was discovered as one of the mechanisms behind the self-renewal of glioma stem-like cells [22].

Wnt signalling pathway is involved in the processes of cell differentiation, survival, proliferation, and apoptosis [23]. The Wnt pathway consists of 3 subpathways: the non-canonical planar cell polarity (PCP) pathway, non-canonical Wnt/calcium pathway, and canonical pathway [24].

Both canonical and non-canonical pathways have a significant effect on CSCs. The canonical pathway affects the proliferation of those cells, while the non-canonical pathways enable the induction of CSC's dormancy. The Wnt/β-catenin pathway stimulates the reactivation of dormant CSCs, which promotes tumour recurrence [25].

The Notch signalling pathway performs a crucial function in the process of organ formation and tissue repair, and its disturbances broadly contribute to the development of cancer. This pathway interacts

with 4 NOTCH receptors [26]. The family of those receptors mediates the transmission of information in the cell over a short distance, and acts as a transcription factor activated by a family of ligands: Delta, Lag-2, and Serrate [27].
Increased activity of the Notch pathway is observed in CSCs, and some even claim that this signalling allows the tumour to achieve its native cancer phenotype. Consequently, this pathway causes these cells to show a lower level of proliferation, which allows them to survive in a dormant state and trigger tumour recurrence. This translates into resistance to anticancer therapy [28].
The Hedgehog pathway is a ligand-dependent signalling pathway in which the ligand can be Desert hedgehog, Sonic hedgehog, and Indian hedgehog. It plays an important role in the process of cell differentiation, proliferation, tissue polarity, cell survival, and stem cell formation [29].
There are indications that this pathway regulates CSCs in pancreatic adenocarcinoma, glioblastoma multiforme, and chronic myeloid leukaemia (CML) [30].
The significant impact of the Hedgehog pathway on CSCs has been demonstrated in a study of plasmocytic myeloma (PCM), which consists of a population of stem cells resembling memory B cells and a population of malignantly differentiated plasma cells, which constitute the majority. It turned out that maintaining the signalling of this pathway keeps PCM stem cells in an undifferentiated state, capable of proliferation [31].
Specific markers are probably expressed on the surface of CSCs. Those include the following: CD44, CD24, CD29, CD90, CD133, epithelial-specific antigen (ESA), and aldehyde dehydrogenase-1 (ALDH1) [32].
Other key markers of CSCs are transcription factors like OCT-4 and SOX-2, and drug-efflux pumps like ATP-binding cassette (ABC) drug transporters [33].
Of those, the surface marker CD133 has been observed in the CSC population in variety of neoplasms, among others: breast cancer, brain tumours, lung cancer, pancreatic cancer, prostate cancer, and ovarian cancer [34].
Interestingly, CSCs undergo both the epithelial-to-mesenchymal transition (EMT) and the mesenchymal-to-epithelial transition (MET), both of which promote tumour progression and metastasis [35].
Our review aims to determine the CSC's supposed role in tumour metastasis.

Stem cells and cancer stem cells – similarities and differences

Generating mutations in cellular DNA using carcinogens, i.e. causative agents, contributes to the development of cancer. These substances can include ionising radiation and various types of chemicals such as components of cigarette smoke, alcohol, formaldehyde, oncogenic viruses, and many others [36].
It has been estimated that the number of single-strand breaks and spontaneous base loss in nuclear DNA is approximately 10,000 changes per cell per day [37]. However, overcoming the carcinogenesis barrier, countered by the DNA repair process and apoptosis, requires a cell to accumulate between 3 and 7 mutations [38]. Somatic mutations can undergo clonal amplification, the number of which increases with age in human tissues [39]. Damaged tissues can be repaired through the impact of stem cells, which possess the ability to self-renew and divide throughout their lifespan [40]. The accumulation of genetic disorders at the level of epigenetic regulators may cause the transformation of normal stem-cells into CSCs, which is crucial in carcinogenesis [41].
Stem cells remain undifferentiated both in embryonic and adult stem cells. During the stem cell differentiation process, the stem cell program is silenced, but in the process of developing cancer, epigenetic reactivation of the stem cells leads to tumour multiplication and progression [42]. It may result in the transformation of a normal stem cell (NSC) into a CSC. Therefore, normal haematopoietic stem cells and cancer stem cells should present with similar features [43]. Firstly, both stem cells and CSCs are capable of self-renewal and division [44]. However, the potential for self-renewal is internally limited and reaches the division limit depending on the activity of p53 [45].
CSCs, on the other hand, can unlimited self-renewal and unlimited division, without the risk of differentiation, aging or cellular death [46]. Some differences and similarities also occur in the type of cell division. Stem cells usually divide asymmetrically. As a result of this division, a SC gives rise to another stem cell and a progenitor cell [45].
CSCs divide mainly by symmetric divisions, although it is worth noting that both CSCs and SCs have the capacity for both types of divisions. However, the symmetric division favoured by CSCs promotes tumour growth and is accompanied by a loss of the protective effect of p53 [47].
Another common feature is that they have common surface markers. CD34 is present both on the surface of haematopoietic stem/progenitor cells (HSPCs) and, judging by numerous reports, on the surface of CSCs [48]. An important common feature of both normal stem cells and CSCs is that both types of cells inhabit niches, i.e. specialised microenvironments, which consist of fibroblasts, immune cells,

endothelial cells, perivascular cells, extracellular matrix (ECM) components, cytokines, and growth factors [49].

Therefore, there are reasons to believe that cells in the niche, activated by the tumour, support not only normal stem cells but also CSCs in the development of its characteristic features [50].

It is worth mentioning that mesenchymal stem cells (MSCs) interact with both adult stem cells and CSCs in the microenvironments of both those cell types and mobilise them to secrete cytokines such as IL-10 and TGF-β. These actions are aimed at stimulating CD4+ T-cells to their anti-inflammatory phenotype [51].

Both stem cells and CSCs are characterised by a similar transcriptional profile. Even signalling pathways such as Wnt and Notch are active among both stem cells and CSCs, which increases their ability to progress [52].

Thirdly, normal cells capable of proliferation such as immune cells and stem cells can reprogram their metabolism similarly to cancer cells [53]. Cancer cells show a preference for a metabolism focused on glycolysis in terms of obtaining energy, even in the presence of oxygen [54].

Although this process is less efficient than oxidative phosphorylation in the context of obtaining ATP molecules, cancer cells compensate for this by increasing the rate of glycolysis and thus increasing the rate of obtaining ATP [55]. In addition to ATP, cancer cells require intermediate products and precursors that are important for the biosynthesis of macromolecules necessary to increase tumour mass, with reduced demand for nutrients [56].

A similar metabolic plasticity is demonstrated by haematopoietic stem progenitor cells (HSPCs), which are able to obtain ATP through mitochondrial respiration and glycolysis. Due to their small mitochondrial mass, their metabolism is maintained mainly by glycolysis [57].

Metastatic niche and the role of cancer stem cells in metastasis formation

Cancer cells may spread to distant organs from the primary tumour, leading to cancer dissemination, and consequently to the patient's death. This phenomenon is called metastasis [58]. Metastasis is associated with detachment of the cells from the primary tumour mass. This is followed by local infiltration and angiogenesis. In the next stage, cancer cells move to blood and lymphatic vessels, and from there they begin to invade distant organs [59].

At each stage, the process of cancer metastasis and progression is supported by suppressing the host's immune system. This is possible by stimulating immunosuppressive cells and inhibiting immune effector cells [60].

There are several hypotheses regarding the metastasis process, including: EMT, altered integrin expression, a macrophage facilitation process, and a macrophage origin involving either transformation or fusion hybridisation with neoplastic cells and microRNAs (miRNAs) [61–63]. There are also mutations in genes encoding proteins that determine the invasive potential of the tumour, such as mutation of p53 (TP53), cyclin-dependent kinase inhibitor 2A (CDKN2A), phosphatase and tensin homologue (PTEN), and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA) [64].

Among many models of metastasis, there is also one based on the premise that the metastasis process can be initiated by CSCs [65]. CSCs show increased EMT activity. EMT also contributes to the development of a CSC-like phenotype in cells other than CSCs [66]. EMT also contributes to the loss of epithelial adhesion receptors, such as E-cadherin, occludin, α-catenin, and claudin, and consequently the loss of cell polarity. The consequence of this process is an increase in the invasiveness of cancer cells [67]. Through numerous transcription factors, EMT also influences the maintenance of the proper structure of the cytoskeleton and extracellular matrix degradation [68]. The intensified process of destruction of the normal matrix favours its replacement by the cancer matrix. Additionally, this process may lead to the destruction of the basement membrane, which further enhances the metastatic process [69].

When analysing the metastasis formation process, it is worth taking a closer look at microRNA It is a short sequence RNA, the main purpose of which is post-transcriptional silencing of selected genes [70]. miRNA is involved in cancer progression and metastasis, and it enables contact between cancer cells and the TME [71].

According to the latest research, miRNAs can influence CSC properties such as tumourigenesis, self-renewal, and resistance to cytotoxic treatment, thereby enhancing cancer progression [72]. Increased resistance to chemotherapy and radiotherapy in CSCs is also explained by increased expression of anti-apoptotic proteins and increased levels of ATP-binding cassette transporters [73]. It is currently believed that ATP-binding cassette transporters may be involved at every stage of tumourigenesis, including metastasis [74].

As seen above, the metastasis process is a complex process that consists of many factors and mechanisms. However, the metastasis formation is not only limited to changes that occur in tumour cells, but

it additionally requires interaction with stromal cells at both the local and systemic levels [75]. It is therefore worth looking at the TME, which plays a decisive role in cancer progression. The relationship between cancer cells and the TME is inseparable. This is primarily observed in the context of reprogramming of the TME by tumour-derived factors by which the microenvironment enables its survival [76].

There are reasons to believe that the environment has a special impact on cancer cells, thanks to which they acquire the features of "stemness" [77]. It is even believed that the tumour microenvironment induces a change in the phenotype from differentiated cancer cells to CSCs, and this plasticity particularly influences resistance to therapy [78].

It is well known that CSCs live in a special microenvironment, which can be called the "stem-cell niche", and their survival is conditioned by various factors from the niche, which act directly or in a paracrine manner [79]. The entire tumour microenvironment consists of fibroblasts, endothelial cells, immune cells, and extracellular factors such as hormones, growth factors, cytokines, extracellular matrix, etc. The role of the TME is particularly important in the process of metastasising, and it influences the resistance to anti-cancer therapy [80]. Cancer cells activate stromal cells in the microenvironment, i.e. fibroblasts, smooth cells, adipocytes, mesenchymal, progenitor, and inflammatory cells. They, in turn, stimulate the secretion of growth factors and proteases, creating something like a chain reaction in the carcinogenesis process [81].

Fibroblasts are the key component of the TME. Cancer-associated fibroblasts (CAFs) belong to the stromal cells. They secrete the extracellular matrix components, creating a dense tumour network, and contribute largely to tumour progression [82]. Cancer cells can move along collagen fibres and spread further. Additionally, CAFs and cancer cells support the release of matrix metalloproteinases (MMPs), which degrade of the basement membrane, supporting cancer spread [83]. According to some studies, especially concerning breast cancer and hepatocellular cancer, it was observed that CAFs can produce mechanisms of resistance to chemotherapy and induction of CSCs [84]. This most likely occurs through the interaction of hepatocyte growth factor (HGF), IL-6, TGF-β, chemokines, and factors activating Wnt signalling provided by CAFs [85].

One of those factors is periostin, which is secreted, among others, by CAFs. It can activate the PI3K/Akt and/or Wnt/β-catenin oncogenic pathways. Therefore, because those pathways are pathologically dysregulated in CSCs, it may be speculated that periostin, and thus CAFs, enhance the activation of CSCs [86]. The tumour microenvironment is, as we know, a place rich in factors promoting tumour proliferation and metastasis. Uncontrolled growth is a natural consequence of tumorigenesis, but it is also associated with an increased demand for oxygen and generates problems in its supply. It therefore appears to be a natural phenomenon that the tumour environment is in a state of chronic hypoxia [87]. To counteract hypoxia and acidification, TME stimulates angiogenesis, a process that allows the tumour to create vessels supplying the tumour with oxygen and nutrients, while removing unnecessary and harmful metabolic products [88].

Because this phenomenon is crucial in tumour progression, it has become the subject of many studies. It was hypothesised that even if angiogenesis inhibitors were used and oxygen-poor conditions were created, the tumour mass would continue to grow due to CSCs and the associated activation of the Akt/β catenin pathway. Later studies drew attention to the fact that cancer stem cells produce vascular endothelial growth factor (VEGF) at much higher levels under various environmental conditions compared to SCs [89]. It promotes angiogenesis and has mitogenic and anti-apoptotic effects. Additionally, it increases vascular permeability and promotes cell migration [90].

However, the functions of VEGF go beyond the ability to perform angiogenesis. It exhibits paracrine and autocrine signalling because it can modulate the host response to cancer by influencing immune cells in the microenvironment, and VEGF receptors modulate the function of fibroblasts in the tumour stroma. This is believed to be one of the mechanisms for maintaining the capacity of CSCs [91]. There is a correlation between the number of CSCs in tumour tissue and the number of immune cells that infiltrate the tumour. It is suspected that CSCs impair the process of antigen presentation to T lymphocytes using MHC-I. This leads to the development of resistance to the cytotoxic effects of CD8+ T lymphocytes [92].

Dendritic cells (DCs) are the main elements of the adaptive immune response and have the ability to activate T lymphocytes, which is a defence mechanism against cancer. Of course, cancer can affect DC functions by preventing their maturation or through molecules originating from the microenvironment, inhibiting their activation [93].

Moreover, cancer-altered DCs may even promote tumourigenesis. Recent studies based on renal cancer draw attention to the role of CSCs in this process. Renal cancer cells that expressed the surface marker CD105 blocked the maturation of monocyte-derived DCs *in vitro* at a higher rate than tumour cells

negative for this marker. This is important because CD105 is also a surrogate marker for CSCs [94].
We can also distinguish tumour-associated macrophages (TAMs) among the immune cells in the TME. They can enhance immunosuppression, the process of vascular formation, and the proliferation of cancer cells, and thus may increase the tumour mass and the probability of metastasising [95]. TAMs have protective functions towards CSCs against chemotherapy, thus driving therapy resistance. Additionally, TAMs increase the ability of CSCs to initiate tumourigenesis [96].
The state of the CSC is regulated by many different signals from the niche. One of them is transforming growth factor β (TGF-β), a cytokine that gives CSCs drug resistance [97]. TGF-β determines the low immunogenicity of CSCs compared to non-CSCs. The same effect is exerted by the other cytokines like IL-4 and IL-10, which also have immunosuppressive effects, and high expression of which is observed in CSCs [98]. One of its many functions is to stimulate the production of T-reg cells, the function of which is to suppress the immune response against the tumour [99].

Bladder cancer

In 2018, bladder cancer was the 10th most common cancer in the world, with a predominance among men. In this sex, bladder cancer was the sixth most common cancer [100]. In the case of non-muscle invasive bladder cancer (NMIBC), the first-line treatment is bladder-sparing therapy. If the muscle layer becomes infiltrated, as in the case of muscle-invasive bladder cancer (MIBC), cystectomy is performed along with lymph node dissection (LND). The inclusion of neoadjuvant chemotherapy further improves the prognosis. Although the combination of these 2 methods is a standard approach in the case of MIBC, it remains controversial [101].
If metastases occur, cytotoxic chemotherapy based on cisplatin is initiated. Despite that, some patients experience relapses and develop resistance to therapy. The utilisation of chemotherapy itself is associated with many side effects, most importantly with renal toxicity [102].
Therefore, it seems extremely important to search for novel, targeted methods of treatment of bladder cancer, based on better understanding its biology. It is crucial to look at its development and progression, including metastatic pathways.
The presence of CSCs was also discovered in bladder cancer, where they showed typical CSC surface markers such as CD44, CD133, ALDH1, SOX2, and SOX4, and a set of characteristic features such as self-renewal or the ability to initiate cancer, and an increased expression of genes involved in EMT [103].
In superficial papillary carcinomas, the origin is sought among intermediate cells. But even their morphological structure resembles a normal epithelium with a basal layer. This involves questioning the theory that the intermediate cell is a universal cell in the context of cancer formation. Believing in the existence of a universal cell and trying to explain the existence of a basal-like target in NMIBC, it should be noted that in the basal layer of papillary carcinoma regeneration occurs through a process of dedifferentiation, and the microenvironment most likely contributes to this process [104].

Colon cancer

In the United States, colorectal cancer is the third most frequently diagnosed cancer and the third leading cause of cancer death among men and women [105]. It is expected that by 2035 in the world there will be up to 2.5 million new cases of this type of cancer, which makes it a significant global problem that should be a focus of attention of researchers in the context of searching for new therapeutic methods [106]. Therefore, it seems important to understand the structure of cancer and the mechanisms that contribute to its development.
It is highly probable that CSCs are present in colorectal cancer, and further, they have been identified as one of the causative factors capable of developing the tumour [107]. It is even believed that CSCs are mainly responsible for cancer progression, including the ability to metastasise and their resistance to therapy [108].
Under normal conditions, stem cells present at the bottom of the colon crypts divide and give rise to daughter cells. These cells migrate upwards, differentiate, and replace old epithelial layer cells. Sometimes, however, this process may be disturbed. It is suspected that CSCs are formed under the influence of the microenvironment, leading to abnormal divisions and thus tumourigenesis [109].
When considering CSCs and their role in the development of colorectal cancer, it is worth taking a closer look at leucine-rich-repeat-containing G-protein-coupled receptor 5 (Lgr5), which is a protein that is expressed in columnar cells in intestinal crypts. Crypt base columnar cells with positive Lgr5 show features of stem cells, and they were identified as a potential stem marker in cancer [110]. Moreover, in the progression of colorectal cancer and its metastases, a hierarchical structure characteristic of CSCs was noticed, while functional CSCs themselves showed expression of Lgr5 [111].

Based on the research, it was also discovered that Lgr5-negative tumours sometimes progress, but even then, those cells, just like Lgr5-positive cells, have increased rDNA transcription and protein synthesis at a level similar to that of stem cells. This is related to microenvironmental signalling and the induction of a certain plasticity of tumour cells [112].
It is worth mentioning that Lgr5 stimulates canonical Wnt/β-catenin signalling [113], which has a proven impact on the formation of CSCs.
Baker et al. noticed that the introduction of the APC (adenomatous polyposis coli) mutation into Lgr5-positive cells in mouse bodies resulted in the formation of adenomas in the small and large intestine. Thanks to this, the process was better understood, and it was demonstrated that mutated CSCs with a positive Lgr marker may be cancerous [114].
The existence of the 22-kDa transmembrane-4-L-six-family member-1 (TM4SF1) protein, which consists of 4 transmembrane domains, may also be crucial in explaining this process. The protein itself is often called tumour-associated antigen L6, and its increased expression is observed in many cancers, including colon cancer [115]. TM4SF1 is associated with many tumour characteristics, such as growth, invasiveness, and metastatic ability [116].
A study was conducted to look for the function of TM4SF1 in the process of chemotherapy resistance with the help of fluorouracil. Silencing TM4SF1 resulted in reduced expression of surface markers CD133, CD44, SOX2, and ALDHA1. When this intermembrane protein was overexpressed, CD133 and SOX2 were increased [117].
The aforementioned markers are characteristic of CSCs. The ability of TM4SF1 to interfere with the expression of these surface molecules demonstrates that it has an impact on maintaining tumour stemness and is important for CSCs-dependent CRC progression.
Further research using RNA-Seq to identify the differentially expressed genes (DEGs) attempted to discover the connection between TM4SF1, EMT, and cell stemness. It turned out, based on gene set enrichment analysis (GSEA), that CRC with suppressed TM4SF1 showed significant disruptions in the Wnt pathway and a reduction in β-catenin levels, but stimulation of the Wnt/β-catenin pathway could reverse the effects of TM4SF1-deficiency [117]. Therefore, both this signalling pathway and the protein seem to be closely related.
The Wnt/β-catenin pathway is one of the main pathways involved in the development of colorectal cancer. This pathway also regulates CSCs and is an important factor for maintaining the self-renewal capacity of CSCs. It influences the production of CSCs from normal stem cells or non-CSCs cell populations, contributing to their resistance to anticancer therapy [118].
Moreover, myofibroblasts present in the niche are able to stimulate the Wnt pathway, thereby restoring the CSCs phenotype in more differentiated tumour cells by secreting HGF [119].

Breast cancer

Breast cancer is the most frequently diagnosed cancer in the world and is currently the leading cause of cancer-related death among women [120]. According to statistics from 2017 approximately 1.7 million new cases of breast cancer are diagnosed worldwide each year [121].
Its treatment depends on the stage. There are many treatment methods, such as radical surgery, radiotherapy, hormone therapy depending on the receptor status, immunotherapy, chemotherapy, or a combination of several methods [122].
Randomised, controlled trials show that widespread screening with and introduction of novel, targeted therapies have led to a reduction in breast cancer mortality [123].
However, this does not change the fact that breast cancer is a significant social problem, and every effort should be made to ameliorate our understanding of the mechanisms of cancer formation and metastasis in order to further develop novel therapeutic methods.
Despite all the abovementioned, resistance to therapy is still observed. In the course of diagnostics on the specificity of this cancer, a small group of cancer stem cells was identified in the breast tumour – breast cancer stem cells (BCSC). It is suspected that those cells are the cause of resistance and recurrence of cancer [124].
In BCSCs, we are able to observe the functioning of signalling pathways such as Notch, Hedgehog, Wnt, etc. [125]. Those pathways are important and recognised modulators of CSCs function. Thanks to them, BSCSs are able to maintain their unique characteristics.
Moreover, BCSCs interact with immune system cells and host cells, creating a picture of the tumour microenvironment. This complex signalling between microenvironmental cells and BCSCs leads to tumour initiation and progression [126].
Cancer cells are surrounded by normal tissue and CAFs, endothelial cells, and ECM, which together with immune cells create the abovementioned evolving environment. Breast tumours show a certain phenotypic plasticity, which is achieved

thanks to numerous signals from the tumour microenvironment that influence their adaptation to the ecological niche of the tumour [127].

Long non-coding RNA (lncRNA) represents the largest class among non-coding RNA subtypes. It plays an important role in the regulation of transcription and post-transcriptional mechanisms. Its participation is observed in processes such as proliferation, apoptosis, and cellular differentiation [128].

IncRNAs are hypothesised to be involved in "stemness" maintenance. Their dysregulation is observed in CSCs. Although they are found in low quantities, there are reports describing lncRNAs that are specific to BCSCs. When analysing IncRNA, one may come across IncROPM (a regulator of phospholipid metabolism), related to PLA2G16 and thus to phospholipid metabolism. Some studies suggest that lncROPM may up-regulate the expression of PLA2G16 by stabilising PLA2G16 mRNA. This causes a change in phospholipid metabolism and the production of free fatty acids, in particular arachidonic acid, which is able to activate PI3K/AKT, Wnt/β-catenin, and Hippo/YAP signalling pathways [129]. These pathways are, of course, closely related to CSCs, determining their specific features.

The group of researchers also noted that luminal or basal-like breast tumour may arise from mutations of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA) in mammary luminal stem cells. Moreover, it is believed that basal-like cancer with a BRCA1 mutation could arise from basal stem cells, and the loss of BRCA1 leads to uncontrolled division of stem cells, and the population of cells with this mutation gives a chance to develop cancer [130].

When analysing the process of breast cancer development, it is crucial to mention the EGFR-HER2 module. HER2 is an epidermal growth factor whose overexpression is observed in 20% of breast cancers. It can form heterodimers with EGFR, HER3, or HER4. The heterodimerisation process is believed to be one of the mechanisms of resistance to anticancer therapy in cancers with HER2 overexpression [131].

This module is an important marker of clonal expression of transit-amplifying cells (TACs) and their interaction with stem cells. The active EGFR–HER2 complex of TACs modulates the functioning of progenitor cells and promotes their dedifferentiation into stem cells. TAC itself is an intermediary in the transition between stem cells and differentiated cells, and therefore participates in the process of tumour formation [132].

There are reports that under the influence of fractional irradiation, the level of reactive oxygen species decreased in cancer stem cells in breast cancer compared to the level in differentiated cancer cells, which suggests a certain mechanism of resistance to radiotherapy. However, treatment with multidrug CT, in addition to promoting CSCs markers, led to an increase in the number of CSCs of non-stem origin. This process was probably dependent on the influence of CSCs [133].

CONCLUSIONS

The sole existence of cancer stem cells has been proven beyond reasonable doubt. In a variety of cancers CSCs have been identified and phenotypically characterised. It is evident that they are an important part of the complex interplay between signal transduction pathways, cytokine and chemokine interactions, and epithelial-mesenchymal transition. However, their exact role in the carcinogenesis remains unclear. Similarly, they evidently play part in the metastasis formation process; however, data are scarce and more speculative than conclusive. The question of whether CSCs play a key role in carcinogenesis and metastasis requires further extensive research.

CONFLICT OF INTERESTS

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References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023; 73: 17-48.
2. Walcher L, Kistenmacher AK, Suo H, et al. Cancer Stem Cells – Origins and Biomarkers: Perspectives for Targeted Personalized Therapies. Front Immunol. 2020; 11: 1280.
3. Yu Z, Pestell TG, Lisanti MP, Pestell RG. Cancer stem cells. Int J Biochem Cell Biol. 2012; 44: 2144-2151.
4. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001; 414: 105-111.
5. Capp JP. Cancer Stem Cells: From Historical Roots to a New Perspective. J Oncol. 2019; 2019: 5189232.
6. Stevens LC, Little CC. Spontaneous Testicular Teratomas in an Inbred Strain of Mice. Proc Natl Acad Sci U S A. 1954; 40: 1080-1087.

7. Kleinsmith LJ, Pierce GB Jr. Multipotentiality of single embryonal carcinoma cells. Cancer Res. 1964; 24: 1544-1551.

8. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997; 3: 730-737.

9. Hermann PC, Bhaskar S, Cioffi M, Heeschen C. Cancer stem cells in solid tumors. Semin Cancer Biol. 2010; 20: 77-84.

10. Skvortsova I. Cancer Stem Cells: What Do We Know about Them? Cells. 2021; 10: 1528.

11. Biserova K, Jakovlevs A, Uljanovs R, Strumfa I. Cancer Stem Cells: Significance in Origin, Pathogenesis and Treatment of Glioblastoma. Cells. 2021; 10: 621.

12. Clarke MF, Dick JE, Dirks PB, et al. Cancer Stem Cells-Perspectives on Current Status and Future Directions: AACR Workshop on Cancer Stem Cells. Available at: www.aacrjournals.org.

13. Najafi M, Mortezaee K, Majidpoor J. Cancer stem cell (CSC) resistance drivers. Life Sci. 2019; 234.

14. Lathia JD, Mack SC, Mulkearns-Hubert EE, Valentim CLL, Rich JN. Cancer stem cells in glioblastoma. Genes Dev. 2015; 29: 1203.

15. Prager BC, Xie Q, Bao S, Rich JN. Cancer Stem Cells: The Architects of the Tumor Ecosystem. Cell Stem Cell. 2019; 24: 41-53.

16. Dianat-Moghadam H, Heydarifard M, Jahanban-Esfahlan R, et al. Cancer stem cells-emanated therapy resistance: Implications for liposomal drug delivery systems. J Control Release. 2018; 288: 62-83.

17. Batlle E, Clevers H. Cancer stem cells revisited. Nat Med. 2017; 23: 1124-1134.

18. Ajani JA, Song S, Hochster HS, Steinberg IB. Cancer stem cells: the promise and the potential. Semin Oncol. 2015; 42 Suppl 1: S3-S17.

19. Chen K, Huang YH, Chen JL. Understanding and targeting cancer stem cells: therapeutic implications and challenges. Acta Pharmacol Sin. 2013; 34: 732-740.

20. Xin P, Xu X, Deng C, et al. The role of JAK/STAT signaling pathway and its inhibitors in diseases. Int Immunopharmacol. 2020; 80: 106210.

21. Hu X, Li J, Fu M, Zhao X, Wang W. The JAK/STAT signaling pathway: from bench to clinic. Signal Transduct Target Ther. 2021; 6: 1-33.

22. Yang L, Shi P, Zhao G, et al. Targeting cancer stem cell pathways for cancer therapy. Signal Transduct Target Ther. 2020; 5: 8.

23. Willert K, Jones KA. Wnt signaling: is the party in the nucleus? Genes Dev. 2006; 20: 1394-1404.

24. Duchartre Y, Kim YM, Kahn M. The Wnt signaling pathway in cancer. Crit Rev Oncol Hematol. 2016; 99: 141-149.

25. Katoh M, Katoh M. WNT signaling and cancer stemness. Essays Biochem. 2022; 66: 319-331.

26. Zhou B, Lin W, Long Y, et al. Notch signaling pathway: architecture, disease, and therapeutics. Signal Transduct Target Ther. 2022; 7: 95.

27. Ehebauer M, Hayward P, Martinez-Arias A. Notch signaling pathway. Sci STKE. 2006; 2006: cm7.

28. Janghorban M, Xin L, Rosen JM, Zhang XHF. Notch Signaling as a Regulator of the Tumor Immune Response: To Target or Not To Target? Front Immunol. 2018; 9: 1649.

29. Banaszek N, Kurpiewska D, Kozak K, Rutkowski P, Sobczuk P. Hedgehog pathway in sarcoma: from preclinical mechanism to clinical application. J Cancer Res Clin Oncol. 2023; 149: 17635.

30. Merchant AA, Matsui W. Targeting Hedgehog – A cancer stem cell pathway. Clin Cancer Res. 2010; 16: 3130-3140.

31. Peacock CD, Wang Q, Gesell GS, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proc Natl Acad Sci U S A. 2007; 104: 4048-4053.

32. Yu Z, Pestell TG, Lisanti MP, Pestell RG. Cancer stem cells. Int J Biochem Cell Biol. 2012; 44: 2144-2151.

33. Murar M, Vaidya A. Cancer stem cell markers: premises and prospects. Biomark Med. 2015; 9: 1331-1342.

34. Kim WT, Ryu CJ. Cancer stem cell surface markers on normal stem cells. BMB Rep. 2017; 50: 285-298.

35. Han J, Won M, Kim JH, et al. Cancer stem cell-targeted bio-imaging and chemotherapeutic perspective. Chem Soc Rev. 2020; 49: 7856-7878.

36. Huang M, Xia Y, Li K, et al. Carcinogen exposure enhances cancer immunogenicity by blocking the development of an immunosuppressive tumor microenvironment. J Clin Invest. 2023; 133: e166494.

37. Hoeijmakers JHJ. DNA damage, aging, and cancer. N Engl J Med. 2009; 361: 1475-1485.

38. Yin W, Wang J, Jiang L, James Kang Y. Cancer and stem cells. Exp Biol Med (Maywood). 2021; 246: 1791-1801.

39. Vijg J, Schumacher B, Abakir A, et al. Mitigating age-related somatic mutation burden. Trends Mol Med. 2023; 29: 530-540.

40. Clarke MF. Clinical and Therapeutic Implications of Cancer Stem Cells. N Engl J Med. 2019; 380: 2237-2245.

41. Kreso A, Dick JE. Evolution of the cancer stem cell model. Cell Stem Cell. 2014; 14: 275-291.

42. Lytle NK, Barber AG, Reya T. Stem cell fate in cancer growth, progression and therapy resistance. Nat Rev Cancer. 2018; 18: 669-680.

43. Vlaski-Lafarge M, Labat V, Brandy A, et al. Normal Hematopoetic Stem and Progenitor Cells Can Exhibit Metabolic Flexibility Similar to Cancer Cells. Front Oncol. 2020; 10: 713.

44. Das D, Fletcher RB, Ngai J. Cellular mechanisms of epithelial stem cell self-renewal and differentiation during homeostasis and repair. Wiley Interdiscip Rev Dev Biol. 2020; 9: e361.

45. Verga Falzacappa MV, Ronchini C, Reavie LB, Pelicci PG. Regulation of self-renewal in normal and cancer stem cells. FEBS J. 2012; 279: 3559-3572.

46. Chen HY, Cheng AJ, Chen HY, Cheng AJ. Potential to Eradicate Cancer Stemness by Targeting Cell Surface GRP78. Biomolecules. 2022; 12: 941.

47. Cicalese A, Bonizzi G, Pasi CE, et al. The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. Cell. 2009; 138: 1083-1095.

48. Kapoor S, Shenoy SP, Bose B. CD34 cells in somatic, regenerative and cancer stem cells: Developmental biology, cell therapy, and omics big data perspective. J Cell Biochem. 2020; 121: 3058-3069.

49. Plaks V, Kong N, Werb Z. The Cancer Stem Cell Niche: How Essential is the Niche in Regulating Stemness of Tumor Cells? Cell Stem Cell. 2015; 16: 225.

50. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012; 21: 309-322.

51. Aponte PM, Caicedo A. Stemness in Cancer: Stem Cells, Cancer Stem Cells, and Their Microenvironment. Stem Cells Int. 2017; 2017: 5619472.

52. Luo Q, Liu P, Yu P, Qin T. Cancer Stem Cells are Actually Stem Cells with Disordered Differentiation: the Monophyletic Origin of Cancer. Stem Cell Rev Rep. 2023; 19: 827-838.

53. De Berardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Sci Adv. 2016; 2: e1600200.

54. Zhang Y, Yang JM. Altered energy metabolism in cancer: a unique opportunity for therapeutic intervention. Cancer Biol Ther. 2013; 14: 81-89.

55. Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol. 2011; 27: 441-464.

56. Ganapathy-Kanniappan S, Geschwind JFH. Tumor glycolysis as a target for cancer therapy: progress and prospects. Mol Cancer. 2013; 12: 152.

57. Scapin G, Goulard MC, Dharampuriya PR, Cillis JL, Shah DI. Analysis of Hematopoietic Stem Progenitor Cell Metabolism. J Vis Exp. 2019; 9: 10.3791/60234.

58. Suhail Y, Cain MP, Vanaja K, et al. Systems Biology of Cancer Metastasis. Cell Syst. 2019; 9: 109-127.

59. Duffy MJ, McGowan PM, Gallagher WM. Cancer invasion and metastasis: changing views. J Pathol. 2008; 214: 283-293.

60. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013; 19: 1423-1437.

61. Seyfried TN, Huysentruyt LC. On the origin of cancer metastasis. Crit Rev Oncog. 2013; 18: 43-73.

62. Hamidi H, Ivaska J. Every step of the way: integrins in cancer progression and metastasis. Nat Rev Cancer. 2018; 18: 533-548.

63. Bouyssou JMC, Manier S, Huynh D, Issa S, Roccaro AM, Ghobrial IM. Regulation of microRNAs in Cancer Metastasis. Biochim Biophys Act. 2014; 1845: 255.

64. Fares J, Fares MY, Khachfe HH, Salhab HA, Fares Y. Molecular principles of metastasis: a hallmark of cancer revisited. Signal Transduct Target Ther. 2020; 5: 28.

65. Tang KD, Holzapfel BM, Liu J, et al. Tie-2 regulates the stemness and metastatic properties of prostate cancer cells. Oncotarget. 2015; 7: 2572-2784.

66. Najafi M, Farhood B, Mortezaee K. Cancer stem cells (CSCs) in cancer progression and therapy. J Cell Physiol. 2019; 234: 8381-8395.

67. Chen C, Zhao S, Karnad A, Freeman JW. The biology and role of CD44 in cancer progression: therapeutic implications. J Hematol Oncol. 2018; 11: 1-23.

68. Puisieux A, Brabletz T, Caramel J. Oncogenic roles of EMT-inducing transcription factors. Nat Cell Biol. 2014; 16: 488-494.

69. Cox TR. The matrix in cancer. Nat Rev Cancer. 2021; 21: 217-238.

70. Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol. 2018; 141: 1202-1207.

71. Solé C, Lawrie CH. MicroRNAs in Metastasis and the Tumour Microenvironment. Int J Mol Sci. 2021; 22: 4859.

72. Takahashi R u., Miyazaki H, Ochiya T. The role of microRNAs in the regulation of cancer stem cells. Front Genet. 2013; 4: 72858.

73. Zhang J, Li Q, Chang AE. Immunologic Targeting of Cancer Stem Cells. Surg Oncol Clin N Am. 2019; 28: 431-445.

74. Nobili S, Lapucci A, Landini I, Coronnello M, Roviello G, Mini E. Role of ATP-binding cassette transporters in cancer initiation and progression. Semin Cancer Biol. 2020; 60: 72-95.

75. El-Kenawi A, Hänggi K, Ruffell B. The Immune Microenvironment and Cancer Metastasis. Cold Spring Harb Perspect Med. 2020; 10: a037424.

76. Liu W, Powell CA, Wang Q. Tumor microenvironment in lung cancer-derived brain metastasis. Chin Med J (Engl). 2022; 135: 1781.

77. Sun HR, Wang S, Yan SC, et al. Therapeutic Strategies Targeting Cancer Stem Cells and Their Microenvironment. Front Oncol. 2019; 9: 1104.

78. Clara JA, Monge C, Yang Y, Takebe N. Targeting signalling pathways and the immune microenvironment of cancer stem cells – a clinical update. Nat Rev Clin Oncol. 2020; 17: 204-232.

79. Duan H, Liu Y, Gao Z, Huang W. Recent advances in drug delivery systems for targeting cancer stem cells. Acta Pharm Sin B. 2021; 11: 55-70.

80. Wu T, Dai Y. Tumor microenvironment and therapeutic response. Cancer Lett. 2017; 387: 61-68.

81. van der Pluijm G. Epithelial plasticity, cancer stem cells and bone metastasis formation. Bone 2011; 48: 37-43.

82. Foster DS, Januszyk M, Delitto D, et al. Multiomic analysis reveals conservation of cancer-associated fibroblast phenotypes across species and tissue of origin. Cancer Cell. 2022; 40: 1392-1406.e7.

83. Piersma B, Hayward MK, Weaver VM. Fibrosis and cancer: A strained relationship. Biochim Biophys Acta Rev Cancer. 2020; 1873: 188356.

84. Nallasamy P, Nimmakayala RK, Karmakar S, et al. Pancreatic Tumor Microenvironment Factor Promotes Cancer Stemness via SPP1-CD44 Axis. Gastroenterology. 2021; 161: 1998-2013.e7.

85. Weiland A, Roswall P, Hatzihristidis TC, Pietras K, Ostman A, Strell C. Fibroblast-dependent regulation of the stem cell

properties of cancer cells. Neoplasma. 2012; 59: 719-727.

86. Xu X, Chang W, Yuan J, et al. Periostin expression in intra-tumoral stromal cells is prognostic and predictive for colorectal carcinoma via creating a cancer-supportive niche. Oncotarget. 2016; 7: 798-813.

87. Jing X, Yang F, Shao C, et al. Role of hypoxia in cancer therapy by regulating the tumor microenvironment. Mol Cancer. 2019; 18: 1-15.

88. Anderson NM, Simon MC. The tumor microenvironment. Curr Biol. 2020; 30: R921-R925.

89. Markowska A, Sajdak S, Markowska J, Huczyński A. Angiogenesis and cancer stem cells: New perspectives on therapy of ovarian cancer. Eur J Med Chem. 2017; 142: 87-94.

90. Stanca Melincovici C, Boşca AB, Şuşman S, et al. Vascular endothelial growth factor (VEGF)-key factor in normal and pathological angiogenesis. Rom J Morphol Embryol. 2018; 59: 455-467.

91. Mercurio AM. VEGF/Neuropilin Signaling in Cancer Stem Cells. Int J Mol Sci. 2019; 20: 490.

92. Tsuchiya H, Shiota G. Immune evasion by cancer stem cells. Regen Ther. 2021; 17: 20-33.

93. Gardner A, Ruffell B. Dendritic Cells and Cancer Immunity. Trends Immunol. 2016; 37: 855-865.

94. Bayik D, Lathia JD. Cancer stem cell-immune cell crosstalk in tumour progression. Nat Rev Cancer. 2021; 21: 526-536.

95. Ngambenjawong C, Gustafson HH, Pun SH. Progress in tumor-associated macrophage (TAM)-targeted therapeutics. Adv Drug Deliv Rev. 2017; 114: 206-221.

96. Jinushi M, Chiba S, Yoshiyama H, et al. Tumor-associated macrophages regulate tumorigenicity and anticancer drug responses of cancer stem/initiating cells. Proc Natl Acad Sci U S A. 2011; 108: 12425-12430.

97. Oshimori N. Cancer stem cells and their niche in the progression of squamous cell carcinoma. Cancer Sci. 2020; 111: 3985-3992.

98. Miyata H, Hirohashi Y, Yamada S, et al. GRIK2 is a target for bladder cancer stem-like cell-targeting immunotherapy. Cancer Immunol Immunother. 2022; 71: 795-806.

99. MaruYama T, Chen W, Shibata H. TGF-β and Cancer Immunotherapy. Biol Pharm Bull. 2022; 45: 155-161.

100. Jubber I, Ong S, Bukavina L, et al. Epidemiology of Bladder Cancer in 2023: A Systematic Review of Risk Factors. Eur Urol. 2023; 84: 176-190.

101. Hamad J, McCloskey H, Milowsky MI, Royce T, Smith A. Bladder preservation in muscle-invasive bladder cancer: a comprehensive review. Int Braz J Urol. 2020; 46: 169-184.

102. Xu Y, Luo C, Wang J, et al. Application of nanotechnology in the diagnosis and treatment of bladder cancer. J Nanobiotechnology. 2021; 19: 1-18.

103. Wang H, Mei Y, Luo C, et al. Single-Cell Analyses Reveal Mechanisms of Cancer Stem Cell Maintenance and Epithelial-Mesenchymal Transition in Recurrent Bladder Cancer. Clin Cancer Res. 2021; 27: 6265-6278.

104. Kripnerova M, Parmar HS, Pesta M, et al. Urothelial Cancer Stem Cell Heterogeneity. Adv Exp Med Biol. 2019; 1139: 127-151.

105. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. CA Cancer J Clin. 2023; 73: 233-254.

106. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. The Lancet. 2019; 394: 1467-1480.

107. Pang R, Law WL, Chu ACY, et al. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell. 2010; 6: 603-615.

108. Zheng H, Liu H, Li H, et al. Characterization of stem cell landscape and identification of stemness-relevant prognostic gene signature to aid immunotherapy in colorectal cancer. Stem Cell Res Ther. 2022; 13: 1-20.

109. Gupta R, Bhatt LK, Johnston TP, Prabhavalkar KS. Colon cancer stem cells: Potential target for the treatment of colorectal cancer. Cancer Biol Ther. 2019; 20: 1068-1082.

110. Barker N, Van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature. 2007; 449: 1003-1007.

111. Fumagalli A, Oost KC, Kester L, et al. Plasticity of Lgr5-Negative Cancer Cells Drives Metastasis in Colorectal Cancer. Cell Stem Cell. 2020; 26: 569-578.e7.

112. Vasquez EG, Nasreddin N, Valbuena GN, et al. Dynamic and adaptive cancer stem cell population admixture in colorectal neoplasia. Cell Stem Cell. 2022; 29: 1213-1228.e8.

113. Morgan RG, Mortensson E, Williams AC. Targeting LGR5 in Colorectal Cancer: therapeutic gold or too plastic? Br J Cancer. 2018; 118: 1410-1418.

114. Lei X, He Q, Li Z, et al. Cancer stem cells in colorectal cancer and the association with chemotherapy resistance. Med Oncol. 2021; 38: 43.

115. Peng XC, Zeng Z, Huang YN, Deng YC, Fu GH. Clinical significance of TM4SF1 as a tumor suppressor gene in gastric cancer. Cancer Med. 2018; 7: 2592.

116. Park YR, Lee ST, Kim SL, et al. MicroRNA-9 suppresses cell migration and invasion through downregulation of TM4SF1 in colorectal cancer. Int J Oncol. 2016; 48: 2135-2143.

117. Tang Q, Chen J, Di Z, et al. TM4SF1 promotes EMT and cancer stemness via the Wnt/β-catenin/SOX2 pathway in colorectal cancer. J Exp Clin Cancer Res. 2020; 39: 232.

118. Zhao H, Ming T, Tang S, et al. Wnt signaling in colorectal cancer: pathogenic role and therapeutic target. Mol Cancer. 2022; 21: 144.

119. Du L, Cheng Q, Zheng H, Liu J, Liu L, Chen Q. Targeting stemness of cancer stem cells to fight colorectal cancers. Semin Cancer Biol. 2022; 82: 150-161.

120. Wilkinson L, Gathani T. Understanding breast cancer as a global health concern. Br J Radiol. 2022; 95: 20211033.

121. da Costa Vieira RA, Biller G, Uemura G, et al. Breast cancer screening in developing countries. Clinics (Sao Paulo). 2017; 72: 244-253.

122. Waks AG, Winer EP. Breast Cancer Treatment: A Review. JAMA 2019; 321: 288-300.

123. Coleman C. Early Detection and Screening for Breast Cancer. Semin Oncol Nurs. 2017; 33: 141-155.

124. Palomeras S, Ruiz-Martínez S, Puig T. Targeting Breast Cancer Stem Cells to Overcome Treatment Resistance. Molecules. 2018; 23: 2193.

125. Dandawate PR, Subramaniam D, Jensen RA, Anant S. Targeting cancer stem cells and signaling pathways by phytochemicals: Novel approach for breast cancer therapy. Semin Cancer Biol. 2014; 40-41: 192-208.

126. Guha A, Goswami KK, Sultana J, et al. Cancer stem cell-immune cell crosstalk in breast tumor microenvironment: a determinant of therapeutic facet. Front Immunol. 2023; 14: 1245421.

127. Fico F, Santamaria-Martínez A. The Tumor Microenvironment as a Driving Force of Breast Cancer Stem Cell Plasticity. Cancers (Basel). 2020; 12: 1-28.

128. Akhade VS, Pal D, Kanduri C. Long Noncoding RNA: Genome organization and mechanism of action. Adv Exp Med Biol. 2017; 1008: 47-74.

129. Liu S, Sun Y, Hou Y, et al. A novel lncRNA ROPM-mediated lipid metabolism governs breast cancer stem cell properties. J Hematol Oncol. 2021; 14: 178.

130. Zhang L, Chen W, Liu S, Chen C. Targeting Breast Cancer Stem Cells. Int J Biol Sci. 2023; 19: 552-570.

131. Weinberg F, Peckys DB, de Jonge N. EGFR Expression in HER2-Driven Breast Cancer Cells. Int J Mol Sci. 2020; 21: 1-19.

132. Yang F, Xu J, Tang L, Guan X. Breast cancer stem cell: the roles and therapeutic implications. Cell Mol Life Sci. 2017; 74: 951-966.

133. Bai X, Ni J, Beretov J, Graham P, Li Y. Cancer stem cell in breast cancer therapeutic resistance. Cancer Treat Rev. 2018; 69: 152-163. ■