REVIEW

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CANCER STEM CELLS IN RECURRENCE AND THERAPY RESISTANCE: BIOLOGICAL INSIGHTS AND EMERGING THERAPEUTIC STRATEGIES

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Cancer stem cells (CSCs), a resilient subset of tumor cells, able to evade immune detection and rapidly proliferate, are responsible for the metastasis, recurrence, and therapeutic resistance observed across various cancers. Recent research has concentrated on understanding the molecular networks that support CSCs immune evasion, self-renewal, and adaptability. Signaling pathways (Wnt, Notch, Hedgehog, JAK-STAT) and surface markers (CD44, CD133, ALDH1) that characterize CSC behaviour are compiled in this review. We highlight the expanding usefulness of omics technologies, such as CRISPR functional genomics, single-cell transcriptomics, and spatial proteomics, in determining vulnerabilities unique to CSCs and guiding tailored treatment plans.

K e y w o r d s: cancer stem cells, surface markers, signalling pathways, resistance to treatment, targeted therapy.

Cancer remains a leading global cause of mortality, with prognosis strongly influenced by stage at diagnosis. Cancer, a relentless killer, claims nearly 10 million lives yearly, making it a leading cause of death globally. According to the WHO, new treatment strategies are desperately needed to combat this grim statistic. Cancer is a multi-factorial process in which normal cells change, grow, and spread to form new cells throughout the body [1]. Recently, Research has demonstrated the significance of the tissue microenvironment and specific cell physiology in cancer [2]. The concept of phenotypic plasticity refers to the ability of individual genotypes to exhibit different physical traits in response to varying environmental conditions (phenotypic plasticity) and dedifferentiation are unique abilities, both non-epigenetic reprogramming and polymorphic microbiomes creating specific traits that lead to the acquisition of potential characteristics [3]. Many genes are mutated in cancer cells, causing cellular defects. Cancer involves two main genetic mutation types: dominant mutations, impacting a single gene allele to cause effects, and recessive mutations, requiring both alleles of a tumor suppressor gene to be disabled before having any effect [4]. Two interconnected forms of cellular plasticity that contribute to tumor cell differentiation and heterogeneity are epithelial-to-mesenchymal transition (EMT) and the emergence of cancer stem cells (CSCs). It is a “death companion” that can support tumor progression, metastasis, tumor recurrence and treatment resistance in cancer. Among the many cell types, a distinct subpopulation termeds CSCs. Understanding the properties and properties of CSCs is important for future cancer research [5]. CSCs are increasingly recognized for their roles in tumor initiation, metastatic progression, and therapeutic resistance. Metastatic disease remains a major challenge in cancer treatment due to late diagnosis and limited curative options [6]. The reasons behind this are that the asymptomatic nature and delayed diagnosis of some cancers can cause the cancer to spread from its original site to another part of the body. The location in the body where cancer originates is

known as the “primary site of cancer”, and the area where the cancer spreads is called the “secondary or metastatic area” [7, 8]. The three primary routes of cancer spread include direct invasion of adjacent tissues, lymphatic dissemination, and hematogenous dissemination (via the bloodstream), with the latter being the most frequent cause of distant metastases. Therefore, when cancer metastasizes, treatment must eliminate not only primary metastases but also secondary metastases. Treatment of metastases is an important problem. There are also specific metastatic events that are too small to detect in cancer. This is called micrometastases [9]. For some types of cancer, blood tests can detect proteins secreted by cancer cells. These marker proteins may indicate cancer, which is difficult to identify through scanning techniques [10]. Many cancers show no symptoms in their early stages, making early detection difficult and delaying treatment. This review examines the complex molecular mechanisms and signaling pathways involved in CSCs, highlighting the development of targeted therapeutic strategies for better cancer patient outcomes.

Cancer stem cell’s features

Tumor-initiating cells, also referred to as CSCs, are a type of cells that are responsible for initiating the growth of tumors. They are thought to contribute to resistance and cancer progression, which is considered to be part of their ability to self-renew themselves and differentiate into a diverse hierarchy of cancer cell types [11]. Two various systems have been anticipated to define the character of CSCs: (a) In the hierarchical theory, CSCs are positioned at the top of the hierarchy. They are capable of generating every clone in a heterogeneous tumor. (b) Tumors are believed to develop from accidental mutations that occur in normal cells according to the stochastic theory. Changes in the environment and mutations that occur later may encourage phenotypic plasticity, which allows certain non-stem cancer cells to adopt stem-like traits and contribute to the diversity of tumors. New models suggest that this plasticity enables cells to switch between stem-like and non-stem-like states in a dynamic way. This means that the classical CSC model and the stochastic model may not be mutually exclusive, but instead represent two different ways that tumors grow [12, 13]. Fig. 1 illustrates the primary characteristics of CSCs, including their ability to self-renew, initiate tumors, resist various treatments, and promote the main characteristics of CSCs, including as their ability to self-renew, initiate tumors, resist various treatments, and cause metastasis and recurrence. These features

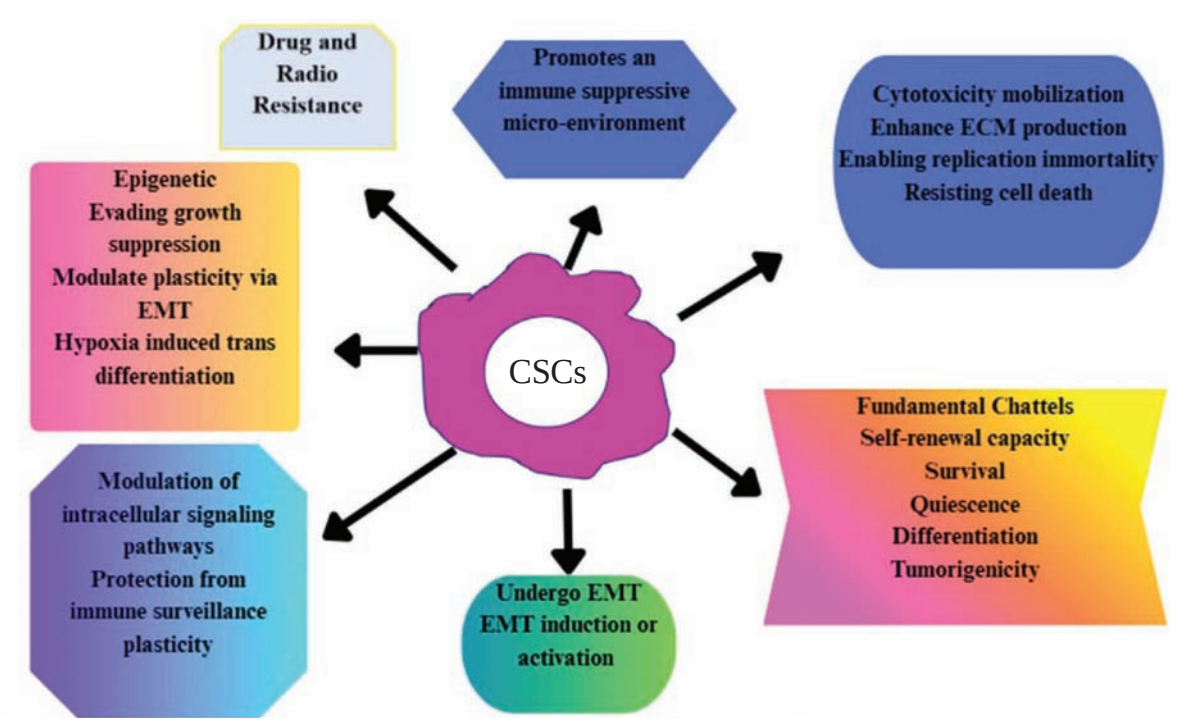

Fig. 1. Key characteristics of CSCs

are often correlated with characteristic molecular markers and unique phenotypic signatures. Some models suggest that CSCs may form when non-stem cells lose their differentiation under particular conditions. However, researchers are still trying to figure out where they come from – whether they come from normal stem cells, progenitor cells, or reprogrammed differentiated cells [14].

CSCs in the tumor niche can renew themselves, differentiate into multiple cell types, and initiate the formation of tumors [15]. Genetic and molecular changes in CSCs during chemotherapy and radiotherapy are responsible for chemotherapy resistance and cancer recurrence [16].

CSCs resemble normal stem cells and have mutations in signaling pathways crucial for their maintenance. The most considered signaling pathways in various types of cancer are Wnt, Notch, Hh, NF-B PI3K, JAK-STAT, and TGF-signaling pathways. These altered pathways, together with immune system–mediated oncogenic mechanisms, contribute to CSCs development. As a result, signaling pathways and immunomodulators have been the focus of cancer treatment. Targeting genetic alterations and dysregulated signaling pathways is being explored to develop new strategies for precision cancer medicine [17].

CSCs vital role in niches

Inside the tumor microenvironment are specific anatomical regions called niches, where CSCs reside. Within their niche, CSCs maintain self-renewal and protect against damage caused by the host immune system, chemotherapy, radiotherapy, etc. These niches preserve the properties of CSCs, shield them from the immune system, maintain their phenotypic plasticity and facilitate their metastatic potential [18]. The development of blood vessels, immune cells, fibroblasts and other cell types with specific features inside the tumor microenvironment is supported by the CSCs niches. Regulation of the stem-like state of CSCs by niche-derived signals is essential for controlling the malignant behavior of their offspring [19].

CSCs may arise from normal stem cells, progenitor cells, or differentiated tumor cells through genetic/epigenetic changes and oncogenic signaling. The tumor's microenvironment, a complex mix of cytokines like IL-6, IL-10, and TGF-β, hypoxia, altered epigenetics, and signaling pathways such

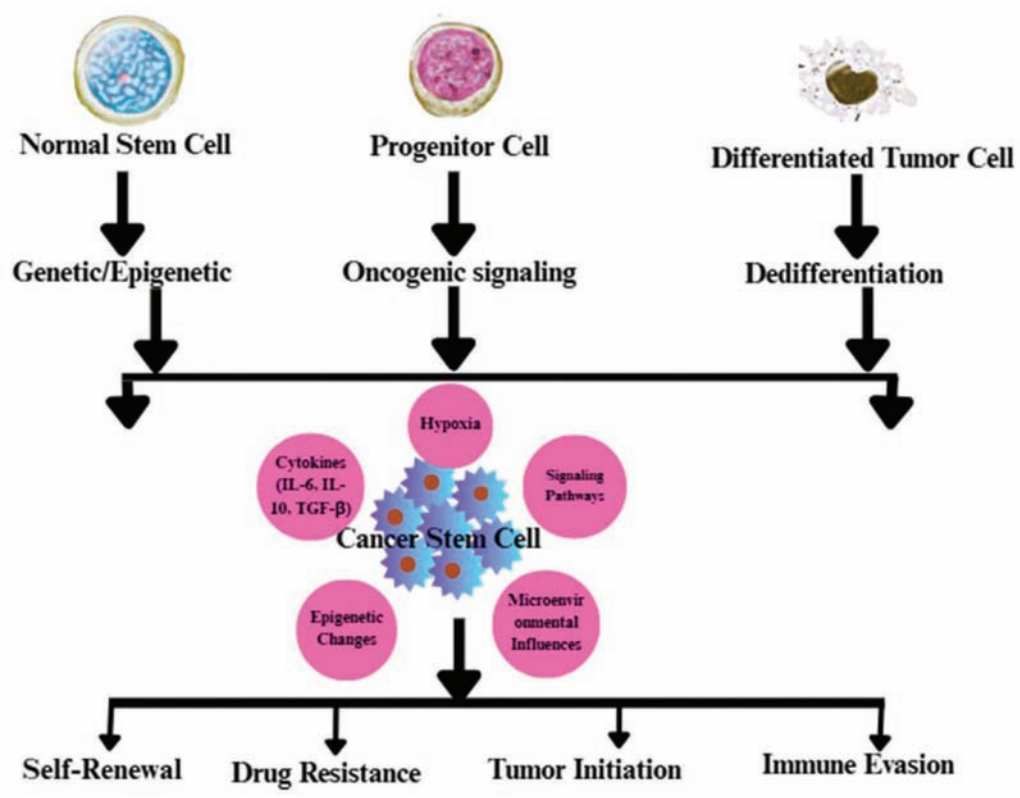

Fig. 2. Origin and regulation of cancer stem cells (CSCs) and their role in tumor progression

as Notch, Wnt, and Hedgehog, actively supports CSCs survival, adaptability, and their crucial roles in self-renewal, immune evasion, drug resistance, and tumor initiation. The intricate interplay of these factors creates a supportive niche for CSCs. Recent research has shown that extracellular vesicles (EVs), such as exosomes, are important for communication in the tumor microenvironment. CSCs can release or respond to EVs containing miRNAs, proteins, and other bioactive compounds that modulate their stemness, resistance, and metastatic potential. These EVs might help CSCs change distant locales, making pre-metastatic niches by changing the immune response or the permeability of blood vessels. So, targeting CSCs-derived EVs or the way they are taken up by target cells could be a way to stop metastasis. Researchers are also looking into liquid biopsy methods to find circulating tumor EVs, which could be non-invasive markers of CSCs activity and the ability of cancer cells to spread. However, we still do not know enough about the specific biomarkers that are linked to CSCs-derived EVs in the establishment of pre-metastatic niches [20].

Immune evasion of CSCs

Cancer therapy faces a major hurdle due to immune evasion, with tumors creating methods to hide from or overcome the immune system [21]. CSCs have special ways of avoiding the immune system, unlike typical tumor cells that use various methods, which explains how they survive and cause tumors to reappear. By secreting cytokines like IL-10 and TGF-β, CSCs establish an environment that suppresses immunity, thereby preventing T-cells and NK-cells from doing their job [22]. Moreover, CSCs boost immune checkpoint ligands like PD-L1, CTLA-4 ligands, TIM-3, and LAG-3, thereby enabling them to directly inhibit T-cell-mediated cytotoxicity. Lei & Lee (2021) found that heightened PD-L1 expression on CSCs-enriched populations is associated with resistance to immune checkpoint blockade therapy [23].

One effective strategy is to decrease the expression of major histocompatibility complex (MHC) class I molecules, leading to decreased antigen presentation and enabling CSCs to evade detection by cytotoxic T lymphocytes. Additionally, CSCs often trigger the STAT3 signaling pathway, which boosts the creation of immunosuppressive elements like indoleamine 2,3-dioxygenase (IDO) and VEGF, thereby suppressing immune reactions.

CSCs also interact with other immunosuppressive cells, which can be found in the dense tumor microenvironment. For example, CSCs-derived exosomes filled with miRNAs and proteins have the ability to convert macrophages into tumor-associated macrophages (TAMs). As described by Verona et al., these TAMs release immunosuppressive molecules that shield CSCs. CSCs, in the same way, attract regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) to stop effector immune cells from functioning [24].

These strategies together emphasize how CSCs are not just passively enduring but actively building immune evasion, giving them the tools to live through treatment, cause relapse, and withstand immune-based therapies [25]. Knowing the immune evasion tricks of CSCs helps develop future immunotherapies that use checkpoint inhibitors with CSCs-focused treatments.

Role of CSCs in metastasis and dissemination

In this review, the roles of CSCs in tumor initiation, progression, and metastasis, as well as in therapeutic resistance, are examined, with an emphasis on the cellular and molecular controllers that affect the phenotypic alterations and activities at various stages of cancer growth [26]. Therefore, even after receiving adequate treatment, this population of cells still plays a crucial role in the tumor mass's ability to expand and promote tumor aggression. As a result of the numerous steps required to prevent it from different immune systems and cancer therapies, metastatic disease is also known as a highly inefficient disease [27]. The circulatory system can be penetrated by even the distribution from a primary tumor that is 1 cm in size (or approximately 1×10^9 cancer cells) and can penetrate the vascular system with one million cancer cells every day [28].

Recent results from *in vivo* models and *in vitro* experiments have provided new insights into the importance of metastatic CSCs. The existence of CSCs in metastasis is not well understood and is mostly predicated on conjecture. For instance, breast CSCs that metastasize to the lung have been shown to express markers similar to those found in lung-resident CSCs, suggesting a possible phenotypic convergence driven by the metastatic niche [29]. Other tumor types, such as pancreatic and colorectal cancer, have also been found to contain similar CSC populations that can induce metastatic growth [30]. The inherent

tumorigenicity of CSCs is characterized by their ability to efficiently repopulate the original tumor even at low clonal density when transplanted into immunodeficient mice. The sphere-forming assay is typically used to detect the presence of CSCs in a tumor sample from a cancer patient [31]. Overcoming hindrances in identifying CSCs targets due to a lack of specific markers and CSCs plasticity is a significant challenge.

The CSC's signaling pathway

Several regulatory factors control the self-renewal of CSCs, including Notch, Wnt/β-catenin, Hedgehog signaling, chromatin remodeling complexes, transcription factors, and non-coding RNAs. The activation of the Wnt/β-catenin pathway is facilitated by β-catenin and T cell factor (TCF) [32]. Physiological and pathological processes are significantly influenced by signaling pathways, including growth, organogenesis and tumorigenesis. Wnt signaling pathways play a critical role in embryonic patterning and adult tissue homeostasis in many ways. Studies have shown that mutations leading to constitutive activation of the transcriptional response of the Wnt pathway are related to a high incidence of certain human cancers [33]. The self-renewal of CSCs is significantly influenced by the Wnt pathway. Fig. 3 shows the main signals of CSCs. Here, Wnt/β-catenin signaling is stimulated by TCF and β-catenin, and ASCL2, LGR5, Axin2, CCND1/2, TCF7, SOX4, c-MYC, etc. The process results in the activation of specific genes, known as target genes, which then get expressed [34]. Two different WNT pathways have been recognized, one considered as the non-canonical pathway and the other in which the canonical pathway is associated with β-catenin activation. β-catenin is an intracellular signaling molecule encoded by CTNNB1 and plays a crucial role in cancer. Meanwhile, the non-canonical pathway operates through β-catenin-independent activity and affects cell signaling and target gene expression [35].

In many tissues and cell types, the Notch signaling system controls self-renewal and differentiation. Notch is a binary cell-fate factor, and its hyperactivation is considered oncogenic in various cancers, including T-cell acute lymphoblastic leukemia (T-ALL) and breast cancer. For example, in breast and colon cancer models, Notch signaling—particularly via Notch4 and its downstream targets HES1 and HEY1—has been directly implicated in sustaining the self-renewal and survival of CSCs populations [36, 37]. Fig. 3 explains the Notch signaling pathways. When the Notch ligands DLL1-4 bind to the Notch receptors, the receptors are cut by the γ-secretase enzyme [38]. This process forms a stable intra-cellular domain also known as NICD, the NICD can move into the nucleus and stimulate the transcription of genes that are targeted by Notch, such as the HEY and HES families of genes, as well as NRARP and others [39].

The Hedgehog (Hh) pathway is a signaling cascade that is essential for many basic functions, such as tissue homeostasis and embryonic development. Importantly, hyperactivation of Hh is connected with malignancy, drug resistance and neoplastic transformation, in many cancers [40, 41]. Mechanistically, Hh signaling promotes cancer by regulating tumorigenesis, malignancy, metastasis, and CSCs proliferation [42]. Stimulation of Hh signaling is regulated by two receptors Patched and Smo. While patched receptors prevent initiation of the Hedgehog pathway, Smo receptors have the opposite effect. When bound to the ligand (ihh, shh and dhh), Patched's inhibitory effect is released, Smo is triggered, and Hedgehog target genes are stated as shown in Fig. 3 [43].

The Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway is very important for controlling the growth, maintenance, and immune evasion of CSCs. In particular, activating STAT3 has been linked to encouraging CSCs self-renewal in breast, colorectal, and cervical cancer, among others. In cervical cancer, the oncoproteins E6 and E7 from HPV disrupt normal cell cycle regulation and aberrantly activate the JAK/STAT pathway. This atypical activation occurs independently of canonical cytokine-receptor signaling, thereby creating a tumor-promoting environment favorable for CSCs survival. MicroRNAs (miRNAs) are also becoming more well-known as important post-transcriptional regulators of CSC behavior. Some miRNAs, such as miR-21 and let-7, change pathways like Wnt, Notch, and STAT3 to either boost or lower CSCs characteristics. Their dysregulation may increase the likelihood of tumor development, cause resistance to treatment, and promote spread to other parts of the body. Researchers are looking at these chemicals not only as diagnostic markers but also as therapeutic targets to stop CSCs-driven tumor growth [44].

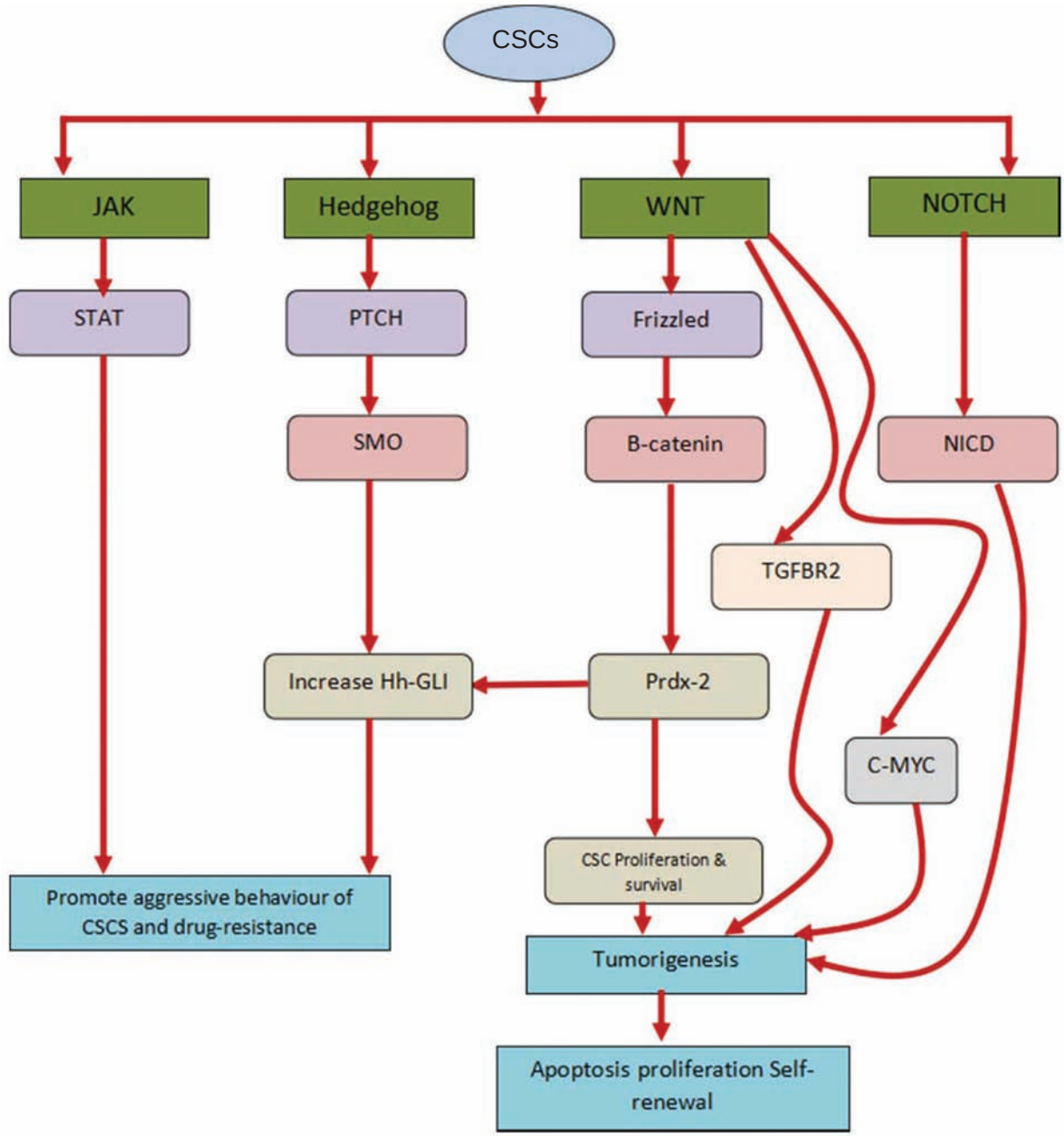

Fig. 3. Signaling pathways driving cancer stem cells (CSCs) maintenance and therapy resistance

Surface marker role in CSCs identification and cancer diagnosis

Cluster of differentiation (CD) markers are the main instruments used to identify and sort cells. Researchers have found a number of surface markers that can tell CSCs apart from differentiated tumor cells. Still, targeting CSCs is hard because they are different from each other and might change. Stem cell-related surface markers such as CD44, CD90, CD133, CD24, EpCAM, and side population (SP) markers are present on CSCs [45]. These markers make it possible to isolate and enrich CSCs both *in vitro* and *in vivo*. For instance, Fan's group found that liver CSCs that express both CD133 and CD13 had a higher ability to renew themselves and make tumors than cells that just express one of these markers [46].

While most studies focus on CSCs markers in solid tumors, similar principles apply in hematological malignancies. In blood cancers, CD34+CD38− cells are linked to leukemic stem cell activity,

whereas CD34−CD38+ cells are not. Many of these markers are also found in normal stem cells; however, variations in their expression levels and combinations can aid in the diagnosis and prediction of different malignancies. For instance, markers such as EpCAM and CD44 are also expressed on normal tissue stem cells. Therefore, these markers are often used in combination (e.g., CD44^high/CD24^low) to increase CSCs specificity and enrichment [47]. Flow cytometry or antibody-based enrichment approaches are often used to find CSCs surface markers such CD44, CD133, and EpCAM [48, 49]. These methods assist in finding CSCs subpopulations and sorting patients into groups for targeted therapy. Table shows a list of common surface markers found on different types of tumors. The ALDH1A1 is a functional cytoplasmic marker, not a membrane-bound surface marker. But it is widely used in CSCs research for isolating stem-like cells using ALDEFLUOR assays [50].

Resistance mechanisms of cancer stem cells to conventional therapies

CSCs are a big problem for treating cancer because they are naturally resistant and have the ability to acquire therapy-induced resistance to chemotherapy, radiation, and targeted therapies. CSCs are different from most tumor cells in that they can stay alive after therapy and cause relapse. They do this by being quiescent, repairing DNA quickly, having a lot of drug efflux pumps, and being resistant to apoptosis. Quiescence is one of the most important things that makes CSCs unique. In this state, cells are quiescent or slowly cycling, which means they can avoid medications that target cells that divide quickly. This allows CSCs to survive through chemotherapy and then resume the tumor growth. Also, overexpression of ATP-binding cassette (ABC) transporters, such as ABCG2, expels chemotherapeutic drugs from the cell, which lowers their concentrations inside the cell and makes them less hazardous. CSCs also have better ways to fix DNA, which keeps them safe from DNA damage induced by radiation therapy or alkylating chemicals. For instance, CSCs quickly activate DNA damage response (DDR) mechanisms, such as ATM/ATR and RAD51-mediated homologous recombination. At the same time, CSCs often have higher levels of anti-apoptotic proteins, such as Bcl-2 and survivin, which help them survive even when they are under stress from cytotoxic drugs.

Another factor that makes therapy less effective is epithelial-mesenchymal transition (EMT). Transcription factors that are connected to EMT, like Snail, Twist, and Zeb1, help cells become more like stem cells, move about, and resist drugs. EMT also causes phenotypic plasticity, which means that non-CSCs can change into CSC-like cells when they are under treatment pressure [68]. CSCs also do well in a tumor microenvironment (TME) that protects them, such as one with low oxygen levels, an acidic pH, and immune-suppressive niches. The TME helps keep CSCs alive and protects them from being cleared by the immune system and drugs. For instance, hypoxia-inducible factors (HIFs) turn on stemness genes and change metabolic states, which make resistance more likely. Fig. 4 shows a full picture of these processes, including drug efflux, anti-apoptotic signaling, DNA repair enhancement, EMT, and TME-mediated support. These are all key CSCs-specific resistance pathways.

Effective cancer research requires a focus on specific areas such as identifying and isolating CSCs, improving cancer diagnosis, and developing effective cancer treatment methods. CSCs are particularly important as they can cause cancer recurrence, metastasis, multidrug resistance, and radioresistance by arresting intermediate stages and promoting the formation of new tumors. Therefore, CSCs are the most optimal targets for emerging successful cancer treatments. The development of therapies specifically targeting CSCs and the supportive niches within the tumor microenvironment is of critical importance. While conventional cancer therapies effectively target the fast-growing majority of tumor cells, they fail to address the stubbornly dormant and resistant CSCs, leaving these cells to potentially cause recurrence. The absence of this therapeutic approach is linked to the return of tumors and the ineffectiveness of treatments in the long run [69]. The use of targeted CSCs-specific agents in combination with traditional therapies may provide a promising lifelong treatment strategy and potential cure for cancer [70]. CSCs can enter a quiescent state, also known as cell cycle arrest, which supports their resistance to chemotherapy and radiation. Chemotherapeutic drugs induce apoptosis in proliferating cells. Despite advancements in cancer therapies that effectively shrink tumors, CSCs often survive due to their intrinsic resistance mechanisms, including enhanced drug pumping, dormancy, and superior DNA repair, allowing them to evade treatment. These surviving CSCs, often characterized by their quiescence

T a b l e. Representative CSCs markers across human tumor types

Types of tumor	CSCs-markers	Reference
Bronchogenic carcinoma	ABCG2, CD90+ ALDH, CD133+, CD44+, CD87+, SP	[51, 52]
Colorectal cancer	ALDH, CD24+, ESA EpCAM+, CD44+, CD133+, CD166+,	[53, 54]
Hepatocellular carcinoma	CD90+, CD44+, ALDH, ABCG2, CD24+, CD133+, CD49f+, ESA	[55, 56]
Invasive ductal carcinoma/ invasive lobular carcinoma	ALDH-1,*EpCAM+, CD133+, CD44+, CD24−	[57, 58]
Pancreatic ductal adenocarcinoma	EpCAM+, CD44+, ESA CD24+, ABCG2, CD133+, ALDH	[57, 58]
Glioblastoma/medulloblastoma	CD133+/nestin+/Lin--	[59]
Gastrointestinal stromal tumor (GIST)	CD133+, CD44+, CD24+	[60]
Chronic myelogenous leukemia	CD34+, CD38−, CD44+, CD13+	[61]
Acute myeloid leukaemia	CD34+/CD38--/CD44+/IL3R+/CD33+/CD131+	[61, 62]
Glandular prostate cancer	ABCG2, α2β1, ALDH, CD44+, CD133+	[63]
Melanoma	ABCB5+, CD20+	[64]
Squamous cell carcinoma	SSEA-1+, CD44+, CD133+	[65, 66]
Neuroblastoma	ABCG2/BCRP1+	[67]

Note. Ep-CAM, although not exclusive to CSCs marker, is commonly used alongside others (e.g., the CD44^high/ CD24^low combination) to increase CSC populations across multiple tumor types, including breast, colorectal, liver, gastric, and pancreatic cancers

and drug resistance, are believed to be the primary drivers of cancer recurrence and spread to distant sites [71]. Although current treatments offer better patient outcomes and disease management, eradicating CSCs is still a significant hurdle to achieving durable remission. The persistent presence of CSCs necessitates innovative treatment strategies.

As previously discussed, CSCs and normal stem cells follow the same control signaling pathways, such as the Wnt/β-catenin pathway [73]. The Sonic Hedgehog (Hh) and Notch pathways are also intricate in the self-renewal process. Additionally, PTEN and polysome family members, which are other signaling molecules, have vital roles in regulating CSCs growth. Understanding the parameters of CSCs self-renewal is a crucial aspect of comprehending tumorigenesis [74].

Targeting signaling pathways that control the persistence of CSCs is a critical area of cancer therapy. The most significant signaling pathways in this regard are the Notch, Wnt and Hh pathways and the TGF-β, JAK-STAT, PI3K, and NF-κB pathways. In developing tumors and CSCs, these pathways frequently interact. Notch and Hh pathway inhibitors have shown notable progress in primary clinical trials, but targeting the Wnt pathway has proven to be challenging [75]. The California Institute for Regenerative Medicine has initiated two cancer patient-based trials aiming at CSCs, based on an early database. One patient was treated with an antibody-mediated immunotherapy targeting CD47, while the other was targeted with ROR1. In the first case, a humanized anti-CD47 antibody called Magrolimab (originally known as Hu5f9-G4 or 5f9) was tested. The efficiency results of four phase 1 trials using Magrolimab (monoclonal antibody) as monotherapy or in combo with rituximab or azacytidine for AML (acute myeloid leukemia), NHL (Non-Hodgkin's lymphoma), and solid malignancies showed considerable results. In the second case, the focus was on treating anti-ROR1, a receptor for Wnt5a signaling associated with the self-renewal, maintenance, and metastasis of CSCs. The experiment involved using an anti-ROR1 antibody called Cirmtuzumab, which blocks ROR1-dependent Wnt5a signaling. The results of this experiment are promising [76]. Vac-

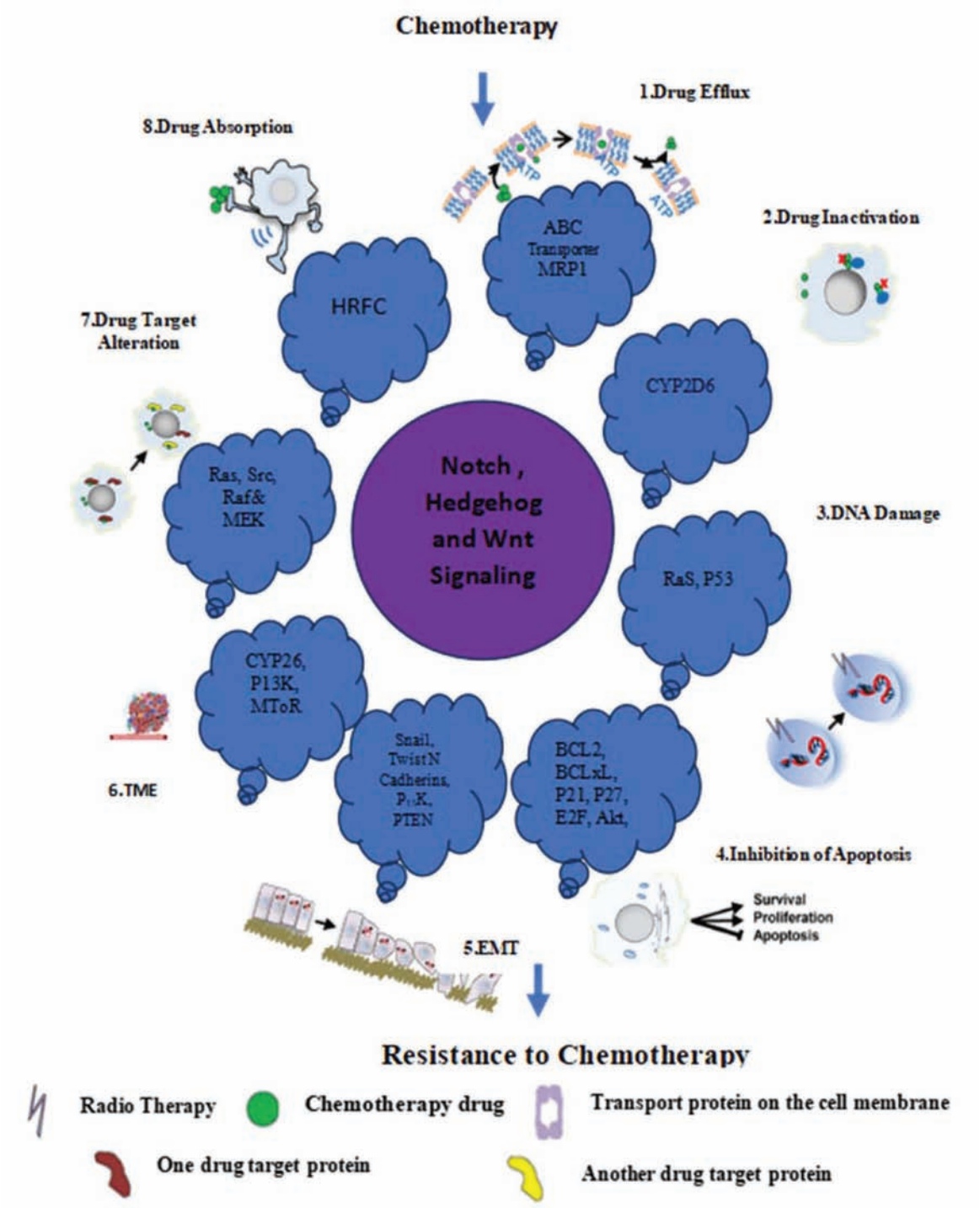

Fig. 4. Mechanisms of CSC's resistance to therapy. This figure was redrawn and modified by the authors based on the concept presented by Liu et al. (2021) [72]. It illustrates the key mechanisms of chemoresistance in CSCs, including drug efflux, DNA damage repair, apoptosis inhibition, and EMT

cines, antibodies, and chimeric antigen receptor T cells (CAR-T) have been established to precisely target CSCs, and few of these features have previously undergone clinical trials [77].

Emerging therapeutic strategies and omics-guided target discovery against CSCs

New paths for understanding the molecular complexity of CSCs and locating targets for action have been made possible by recent developments in multi-omics technologies. The transcriptional programs underlying self-renewal and immune evasion, as well as CSC subpopulations, can be resolved by single-cell RNA sequencing (scRNA-seq). The expression of signaling adaptors (e.g., STAT3, WNT modulators) and chromatin regulators (e.g., EZH2, BMI1) varies throughout CSCs habitats, according to integrative transcriptome and epigenomic profiling. Drug resistance is further clarified by proteomic and phosphoproteomic techniques, such as mass spectrometry-based kinase activity mapping [78].

For instance, glioblastoma single-cell proteogenomic investigations have identified metabolic requirements on oxidative phosphorylation that are particular to CSCs and can be therapeutically exploited through the use of mitochondrial inhibitors. In a similar vein, spatial transcriptomics has made it possible to identify clusters of stem-like cancer cells that express therapy-persistent markers like CD44v6 and LGR5, opening the door to spatially tailored drug administration. The use of patient-derived xenografts (PDXs) and high-throughput CRISPR screening in organoids to confirm omics-derived CSCs targets is growing [79]. The promise of omicsdriven approaches in creating precision treatments that specifically kill CSCs while preserving healthy stem cells is highlighted by these findings. Integrating systems biology with drug screening platforms and delivery methods afforded by nanotechnology is necessary to turn these discoveries into clinically feasible therapies.

CAR-T cells, monoclonal antibodies, nanoparticles, and pathway inhibitors are examples of novel CSCs-targeted treatments. Preclinical models indicate potential for antibodies such as anti-CD133 conjugates and Magrolimab (anti-CD47) [80]. CAR-T treatments that target CSCs markers, such as ROR1 and CD133, are being evaluated in early clinical stages [81]. Drugs such as doxorubicin are delivered to CSCs more effectively and selectively thanks to nanoparticles. Chemotherapy with Wnt, Notch, and Hedgehog pathway inhibitors (such as vismodegib and GSIs) is being studied separately or in combination [82]. Although these tactics provide hope for preventing resistance and relapse, therapeutic success hinges on accurately identifying CSCs and overcoming their adaptability.

Conclusion. CSCs are very important in initiating tumors, making them resistant to treatment, causing them to recur, and spreading to other parts of the body. Their natural traits, such as being able to remain dormant, repair DNA more effectively, resist drugs, and evade the immune system, let them survive standard treatments. To provide targeted therapies, we need to know how the signaling cascades (including Wnt, Notch, Hedgehog, and JAK-STAT) that control CSCs maintenance work. Also, identifying CSCs-specific surface markers, such as CD44, CD133, and ALDH1A1, opens new ways to diagnose and target treatments more accurately. Normal stem cells share many features (markers) with CSCs; however, they could be useful in combination panels or with drug-conjugated antibodies. Recent improvements in omics technology have made it easier to find vulnerable molecular targets in CSCs. This has opened the door for combination therapies that attack both CSCs and the tumor bulk. These kinds of integrative techniques could lower the risk of relapse and increase long-term survival. Future research should focus on improving CSCs-targeted delivery systems, finding ways to overcome resistance mechanisms, and testing CSCs biomarkers in real-life situations. By developing treatments that specifically kill CSCs while leaving healthy tissue alone, it may be possible to get long-lasting results and stop cancer from spreading to other parts of the body.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

РАКОВІ СТОВБУРОВІ КЛІТИНИ ПРИ РЕЦИДИВІ ПУХЛИНИ ТА ТЕРАПЕВТИЧНІЙ РЕЗИСТЕНТНОСТІ

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Ракові стовбурові клітини (CSCs) – це резистентна субпопуляція пухлинних клітин, здатна уникати імунного нагляду та швидко проліферувати, яка відповідає за метастазування, рецидивування та терапевтичну резистентність, що спостерігаються при різних типах раку. Нещодавні дослідження зосереджені на розумінні молекулярних мереж, які забезпечують імунне ігнорування, самооновлення та адаптивність CSCs. У цьому огляді узагальнено сигнальні шляхи (Wnt, Notch, Hedgehog, JAK-STAT) та поверхневі маркери (CD44, CD133, ALDH1), що характеризують поведінку CSCs. Особливу увагу приділено зростаючій ролі omics-технологій, зокрема CRISPR функціональної геноміки, одноклітинної транскриптоміки та просторової протеоміки, у виявленні вразливостей, специфічних для CSCs, та розробки індивідуальних терапевтичних підходів.

Ключові слова: ракові стовбурові клітини, поверхневі маркери, сигнальні шляхи, стійкість до лікування, цілеспрямована терапія.

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