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Biology of stem cell paradox: a double-edged sword-implications for cancer therapy

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Abstract

This comprehensive review investigates the complex interactions between oncogenesis and stem cells, underscoring the crucial importance of cancer stem cells (CSCs) in the mechanisms of tumorigenesis, metastatic dissemination, and resistance to therapeutic interventions. CSCs constitute a distinct subset within neoplasms, defined by unique characteristics such as the ability to self-renew, the potential to differentiate into various cellular phenotypes, and an inherent resistance to conventional treatment strategies. It is hypothesized that these properties are regulated by a multitude of signaling pathways, including Wnt/β-catenin, Notch, and Hedgehog signaling cascades. The tumor microenvironment (TME) significantly influences CSC dynamics by providing a nurturing niche that enhances CSC survival, enables immune evasion, and contributes to resistance against therapies. Key components of the TME, such as cancer-associated fibroblasts and immune-modulating exosomes, play a crucial role in augmenting CSC plasticity and metastatic capabilities. Furthermore, the hypoxic environment prevalent within the TME enhances CSC stemness through both metabolic and epigenetic alterations. Despite the existence of promising CSC-targeted therapeutic strategies, including immunotherapy, small-molecule inhibitors, and combinatorial approaches, notable challenges persist. These obstacles include CSC plasticity and heterogeneity, as well as the potential for immune rejection of CSC-targeted therapies. Advancements in precision medicine, genomics, and novel therapeutic interventions instill hope for the improvement of CSC-targeted strategies. A comprehensive understanding of the fluid interactions between CSCs, standard stem cells, and the TME is vital for advancing personalized cancer treatments that enhance patient success and reduce recurrence rates. This review highlights the need for continued research to bridge existing knowledge gaps and facilitate the translation of foundational scientific discoveries into clinical practices.

Keywords Stem cell biology, Cancer biology, Cancer stem cells, Normal stem cells, Cancer therapy

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Introduction

Cancer is a complex, multifactorial disease characterized by the uncontrolled proliferation of abnormal or immature cells. It arises from a dynamic interplay of genetic, environmental, and Lifestyle factors, and remains one of the most pressing global Health challenges of the 21st century [1–3]. As of 2025, cancer is responsible for approximately 10 million deaths annually, making it the second leading cause of mortality worldwide, following cardiovascular disease [4, 5]. The global Burden continues to rise, with an estimated 19 million new case**s** diagnosed each year—a figure projected to reach 33 million cases and 18 million deaths by 2050 if current trends persist [6]. In the United States alone, cancer accounts for an estimated 618,000 deaths in 2025, with over 1.9 million new diagnoses, underscoring its significant impact on public health and healthcare systems [5]. Common malignancies include lung, colorectal, breast, and prostate cancers, with incidence rates increasing markedly with age. This age-related rise is likely due to cumulative genetic mutations and a decline in immune surveillance mechanisms over time [7]. However, recent epidemiological data reveal a troubling surge in early-onset cancers—those diagnosed in individuals under 50—particularly among younger generations. These include rising rates of liver, colorectal, and breast cancers, often detected at more advanced stages [7].

The etiology of cancer is deeply rooted in both modifiable risk factors and genetic predispositions. Lifestyle and environmental influences—such as tobacco use, alcohol consumption, obesity, poor diet, physical inactivity, and exposure to carcinogens—are collectively responsible for nearly 50% of all cancer deaths worldwide [6, 8]. Tobacco remains the leading preventable cause of cancer, while obesity and dietary factors contribute significantly to the development of colorectal, breast, endometrial, and Pancreatic cancers. Notably, obesity alone is implicated in up to 60% of preventable endometrial cancer cases [8]. In addition to these external factors, genetic mutations play a pivotal role in tumorigenesis. While 5–10% of cancers are attributed to inherited genetic mutations, such as those associated with BRCA1/2 or Lynch syndrome, the vast majority—90–95%—result from acquired genetic alterations that accumulate over a person's lifetime [9, 10]. Advances in genomic profiling have expanded our understanding of cancer susceptibility, identifying novel loci and molecular pathways that may inform future strategies for early detection, risk stratification, and targeted therapy.

For deeper insights, advances in understanding cancer biology have revealed key hallmarks of the disease, such as sustained proliferative signaling and resistance to apoptosis, which explain how malignant cells survive and spread [11, 12]. Early detection remains crucial for enhancing patient outcomes, and traditional treatment options—surgery, chemotherapy, and radiation—continue to be widely employed with varying degrees of success [13, 14]. More recently, innovations in nanotechnology and targeted therapy have introduced new treatment possibilities with higher specificity and potentially fewer side effects [12]. Despite these advances, disparities in cancer incidence and prognosis persist across different populations. Continued research is essential to develop more effective prevention strategies, close healthcare gaps, and reduce the global burden of cancer.

For the above-mentioned existing problems, CSCs play a pivotal role, and indeed, they are small but indispensable cells within tumors that can self-renew and drive processes like tumorigenesis, metastasis, and tumor heterogeneity [15]. The ability of CSCs to self-renew and differentiate is central to the growth and progression of tumors [16]. In addition to causing metastasis and tumor heterogeneity, these cells also make conventional cancer treatments less effective [14, 16]. In cancer therapy, genes have been identified as critical targets due to their involvement in treatment resistance and recurrence. CSC biology and its regulatory pathways are essential for developing effective treatments. Several signalling pathways are involved in regulating CSCs, including the WNT/β-catenin, Notch, and PI3K/AKT [18, 19]. There are many challenges associated with targeting CSCs. Standard therapies are ineffective against these cells because they exhibit intrinsic resistance [16]. As well, immunological rejection and recurrence complicate CSC-targeted therapies [14]. Moreover, the tumour microenvironment contributes to CSC survival and activity, further complicating therapeutic interventions [16]. Despite these obstacles, CSC-targeted therapies hold considerable promise in improving cancer outcomes. The future of cancer treatment could be transformed by these strategies, which overcome resistance to treatment and prevent tumour recurrence [14, 16]. For CSC-targeted treatments to be more effective, new approaches are being explored, such as exosome-based therapies and stem cell genetic modification [20]. New therapeutic targets might be discovered by investigating the transcriptional programs and signaling pathways of CSCs [18].

Tissue stem cell modifications can lead to unchecked growth and tumors [15]. Regenerative medicine uses stem cells for self-renewal and differentiation, treating degenerative diseases and injuries. Understanding their types, applications, and challenges is crucial for safe and effective use. Stem cells are categorized by differentiation potential [21]. Totipotent stem cells, including placental cells, derive from early embryonic development. Pluripotent stem cells from embryonic blastocysts can generate almost all cell types except placental cells [22]. Multipotent adult tissue stem cells have limited differentiation

but are vital for specific functions, like hematopoietic stem cells, which produce blood cells [23]. Stem cells have diverse and expanding medicinal applications, including treating autoimmune, spinal cord injuries, and neurodegenerative diseases [23, 24]. In tissue engineering, stem cells repair damaged tissues and create artificial organs [25]. Clinical trials show stem cell therapies are effective for macular degeneration and epidermolysis bullosa [26].

Despite progress, stem cell research and treatment face challenges and ethical dilemmas. Immune rejection, requiring immunosuppressants with side effects, is a major challenge [19]. Precise control of cell differentiation is essential to prevent tumourigenesis [18]. Ethical concerns, particularly regarding embryonic stem cell use, complicate research [17]. Addressing these challenges is vital for realizing the full potential of stem cell research. Advances in precision medicine and gene editing, like CRISPR, may overcome safety and efficacy barriers, enhancing ethical acceptance. Stem cell medicine research and interdisciplinary collaboration are poised to revolutionize modern medicine [19].

The cancer stem cell (CSC) hypothesis suggests that a small subset of tumor cells initiates and propagates cancer [27]. CSCs' role in recurrence and resistance has garnered significant attention, highlighting the need for CSC-targeting therapies. This hypothesis posits that tumors are heterogeneous with a hierarchical structure where CSCs drive initiation and progression [22]. Their self-renewal and differentiation may contribute to tumor growth and recurrence after conventional therapy [28]. Standard cancer treatments often fail against CSCs, leading to relapse [29, 30]. Identifying biomarkers like CD44 and CD133 is crucial for targeted therapies [28, 31]. Understanding Wnt, Notch, and Hedgehog pathways can aid in effective CSC targeting [33–35]. Disrupting these pathways may enhance treatment by eliminating tumor-forming cells [16, 35]. While insightful, the CSC hypothesis may not apply to all cancers [22]. Such strategies may improve treatment outcomes by addressing tumor persistence and resistance. Environmental stress and therapeutic pressure can induce non-CSCs to adopt CSC-like states [36]. Given the evolving understanding of CSC plasticity, developing adaptable therapeutic approaches is crucial [31]. Despite its value, the CSC hypothesis may not uniformly apply to all cancers, as some tumors may lack a hierarchical CSC structure, necessitating further research into diverse tumor growth mechanisms [22]. CSCs are complex, emphasizing the need for continued exploration.

These cells are thought to drive tumor growth and relapse. Their self-renewal and differentiation necessitate therapies targeting them, as they often resist standard treatments [11]. CSCs' resilience allows them to survive standard therapies, leading to post-treatment growth and recurrence [22]. The CSC model explains tumor heterogeneity, aggressive behavior, and treatment difficulty, increasing invasiveness and relapse risk [37]. Targeting CSCs is a promising avenue for more effective cancer treatments, with researchers identifying exploitable vulnerabilities [16, 38]. Despite its validity, the CSC hypothesis may not fit all cancers, necessitating the investigation of alternative progression mechanisms [22].

CSCs' unique properties offer a promising cancer treatment strategy. Targeting CSCs can combat recurrence and improve patient outcomes. CSCs rely on Wnt/β-catenin, Notch, and mTOR pathways for self-renewal and differentiation [14, 39]. Disrupting these pathways could impair CSC functions and reduce tumor growth. Innovative drug delivery methods like hydrogels are being developed to enhance targeted delivery to CSCs by mimicking the TMEfor controlled release [40]. CSC resistance to conventional treatments is a major cause of recurrence. Combination therapies, integrating CSC-targeted and traditional treatments, may overcome this resistance and reduce relapse [41]. CSC-targeting may also complement immunotherapy by reducing immune evasion [37]. Ongoing research into CSCs may lead to more personalized treatments by identifying specific tumor characteristics for tailored therapies that are more effective and less harmful [42]. Despite the therapeutic potential, challenges remain, including immunological rejection and disease recurrence [9]. Continued research and development are crucial to overcome these challenges and maximize the clinical potential of CSC-targeted therapies.

Due to their unique characteristics, a novel approach is imperative for effective CSC targeting and eradication. Immunotherapies like CAR-T cells, immune checkpoint inhibitors, and dendritic cell vaccines show promise against CSCs [43, 44]. Molecular targeting of markers (e.g., ALDH) and pathways (e.g., Wnt/β-catenin, Notch) can sensitize CSCs to therapy, improving outcomes in recurrent cancers [45, 46] and potentially overcoming resistance to conventional treatments [9, 41]. However, clinical translation is challenging due to immunosuppressive tumor microenvironments hindering CSC detection (39, 40), heterogeneous CSC populations exhibiting differential treatment responses [9, 40], and potential unanticipated side effects requiring rigorous clinical testing [9]. Despite therapeutic potential, tumor biology complexity, immune system interactions, and safety concerns pose significant implementation hurdles, necessitating further research and development.

CSCs a small subset within neoplasms, possess self-renewal and differentiation capacities, driving tumor proliferation, metastasis, and recurrence. Their resistance to standard therapies leads to treatment failure. Overcoming this requires a multidisciplinary framework

encompassing elucidation of CSC regulatory mechanisms, biomarker identification, targeted therapeutic development, and CSC microenvironment examination. This approach will enhance neoplastic biology comprehension, refine therapeutic strategies, and foster personalized oncology paradigms [47, 48]. Their quiescence during therapy enables evasion, significantly contributing to treatment failure and recurrence [49]. Despite advancements, considerable research gaps exist; further investigation is crucial to clarify CSC molecular mechanisms and TME interactions [50]. Intrinsic CSC heterogeneity complicates targeted therapy formulation, underscoring the need for research to pinpoint specific targetable markers and signaling pathways [42]. Addressing these challenges and achieving a nuanced understanding of CSCs is critical for enhancing cancer therapies and ameliorating patient prognoses.

CSCs inherently exhibit self-renewal and differentiation, significantly contributing to tumor heterogeneity and recurrence [29]. These traits enable CSCs to withstand conventional therapies, resulting in resistance and regrowth potential [40], highlighting the necessity for targeted strategies. Identifying specific markers (e.g., CD44, CD133) and critical signaling pathways (e.g., Wnt/β-catenin, Notch) is paramount for formulating targeted therapies [40], providing insights for selective CSC eradication. Novel approaches like CAR T-cell therapy, small molecule inhibitors, and immunotoxins aim to specifically target and eliminate CSCs while preserving normal cells [45, 51], potentially augmenting current oncological treatment efficacy and diminishing recurrence [9]. Focusing on CSCs may curtail tumor recurrence and enhance long-term therapeutic effectiveness. Progress in CSC research is anticipated to yield breakthroughs in refractory cancers, improving patient survival and quality of life [52]. Nevertheless, challenges like immunological rejection and recurrence risk present substantial obstacles, necessitating ongoing investigation and innovative solutions for successful clinical integration of CSC-targeted therapies. Therefore, the principal aims of reviewing the stem cell-cancer relationship include clarifying biological attributes, pinpointing therapeutic targets, and exploring avant-garde treatment modalities to enhance patient outcomes.

Biology of stem cells

Stem cells are distinguished by their extraordinary capacity for self-renewal and their ability to differentiate into a myriad of specialized cell types, rendering them essential for both embryonic development and the maintenance of tissue throughout the lifespan [53]. Their categorization is predicated on their differentiation potential: totipotent cells are capable of giving rise to all cell types, inclusive of extra-embryonic tissues; pluripotent cells, exemplified by embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), can differentiate into nearly all cell types except for extra-embryonic tissues; multipotent cells, such as hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), are confined to specific lineages; and unipotent cells are restricted to generating a singular cell type while retaining their capacity for self-renewal [54, 55]. The fundamental characteristics of stem cells encompass self-renewal, which is the capacity for continuous division while preserving their undifferentiated state, and differentiation, which pertains to the process of evolving into specialized cell types under the influence of both intrinsic and extrinsic cues [54, 56]. Moreover, stem cells manifest plasticity, illustrating their capability to adapt and differentiate into various cell types contingent upon their environmental context [54]. Within the domain of regenerative medicine, stem cells possess substantial potential, particularly concerning conditions such as Parkinson's disease, wherein they may provide a therapeutic strategy for the replacement of damaged neurons [52]. They also fulfill pivotal functions in tissue engineering, offering promising solutions for organ repair and replacement [57]. Nevertheless, the ethical implications associated with the utilization of embryonic stem cells, in particular, persist in inciting considerable debate, thereby shaping the ongoing research and public dialogue regarding stem cell applications [21, 57].

Properties of stem cells: self-renewal and differentiation

Stem cells exhibit remarkable capabilities, including self-renewal, which refers to the ability to sustain their population through continuous division, and differentiation, which denotes the capacity to develop into specialized cell types [58, 59]. These mechanisms are vital for tissue regeneration and development throughout the organism's life. Self-renewal transpires via symmetrical and asymmetrical cell division, ensuring the preservation of the stem cell reservoir while producing differentiated progeny [58, 59]. At the molecular level, pivotal factors such as Oct4, Sox2, and Nanog are instrumental in sustaining pluripotency and self-renewal, particularly in embryonic and induced pluripotent stem cells (iPSCs [56, 60]. The microenvironment of stem cells and various signaling pathways, including the Wnt, Notch, and Hedgehog pathways, exert significant influence on self-renewal mechanisms [60]. Furthermore, stem cells demonstrate plasticity, permitting them to differentiate into diverse cell types based on their origin and the signals they encounter [61, 62]. Notably, iPSCs, which are derived from somatic cells, retain the ability to differentiate into multiple lineages, rendering them promising instruments for regenerative medicine [56, 63]. Despite their therapeutic potential, the effective regulation of stem cell

self-renewal and differentiation for clinical applications remains a formidable challenge. Grasping the intricate equilibrium between these processes is crucial to unlocking their full capabilities within the context of regenerative medicine.

Types of stem cells: embryonic, adult, and induced pluripotent stem cells

Stem cells can be divided into three main types: embryonic stem cells, adult stem cells, and induced pluripotent stem cells (iPSCs). Each type has its unique features and contributes differently to the field of regenerative medicine. Understanding these categories is crucial for developing therapeutic approaches and addressing the ethical issues related to stem cell research. Embryonic stem cells (ESCs) are obtained from the inner cell mass of blastocysts and are known for their pluripotency, allowing them to transform into any cell type in the human body. This incredible ability makes them particularly valuable for regenerative therapies, especially in treating neurodevelopmental disorders and conditions like Parkinson's disease [53]. However, their use raises significant ethical concerns since obtaining them involves the destruction of embryos [22]. On the other hand, adult stem cells, including hematopoietic and mesenchymal stem cells, are multipotent and primarily found in tissues like bone marrow and fat. While their ability to differentiate is more restricted compared to embryonic stem cells, they are vital for tissue repair and regeneration. These cells have a well-established role in therapies, particularly in hematopoietic stem cell transplantation for blood disorders [56, 64].

Induced pluripotent stem cells (iPSCs) represent a groundbreaking advancement in stem cell research. By reprogramming somatic cells to achieve a pluripotent state, iPSCs can develop into various cell types, similar to embryonic stem cells, but without the ethical issues associated with embryo destruction [56]. They offer significant potential for personalized medicine and regenerative therapies. Recent studies suggest that iPSCs may even outperform natural adult stem cells in certain applications, such as tissue engineering [65]. However, despite the vast potential of stem cells in regenerative medicine, their clinical use faces considerable challenges. Ethical concerns, the risk of immune rejection, and the potential for tumor formation are critical issues that need to be addressed to facilitate the wider and safer application of stem cells in medical treatments [64].

Role of stem cells in normal tissue homeostasis and regeneration

Stem cells are essential for maintaining tissue balance and promoting regeneration in various organ systems [66]. Their unique ability to self-renew and differentiate into specialized cell types is vital for repairing tissues and replacing old or damaged cells. Stem cells are key players in maintaining the cellular and metabolic stability within tissues. The stem cell niche, which is the microenvironment surrounding these cells, provides important signals that regulate their activity and is crucial for their function and overall tissue health [67]. The regenerative ability of stem cells is highlighted by their capacity to differentiate and repair tissues. Adult tissue-specific stem cells are particularly important for tissue repair, especially in organs that have limited regenerative abilities [68, 69]. Lineage tracing studies have shown their significant roles in regeneration. However, aging presents a challenge to this process, as it reduces the regenerative potential of stem cells, resulting in decreased tissue homeostasis [70], as depicted in Fig. 1. Future research should focus on strategies to improve stem cell function and explore the complexities of stem cell niches, which could lead to more effective regenerative therapies.

Role of stem and progenitor cells in tumor propagation

Contribution of normal stem cells to tumorigenesis: Regular stem cells maintain tissue health and regeneration but can cause tumors if modified for excessive growth [72, 73], highlighting the importance of understanding stem cell function for novel cancer treatments. Identifying specific changes and signals could lead to therapies inhibiting tumor growth while sparing normal stem cells, reducing side effects. Studies indicate the stem cell microenvironment influences their behavior, potentially inducing normal function or tumorigenesis [72, 74]. Developing treatments that maintain tissue health and prevent cancer requires understanding how the extracellular matrix, cell communication, and soluble factors affect the stem cell niche. Investigating this could elucidate stem cell fate decisions and tumor initiation, leading to improved treatment efficacy and patient outcomes. This research may uncover new methods for cancer detection and prevention of recurrence by stabilizing tissue stem cells [75]. Understanding this delicate balance is key to developing treatments that not only inhibit tumor growth but also promote healthy tissue repair and regeneration, improving patients' overall well-being [67, 69]. This holistic approach to cancer treatment underscores the importance of integrating regenerative medicine concepts with conventional oncology, paving the way for personalized therapies tailored to individual patient needs [68, 70]. These advancements could transform cancer care by focusing not only on eradication but also on promoting bodily repair, establishing a foundation for long-term health (Figs. 1 and 2).

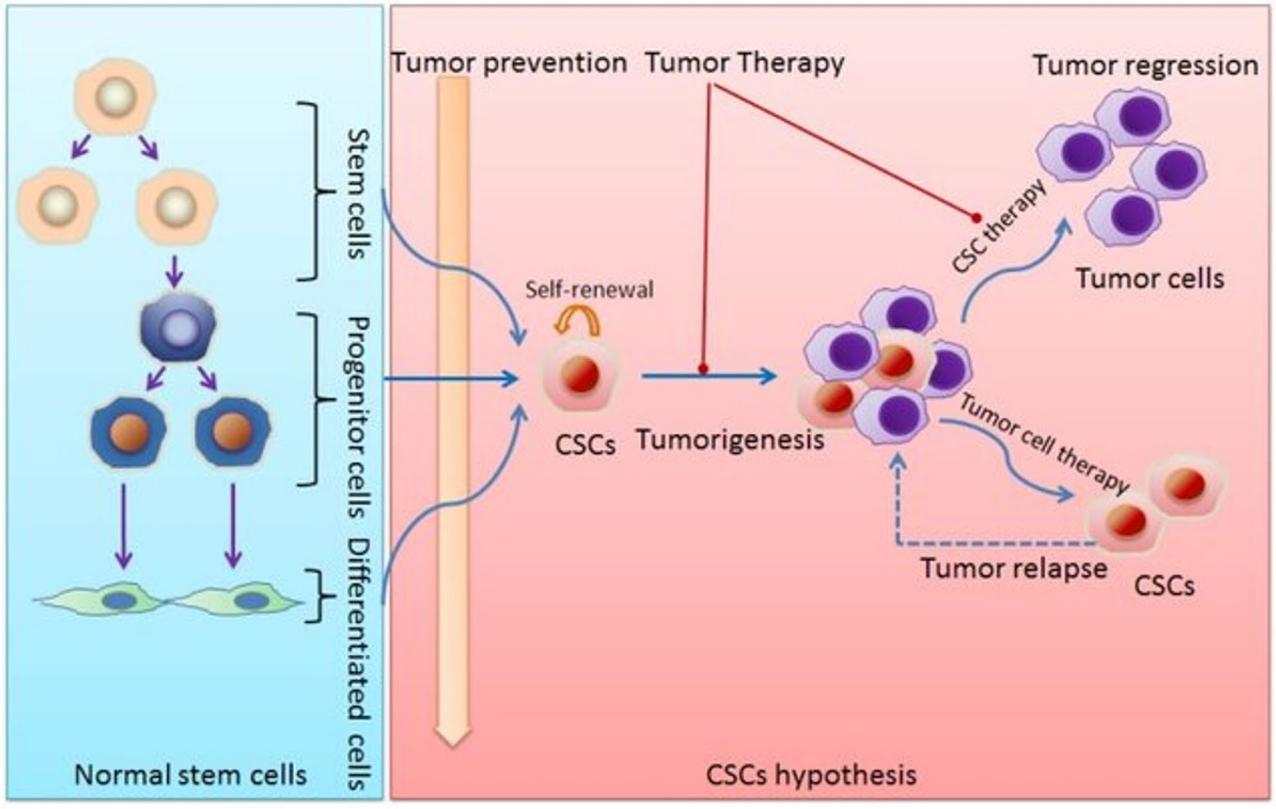

Fig. 1 A schematic representation of stem cells and CSCs. The left panel depicts normal stem cell growth and differentiation. The right panel discusses CSCs and carcinogenesis, as well as their importance for cancer therapy [71]

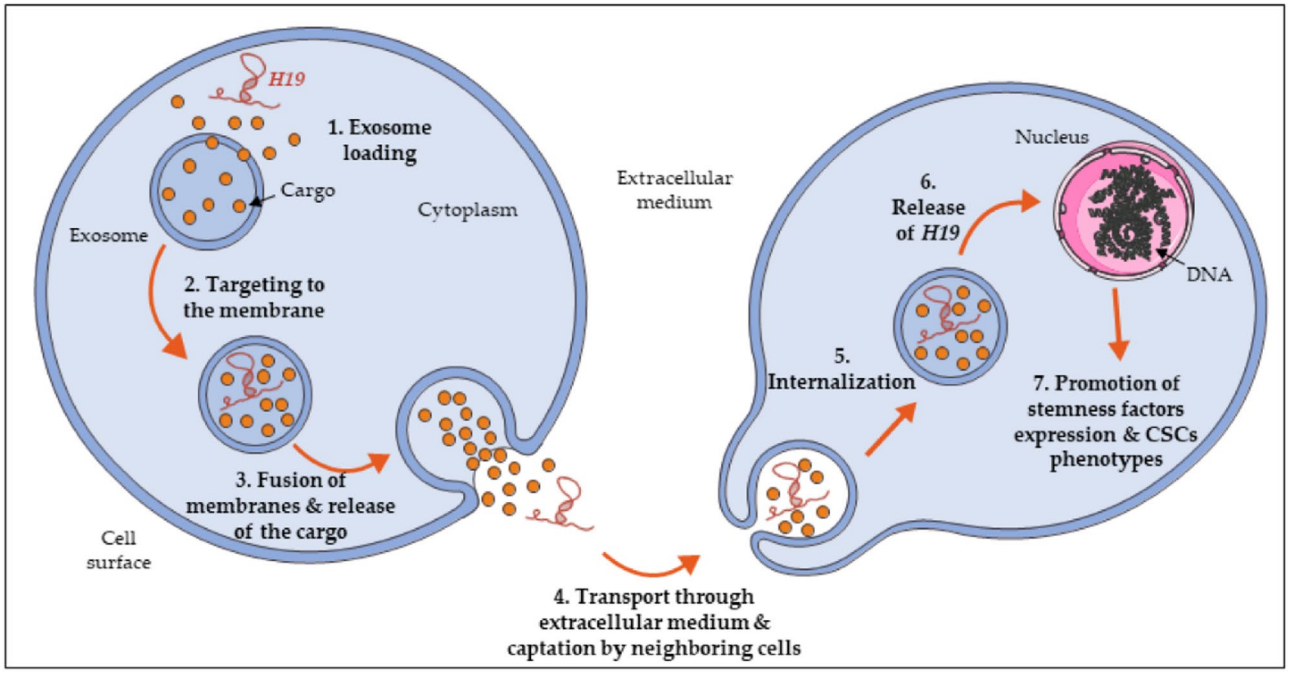

Fig. 2 Exosomes allow the long non-coding RNA H19 to spread throughout the extracellular environment, where it acts on both healthy and malignant cells [76]

Cancer stem cells: characteristics and properties

Cancer stem cells: markers, assays, and controversies: CSCs play a crucial role in tumor growth and treatment resistance. Identifying reliable markers for these cells is essential for enhancing our understanding of cancer biology and creating effective therapies. Common markers used to isolate and characterize CSCs include CD44, CD24, and CD133. CD44, a cell surface protein, aids in cell–cell interactions and is often overexpressed in various cancers [21, 35]. CD24 is a small glycoprotein involved in cell adhesion and is associated with tumor progression. CD133, a marker linked to stemness and self-renewal, is frequently used in studies of brain and colon cancers [18, 35, 77, 78]. However, relying solely on these markers presents challenges. First, the expression levels of these markers can vary significantly across different tumor types, making them less effective as universal identifiers. Second, CSCs are known for their plasticity, meaning their marker expression can change in response to environmental factors, such as chemotherapy [79]. This dynamic nature complicates the consistent identification and tracking of these cells. Finally, CSCs usually represent a small portion of the tumor mass, which makes their isolation and study technically challenging [80, 81]. While these markers provide valuable insights, their limitations underscore the urgent need for further research to develop more robust and reliable methods for identifying and targeting CSCs. This will require a multipronged approach, including the exploration of novel markers, the development of more sophisticated techniques for isolating and analysing CSCs, and a deeper understanding of the underlying mechanisms driving CSC plasticity.

The field of cancer research has made remarkable progress in understanding the role of CSCs in the development of tumors. Various techniques have been created to isolate and characterize these elusive cells, offering valuable insights into their biology and behavior. Methods like sedimentation field-flow fractionation (SdFFF) and ultrahigh-frequency dielectrophoresis (UHF-DEP) allow for label-free isolation, streamlining the process and enhancing its clinical relevance [82]. Additionally, techniques such as flow cytometry and microfluidics play a crucial role in isolating rare CSC populations, enabling detailed analysis of their properties [83, 84]. Characterizing isolated CSCs is essential for gaining insights into their nature. For instance, transcriptomic analysis provides a thorough understanding of the genetic makeup of CSCs, uncovering distinct subpopulations with different characteristics, including tumorigenicity and resistance to therapies [82]. Moreover, lineage tracing techniques allow researchers to monitor CSC behavior within their microenvironment, illuminating their role in tumor initiation and progression [85]. The insights gained from these studies have significant implications for cancer treatment. By understanding the unique properties of CSCs, researchers can develop targeted therapies aimed specifically at eliminating these cells, which could greatly enhance treatment effectiveness and lower the chances of tumor re-emergence [86, 87]. While these assays provide invaluable insights into CSC biology, challenges remain. Standardization of methods and ensuring reproducibility across different tumor types are crucial for broader clinical applications.

Cancer stem cell (CSC) plasticity

Cancer stem cell (CSC) plasticity denotes the capacity of neoplastic cells to reversibly transition between stem-like and non-stem-like states, in addition to shifting among various CSC subpopulations characterized by distinct properties. This dynamic behavior is integral to tumor advancement, resistance to therapeutic interventions, and relapse the of disease. Contrary to the conventional perspective of a fixed CSC population, contemporary research indicates that CSCs are not static entities; rather, they can arise from non-stem cancer cells in response to environmental stimuli or therapeutic stressors. This adaptable identity enables tumors to endure adverse conditions, evade therapeutic measures, and regenerate following apparent regression [88, 89]. Several interrelated mechanisms underpin this plasticity. A fundamental aspect is state switching, whereby cancer cells oscillate between stem-like and differentiated phenotypes. This phenomenon is frequently instigated by alterations in the TME or by exposure to cytotoxic therapies. A pivotal catalyst of this transition is the epithelial-to-mesenchymal transition (EMT), through which epithelial tumor cells acquire mesenchymal traits, thereby enhancing their motility, invasiveness, and stem-like characteristics. EMT not only facilitates metastasis but also equips cells with attributes that confer evasion of therapeutic interventions [91–93].

The impact of the TME is equally profound. Components such as stromal cells, immune elements, hypoxic conditions, and extracellular matrix proteins within the TME collectively activate signaling cascades like Wnt, Notch, and Hedgehog, which bolster stemness and promote plastic transitions. This environment-responsive signaling enables CSCs to persist and adapt even amidst therapeutic pressures [93]. Moreover, CSCs exhibit substantial metabolic and epigenetic versatility. They can transition between glycolysis and oxidative phosphorylation contingent upon their energetic requirements and contextual microenvironment. Epigenetic alterations—such as DNA methylation, histone modifications, and chromatin remodeling—facilitate rapid gene expression modifications that support CSC maintenance and reprogramming [94, 95]. These adaptive characteristics render CSCs particularly proficient at surviving

conventional therapeutic regimens. While numerous anti-cancer agents target rapidly proliferating cells, CSCs frequently enter quiescent or dormant states, allowing them to evade drug-induced mortality. Upon cessation of therapy, these cells can reactivate, contributing to tumor ocurrence. This capacity for survival and subsequent repopulation of tumors highlights the significance of CSC plasticity in treatment resistance and relapse [96]. Nonetheless, CSC plasticity manifests as a double-edged sword. On the one hand, it provides survival benefits to the tumor by augmenting adaptability, diversity, and resilience. Tumor heterogeneity, partially driven by CSC transitions, amplifies the likelihood that certain subpopulations will endure treatment and prosper. Conversely, these identical mechanisms unveil vulnerabilities that can be targeted for therapeutic advantage. By elucidating the pathways that govern CSC plasticity, researchers can identify innovative intervention points that may inhibit state switching or eliminate the supportive signals present within the microenvironment [97].

Numerous experimental methodologies have been devised to specifically target CSCs, although each strategy is accompanied by inherent limitations. Approaches that concentrate on CSC surface markers such as CD44, CD133, or ALDH are designed to selectively eliminate the stem-like cellular population. Nevertheless, the expression of these markers is frequently dynamic and context-sensitive due to the inherent plasticity of CSCs, which diminishes the effectiveness of such targeted therapeutic interventions [98]. An alternative strategy involves the inhibition of epithelial-mesenchymal transition (EMT) to obstruct the acquisition of stem-like characteristics and to mitigate metastasis. However, EMT is a reversible process, and cells that evade such inhibition may reinitiate the transition or discover alternative mechanisms to regain their stemness [90]. Additionally, targeting the TMEdemonstrates potential benefits. Altering hypoxic conditions or disrupting stromal interactions may weaken CSC niches; however, the intricate nature and adaptability of the TMEpose considerable challenges to achieving consistent success [99]. In summary, the plasticity of CSCs is pivotal to the challenges and prospects encountered in contemporary oncology. This plasticity enables tumors to adapt, endure, and recur, while simultaneously offering distinctive therapeutic targets that could revolutionize treatment outcomes. Future therapeutic strategies must extend beyond merely eliminating CSCs to also encompass the dynamic processes that facilitate the conversion of non-stem cancer cells into CSCs. Only through the simultaneous targeting of both the cells and the underlying mechanisms of plasticity can we aspire to attain long-term remission and avert tumor activation.

By and large, the definition and characterization of CSCs remain debated due to their complexity and diverse study methods. Unresolved questions impact our understanding of tumor biology and targeted therapy development. A key debate is the operational definition of CSCs, often described by their tumor-promoting capacity, which doesn't resolve the stem cell versus dedifferentiation origin of cancer [31]. Further complicating this is the potential existence of CSCs derived from adult stem cells or differentiated cells [31]. Methodological challenges also hinder CSC characterization. Inconsistent CSC identification across tumor types arises from varied isolation and characterization techniques. The lack of standardized terminology and criteria impedes understanding and clinical application [100]. Recent findings indicate CSCs may not be a rare subset and can undergo reversible changes, challenging traditional views [101]. Phenotypic variability among patients also questions the generalizability of research and treatment strategies [101, 102]. Despite these challenges, ongoing CSC research holds promise for improved cancer treatments. However, a unified framework with clearer definitions and standardized methods is crucial for advancing the field and its clinical translation.

Self-renewal and tumorigenicity of cancer stem cells

Key molecular mechanisms of cancer stem cell self-renewal and tumorigenicity: The self-renewal and tumor-forming ability of CSCs result from a complex interaction of genetic, epigenetic, and environmental factors. These mechanisms not only help CSCs retain their stem-like characteristics but also contribute to their resistance to standard therapies, making them crucial targets in cancer treatment. Epigenetic regulation is vital for maintaining CSCs, as changes in histone modifications, DNA methylation, and noncoding RNA expression influence gene expression related to stemness and drive the aggressive nature of these cells [48]. Signaling pathways like Wnt/β-catenin, Notch, and Hedgehog are key to sustaining the self-renewal ability of CSCs, reflecting the processes seen in normal stem cells [103, 104]. Additionally, hypoxia-inducible factors (HIFs) trigger pluripotency factors in low-oxygen environments, boosting the self-renewal of breast CSCs [105]. The TMEalso plays a significant role in shaping CSC behavior. CSCs establish specialized niches that shield them from therapies and encourage their self-renewal [104]. Furthermore, the adaptability of CSCs and their capacity to adjust their transcriptional programs allow them to endure various treatment approaches [106]. Although the mechanisms behind CSC self-renewal and tumorigenicity are well understood, the ever-changing nature of these cells presents considerable challenges for effective treatment. Future research must focus on targeting these pathways and understanding the

plasticity of CSCs to develop more effective therapies and improve clinical outcomes.

CSCs- drug & response: CSCs show an impressive ability to withstand treatment, thanks to a mix of internal and external factors that support their self-renewal and tumor-forming capabilities. To start, their cellular plasticity allows CSCs to adjust and gain stem-like traits, helping them survive and grow even when faced with therapies(108). Additionally, the TME is vital for CSC survival and drug resistance. By interacting with stromal and immune cells in the TME, CSCs receive crucial signals that boost their survival and growth [16]. Specific signaling pathways, including Wnt, Notch, and Hedgehog, are essential for preserving CSC traits and influencing their reactions to treatments [108]. On top of that, intrinsic factors also play a role in CSC resilience. For instance, autophagy—a process that helps cells survive under stress—enables CSCs to cope with challenges and maintain balance, aiding their survival during therapy. Likewise, long noncoding RNAs (lncRNAs) manage various cellular functions that bolster CSC maintenance and resistance, further strengthening their resilience [109, 110], as shown in Fig. 3. Despite these strong resistance mechanisms, ongoing studies into CSC weaknesses provide hope for creating targeted therapies that can effectively eliminate these tough cells and lower the chances of cancer returning. A comprehensive understanding of the complex biology of CSCs, including their plasticity, interactions with the TME, and activation of key signalling pathways, is crucial for advancing treatment strategies and improving patient outcomes.

Role of CSCs in tumor initiation, maintenance, and metastasis

CSCs(CSCs) are essential in the initiation, maintenance, and spread of tumors, greatly influencing cancer progression and resistance to treatment [112]. These cells can self-renew and contribute to the diversity within tumors. In the early stages of tumor development, CSCs, often called tumor-initiating cells (TICs), are vital as they evade the immune system and proliferate [112]. Additionally, CSCs help sustain the TME, which aids in their survival and self-renewal, thus promoting tumor persistence [66]. Their dormant state often makes them resistant to standard therapies that mainly target rapidly dividing cells [49]. During metastasis, CSCs enhance their ability

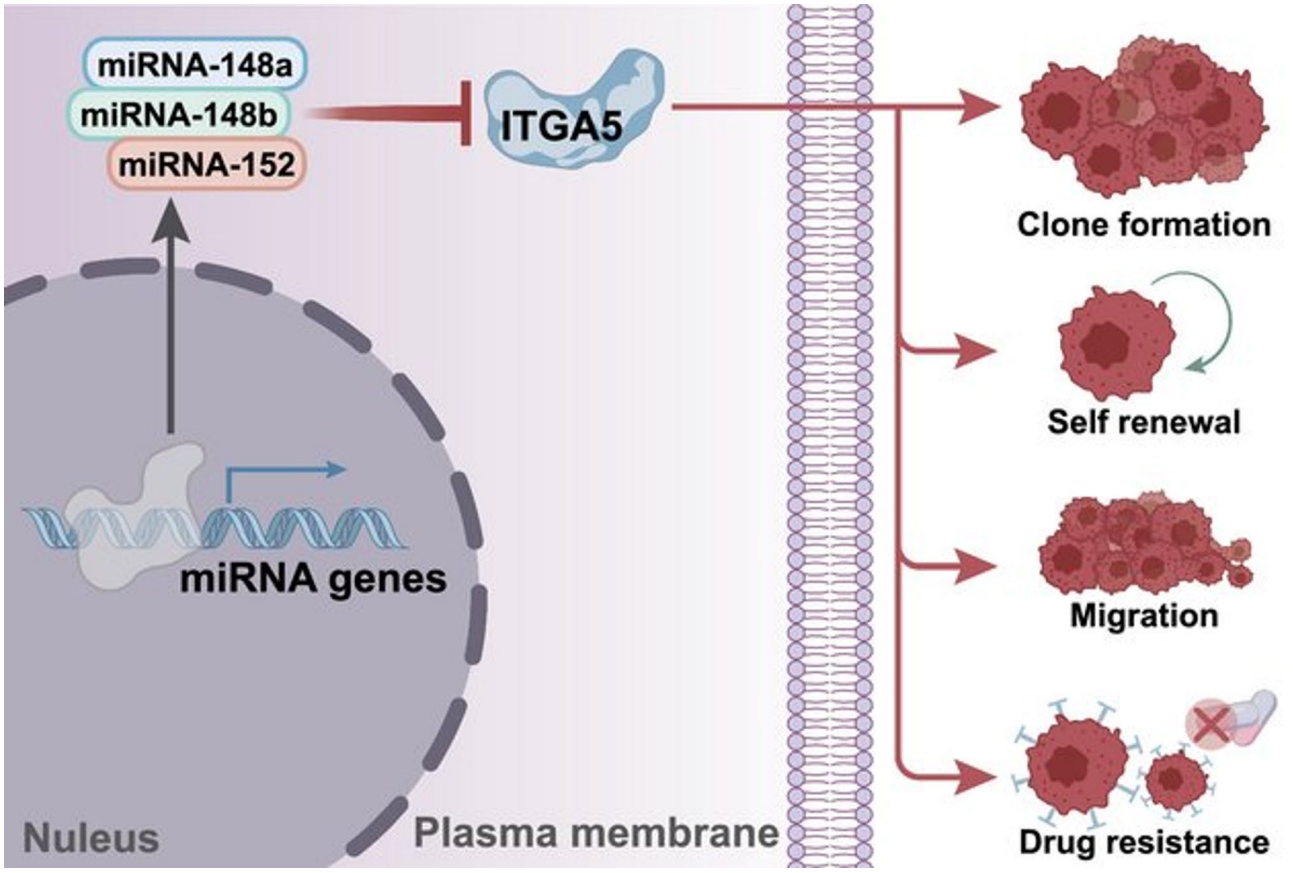

Fig. 3 A schematic representation showing the mechanisms that regulate the stem cell-like features of the miR-148/152 family members in gastric cancer. The miR-148/152 family members target ITGA5 and limit its expression, lowering the self-renewal ability, clonogenic potential, and treatment resistance of gastric CSCs and ultimately inhibiting stem cell-like features in gastric cancer [111]

to migrate and invade through processes like epithelial-mesenchymal transition (EMT) [49]. Circulating CSCs are key players in spreading to distant locations and forming secondary tumors [113]. While the importance of CSCs is clear, it is crucial to recognize that an exclusive focus on them might neglect the roles of nonstem cancer cells (NSCCs) and the complex interactions within the TME, which also significantly affect tumor behavior and treatment results [34], as illustrated in Fig. 4.

Chemoresistance and radioresistance of cancer stem cells: CSCs significantly contribute to chemoresistance and radioresistance in malignancies. Their self-renewal and differentiation capacities facilitate treatment failure and tumor waning necessitating a deeper mechanistic understanding for improved therapeutic strategies. CSCs exhibit multiple mechanisms of chemoresistance, including enhanced DNA damage repair, low proliferative rates rendering them less susceptible to cell cycle-targeting agents, and overexpression of efflux transporters that actively pump chemotherapeutic drugs out of the cell [114]. Furthermore, the activation of specific signaling pathways promoting CSC survival and self-renewal also confers chemo-resistance [115]. The TME also plays a crucial role in mediating CSC resistance to cytotoxic drugs [11]. Similarly, CSCs display resistance to radiation therapy. Fractionated radiation can induce adaptive responses in CSCs, leading to increased radio-resistance [20]. The Nrf2 signaling pathway is pivotal in this context, mediating antioxidant responses and reducing radiation-induced oxidative stress, thereby protecting CSCs from DNA damage [16, 20]. Elevated aldehyde dehydrogenase (ALDH) activity is also associated with increased radioresistance in CSCs [20]. This inherent resistance of CSCs poses a substantial challenge to effective cancer treatment. Ongoing research focuses on developing targeted therapies aimed at eradicating these cells and disrupting their supportive niche. Such efforts offer promising avenues for overcoming these resistance mechanisms and improving patient outcomes.

Molecular mechanisms driving chemoresistance and radioresistance in cancer stem cells: CSCs resist chemotherapy and radiotherapy through complex molecular processes that let them live and grow despite treatment. These processes involve better DNA repair, activation of protective pathways, and interactions with the tumor environment. CSCs can fix DNA damage more, which helps them counter the harmful effects of chemo and radiation [116, 117]. The p53 pathway plays a key role in

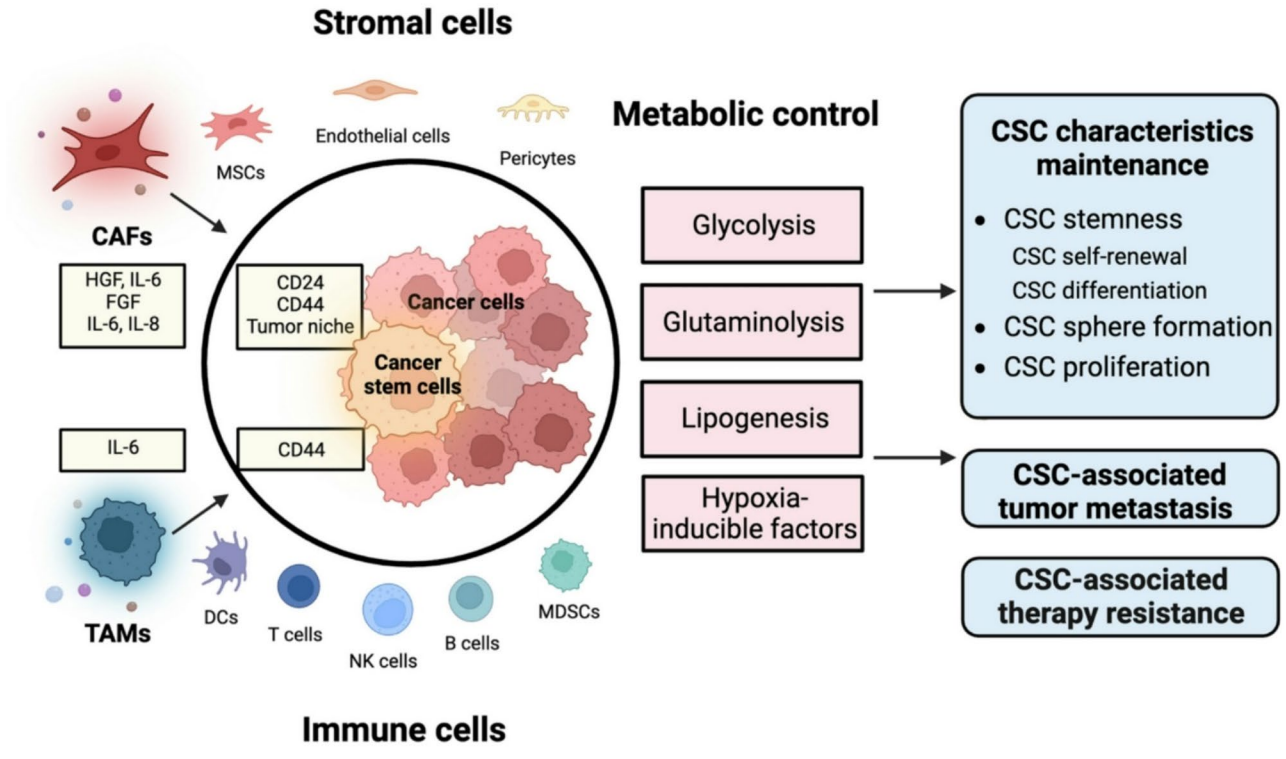

Fig. 4 CSCs are controlled by the tumor microenvironment. The preservation of CSC characteristics, CSC-associated tumor metastasis, and CSC-associated therapeutic resistance are all influenced by different non-tumor cells, such as stromal cells, immune cells, and metabolic control, which are present in the tumor microenvironment. Tumor-associated macrophages (TAMs) are the most vital immune cells, while cancer-associated fibroblasts (CAFs) are the most representative stromal cells. Through the secretion of certain cytokines, they aid in the maintenance of CSC features. The yellow boxes displayed each of the specific cytokines and the factors that they controlled. The four most prominent components of metabolic control that may contribute to the maintenance of CSC characteristics are glycolysis, glutaminolysis, lipogenesis, and hypoxia-inducible factors (red boxes) [108]

this, calling on repair enzymes to lessen treatment damage [116]. Important pathways like JAK/STAT, WNT, NOTCH, and HEDGEHOG help keep CSC traits and boost resistance (116, 119). The p53-IER5-HSF1 pathway aids CSCs to handle protein stress, while the p21-NRF2 pathway ups antioxidant production adding to resistance [116]. The tumor environment, including low oxygen and cancer-linked fibroblasts, affects CSC behavior and resistance [117, 118]. Cell-to-cell talk within the tumor area helps CSCs survive and renew themselves, making treatment harder. Cell-to-cell talk within the tumor area helps CSCs survive and renew themselves, making treatment harder [119].

While these mechanisms highlight the resilience of CSCs, emerging therapies targeting these pathways may offer new avenues for effectively combating chemoresistance and radioresistance in cancer treatment. However, the heterogeneity of tumors poses a significant challenge, as not all CSCs may respond uniformly to such interventions. The development of chemoresistance and radioresistance in CSCs poses significant challenges in cancer treatment, often resulting in treatment failure and tumor return. CSCs have unique characteristics that allow them to evade standard therapies. A major mechanism of resistance is their enhanced ability to repair DNA, which enables them to effectively mend damage caused by chemotherapy and radiotherapy [120]. Additionally, the activation of signaling pathways that promote self-renewal helps CSCs persist after treatment. The TME also plays a vital role in supporting the survival and resistance of CSCs by providing a protective niche [115]. To tackle these challenges, researchers are investigating innovative therapeutic strategies that specifically target CSCs [121]. These approaches include focusing on iron metabolism and exploiting vulnerabilities unique to CSCs. Combining CSC-targeted therapies with conventional treatments may enhance overall outcomes by addressing the core of resistance [122], as presented in Fig. 5. While targeting CSCs presents a promising path for improving cancer treatment results, it is essential to recognize the potential for adaptive resistance mechanisms to emerge. These mechanisms can develop in response to targeted therapies, complicating treatment strategies. Understanding these dynamics is key to creating effective and lasting therapeutic approaches to combat cancer recurrence.

Epigenetic modifications of CSCs *as means of chemoresistance and radioresistance*: Epigenetic modifications are crucial in influencing the resistance seen in cancer cells. These modifications change gene expression without altering the DNA sequence itself and include processes like DNA methylation, histone modifications, and chromatin remodelling. They play a significant role in the distinct characteristics of CSCs, allowing them to survive and grow even when faced with treatments. DNA

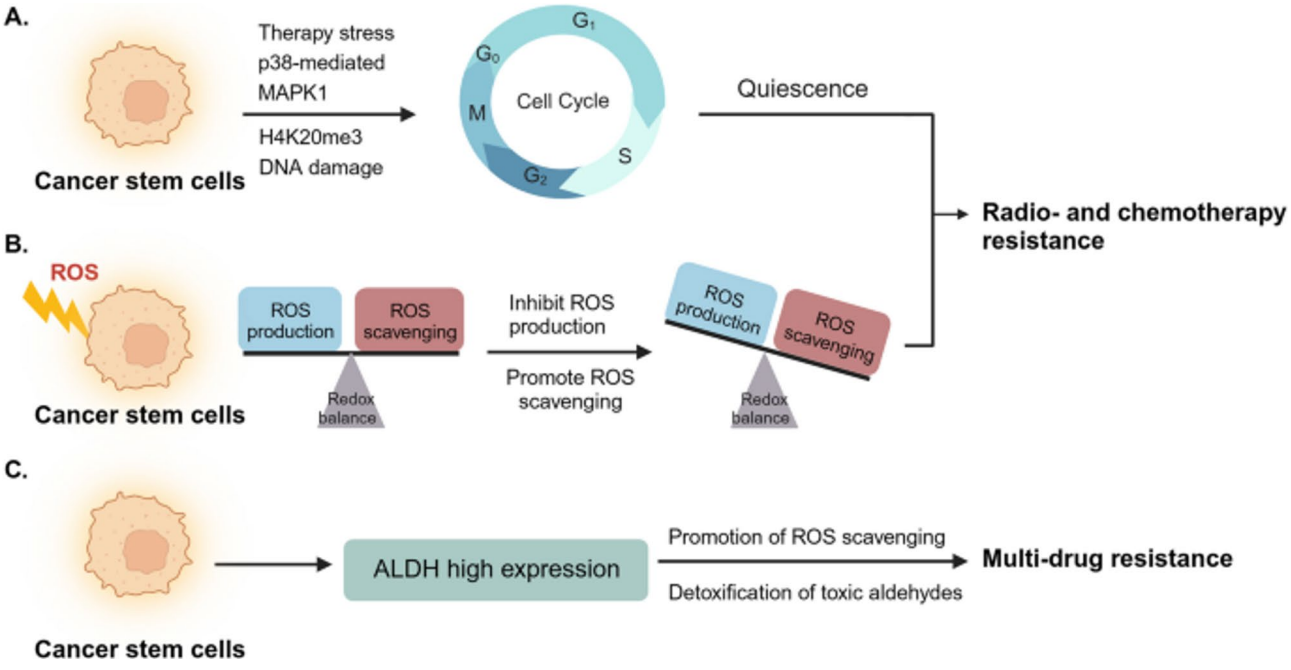

Fig. 5 CSCs in cancer therapy resistance. Compared to ordinary cancer cells, CSCs exhibit higher resistance to cancer treatment, which increases the likelihood that they will avoid chemotherapy and radiation and raises the possibility of tumor recurrence. The development of therapeutic resistance in CSCs is caused by a number of causes. **A** When exposed to environmental stressors like therapy-induced stress, specific molecular stress, or DNA damage stress, CSCs can go into a quiescent state, which allows them to develop resistance to chemotherapy and radiation. **B** CSC-related resistance to chemotherapy and radiation therapy results from a disturbance in the equilibrium between ROS scavenging and ROS generation in CSCs. **C** Multidrug resistance results from increased ROS scavenging and hazardous aldehyde detoxification caused by high ALDH expression in CSCs [108]

methylation, a major epigenetic mechanism, involves adding methyl groups to DNA, which often results in gene silencing. In CSCs, this silencing can affect tumor suppressor genes, hindering their ability to trigger apoptosis and maintain cellular balance [48]. As a result, CSCs can escape the impacts of chemotherapy and continue to multiply. Additionally, changes in histone modifications, such as acetylation and methylation, can greatly influence gene expression in CSCs. These modifications can affect how accessible DNA is to the transcriptional machinery, thus regulating gene expression. For instance, changes in histone modifications can enhance the expression of drug efflux transporters, particularly those in the ATP-binding cassette (ABC) family. These transporters work to actively expel chemotherapeutic drugs from the cell, which limits their accumulation inside and diminishes their effectiveness [48, 123].

Chromatin remodelling is a vital epigenetic mechanism that involves alterations in the structure of chromatin, which is the complex of DNA and proteins that organizes DNA within the cell nucleus. These structural changes can affect how accessible transcription factors are to specific genes, thereby influencing gene expression. In CSCs, chromatin remodelling can enhance the accessibility of transcription factors to genes associated with stemness, thereby supporting the survival and self-renewal of these cells [21, 124]. Given the significant role of epigenetic modifications in promoting CSC resistance, targeting these mechanisms could be a promising therapeutic approach. Clinical trials are currently exploring drugs aimed at reversing these epigenetic changes to make CSCs more susceptible to chemotherapy and immunotherapy [48, 124]. For instance, histone deacetylase inhibitors (HDACis) have shown potential in both preclinical and clinical studies by blocking enzymes that remove acetyl groups from histones, which can lead to increased gene expression and potentially make CSCs more responsive to treatment. Additionally, combining epigenetic therapies with standard treatments like chemotherapy and radiotherapy may provide a synergistic strategy to overcome resistance. For example, the combination of HDAC is with chemotherapy has yielded encouraging results in preclinical models and is under investigation in clinical trials [123], as illustrated in Fig. 6. While epigenetic modifications pose significant challenges in cancer treatment due to their role in resistance, they also present new therapeutic targets. A deeper understanding of these mechanisms is essential for developing effective strategies to target CSCs and enhance patient outcomes.

The microenvironment and cancer stem cells

The TME significantly influences cancer stem cell behaviour: The nature of CSCs is greatly affected by the complex TME [40, 66]. The TME aids CSCs through a variety of cellular and molecular interactions, including the release of extracellular vesicles (EVs) by CSCs. These EVs

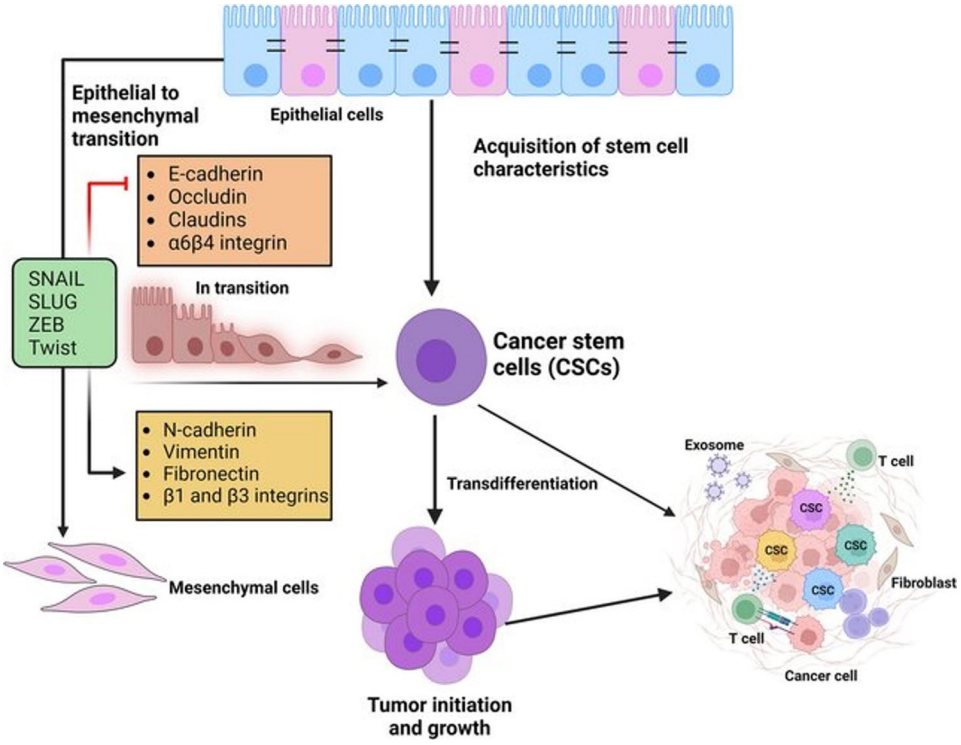

Fig. 6 Epithelial malignant cells acquire stemness features during or after transformation into mesenchymal cells [125]

play a crucial role in modulating the immune response, fostering an immunosuppressive environment that protects CSCs from immune detection [40, 43]. Additionally, the TME contains a wide range of immune cells, such as T lymphocytes and cancer-associated fibroblasts (CAFs), which can either enhance or hinder CSC functions [27, 126, 127]. Surprisingly, CSCs can influence these immune cells, creating a microenvironment that encourages tumor growth and resistance to treatments [126]. These findings indicate that targeting these interactions within the TME could be a promising strategy for developing effective cancer therapies [27, 127, 128], as illustrated in Figs. 7 and 8.

Signalling pathways, niche factors, and therapeutic implications

Regulating Cancer Stem Cells- Signalling and Niche: The regulation of CSCs involves a complex interplay of various signalling pathways that are crucial for their self-renewal and maintenance. Consequently, targeting specific signalling pathways and niche factors has become a promising therapeutic strategy to eliminate CSCs and prevent tumour relapse. CSCs are known for their exceptional self-renewal abilities and resistance to standard therapies, both of which play a significant role in cancer progression and recurrence. Gaining a deeper understanding of these pathways is essential for comprehending how CSCs persist in tumours and contribute to treatment resistance. This area of research is particularly important, as CSCs are often associated with tumour progression, metastasis, and therapeutic failure. Recent

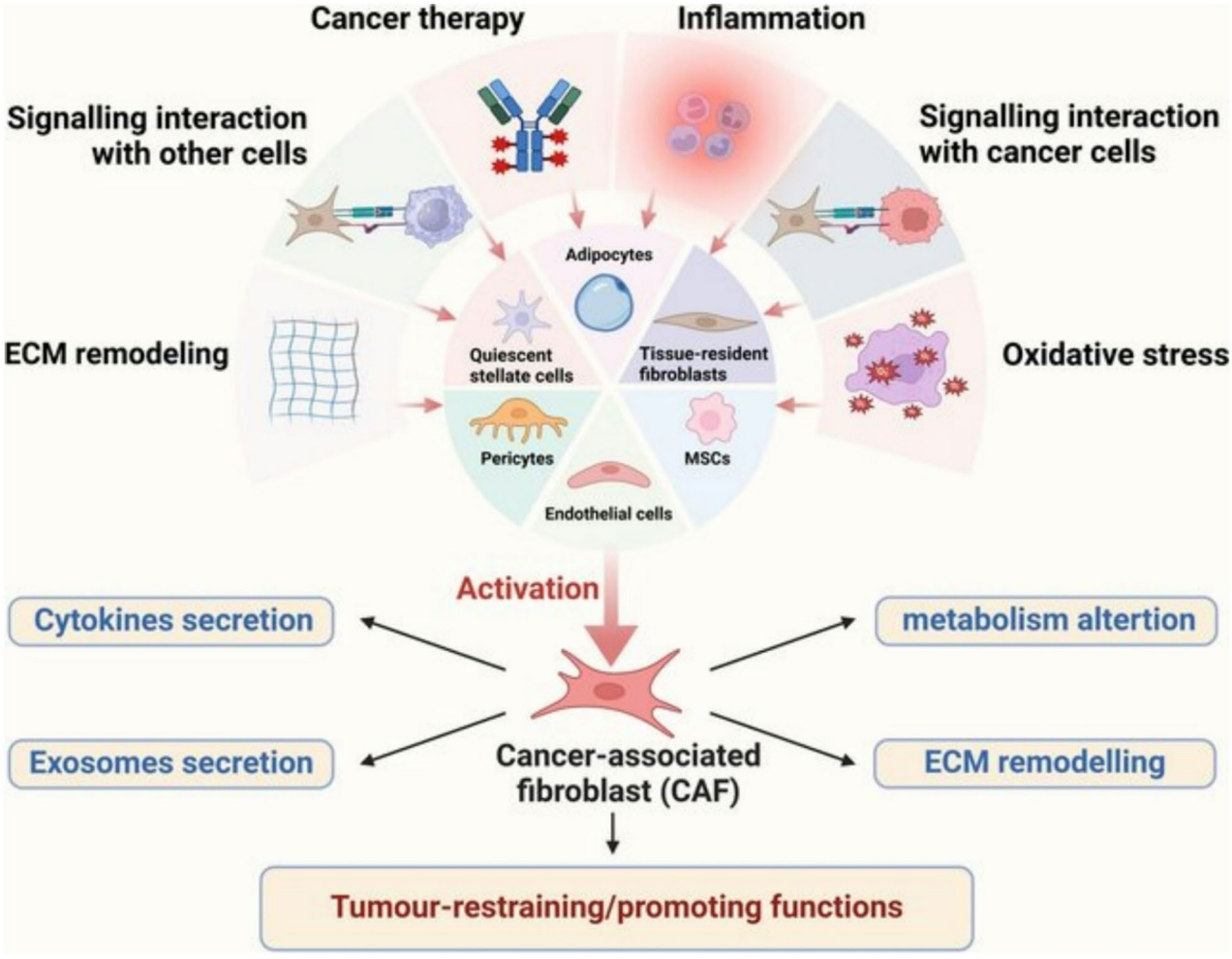

Fig. 7 Overview of CAFs. CAFs can originate from quiescent stellate cells, tissue-resident fibroblasts, mesenchymal stem cells (MSCs), endothelial cells, pericytes, or other cell types. CAFs can be activated via a variety of mechanisms, including ECM remodelling, inflammation, oxidative stress, and signalling interactions with cancer cells or other cells in the tumor microenvironment. Activated CAFs can perform either tumor-restraining or tumor-promoting roles by secreting cytokines and exosomes, modifying the ECM, and altering metabolism

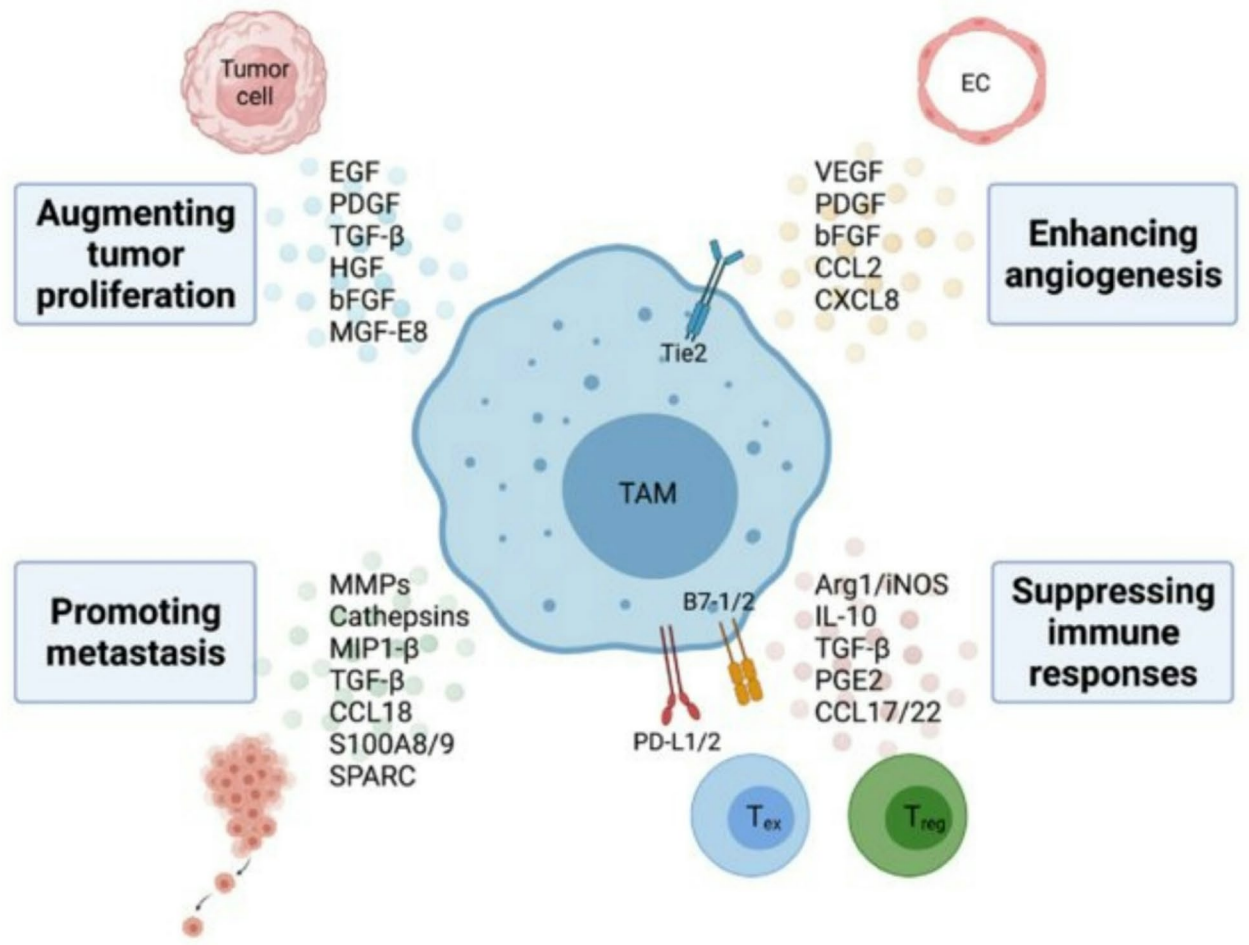

Fig. 8 The functional roles of TAMs are shown. TAMs can promote tumor development through four major mechanisms: [1] increasing tumor proliferation [2], increasing angiogenesis [3], increasing metastasis, and [4] inhibiting adaptive immune responses. Abbreviations: Epithelial growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), milk fat globule-EGF factor 8 (MGF-E8), endothelial cell (EC), and vascular endothelial growth factor (VEGF). CCL2-C motif chemokine Ligand 2, CXCL8-C-X-C motif chemokine Ligand 8, MMP-matrix metalloproteases, MIP1-β-macrophage inflammatory protein 1 beta, CCL18-C motif chemokine Ligand 18, S100A8/9-S100 calcium binding protein A8/9, SPARC-secreted protein acidic and cysteine rich. Arg1: arginase 1; iNOS: inducible nitric oxide synthase; IL-10: interleukin 10; PGE2: prostaglandin E2; CCL17/22: C motif chemokine Ligand 17/22; PD-L1/2: programmed death ligand ½; Tex: exhausted T cell; Treg: regulatory T-cell [129, 130]

advancements in CSC biology, along with insights into their interactions within the tumour microenvironment, have paved the way for innovative therapeutic strategies aimed at disrupting these critical pathways. CSCs depend on several key signalling pathways to uphold their stem-like characteristics and promote tumour growth. Among the most significant pathways are the Wnt/β-catenin, Notch, and Sonic Hedgehog (SHH) pathways, which collectively regulate CSC self-renewal, survival, and differentiation [45, 131].

These pathways are frequently hyperactivated in cancer, leading to increased CSC activity and contributing to therapeutic resistance. The following discussion examines the principal signalling pathways involved in the regulation of CSCs, drawing upon recent research findings. Notably, the Wnt/β-catenin pathway plays a critical role in maintaining the undifferentiated state of CSCs and enhancing their self-renewal capacity. Dysregulation of this pathway has been associated with elevated tumourigenesis, underscoring its pivotal role in CSC dynamics [132, 133] and its potential as a therapeutic target. Notch signalling plays a crucial role in determining cell fate and is vital for maintaining the properties of CSCs. It carefully manages the balance between self-renewal and differentiation, and any disruptions in this pathway have been associated with the persistence of CSCs and their ability to resist standard cancer treatments [132, 133]. The Hedgehog pathway also serves as a key regulator of CSCs, especially in solid tumors. It aids in the growth and survival of CSCs and is frequently hyperactivated

in cancer, making it a potential target for therapy [108, 132]. Furthermore, the TGF-β pathway is closely linked to epithelial–mesenchymal transition (EMT), a process that enhances the stem-like traits and adaptability of CSCs. TGF-β signalling not only boosts the metastatic capabilities of CSCs but also contributes to their resistance against conventional therapies, complicating treatment outcomes [133, 134]. Additionally, the JAK-STAT pathway influences CSC proliferation and survival in response to cytokines and growth factors, with any dysregulation resulting in increased tumor aggressiveness and treatment resistance [108, 134].

Numerous studies indicate that the dysregulation of the Wnt/β-catenin pathway is associated with heightened tumor aggressiveness. Conversely, abnormal Notch and SHH signaling significantly contribute to the maintenance and growth of cancer stem cell (CSC) populations. As a result, therapeutic strategies targeting these pathways are becoming increasingly popular. In particular, small-molecule inhibitors and monoclonal antibodies aimed at Wnt, Notch, and SHH signaling have demonstrated encouraging outcomes in preclinical and early clinical trials. Notably, these treatments not only diminish CSC populations but also enhance the effectiveness of conventional therapies, such as chemotherapy and radiotherapy, by effectively addressing resistance mechanisms [12, 21, 45, 51].

Therefore, the targeted modulation of these signaling pathways offers a promising approach to improving cancer treatment results. Beyond intrinsic signaling pathways, the tumor microenvironment, or CSC niche, plays a vital role in influencing CSC behavior. This niche is composed of various extracellular matrix components and stromal cells, including cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs), which provide critical signals that support CSC self-renewal, facilitate immune evasion, and bolster therapy resistance [135, 136]. Recent therapeutic strategies have concentrated on disrupting the interactions between CSCs and their niche. For instance, targeting specific niche factors, such as cytokines and growth factors released by CAFs and TAMs, has shown promise in reducing tumor relapse driven by CSCs. Additionally, approaches aimed at altering the TME to make it less conducive to CSCs are under investigation. These strategies include therapies that normalize the extracellular matrix or reprogram stromal cells to counteract their protumorigenic roles [135], as illustrated in Fig. 9.

Studies have shown that the tumor environment and how it interacts with the immune system play a key role in controlling CSCs. The way CSCs and their surroundings work together affects how CSCs act, which suggests that focusing on these connections could lead to promising new treatments. These discoveries highlight that CSC biology is complex and that we need treatment plans that do more than just target single signaling pathways [133, 138]. Aiming at CSCs and the factors in their niche could help fight cancer. But there are still hurdles, like understanding tricky interactions and the chance that cancer might resist treatment. We need an approach that can keep up with how tumors change over time. More research is needed to make these methods work well for patients and to help them get better results.

Niche Construction and Immune Evasion: CSCs play a key role in building their supportive microenvironment. They recruit and change normal cells into parts of the TME. This recruitment helps CSCs survive and tumors grow [17]. The TME has an immunosuppressive nature, which cytokines like interleukin-1 (IL-1) and interleukin-6 (IL-6) have an impact on. These cytokines help tumors avoid the immune system and create an environment that helps CSCs multiply [126, 139]. The immunosuppressive setting allows CSCs to escape immune detection. This ensures they stick around and boosts their ability to start and spread tumors. *Metabolic Adaptations*: CSCs demonstrate impressive metabolic flexibility, enabling them to adjust to the harsh conditions of the TME, such as low oxygen levels and scarce nutrients. By alternating between oxidative phosphorylation and glycolysis based on environmental signals, CSCs preserve their stem-like characteristics and survive in adverse conditions. This metabolic adaptability not only sustains CSC stemness but also enhances tumor aggressiveness and resistance to treatments. For instance, the hypoxic environment within the TME often leads to the stabilization of hypoxia-inducible factors (HIFs), which promote CSC survival and growth, making therapeutic interventions even more challenging [140].

The interplay between CSCs and normal stem cells

CSCs and normal stem cells (NSCs) have a complex and interconnected relationship that significantly impacts tumor development, progression, and resistance to therapy. Both types of cells share essential stem-like traits, including self-renewal and differentiation, and their activities are heavily influenced by their unique microenvironments. This intricate relationship highlights the complexity of cancer biology and emphasizes the urgent need for targeted therapies that can effectively eliminate CSCs while preserving the function of normal stem cells. CSCs and NSCs share several important features. For instance, both can self-renew and differentiate into various cell types. This ability contributes to the heterogeneity seen in tumors, which is a key factor in cancer progression and treatment resistance [118]. Additionally, CSCs often enter a quiescent state, similar to that of NSCs, allowing them to evade therapies and persist within tumors. This dormant state can ultimately result in

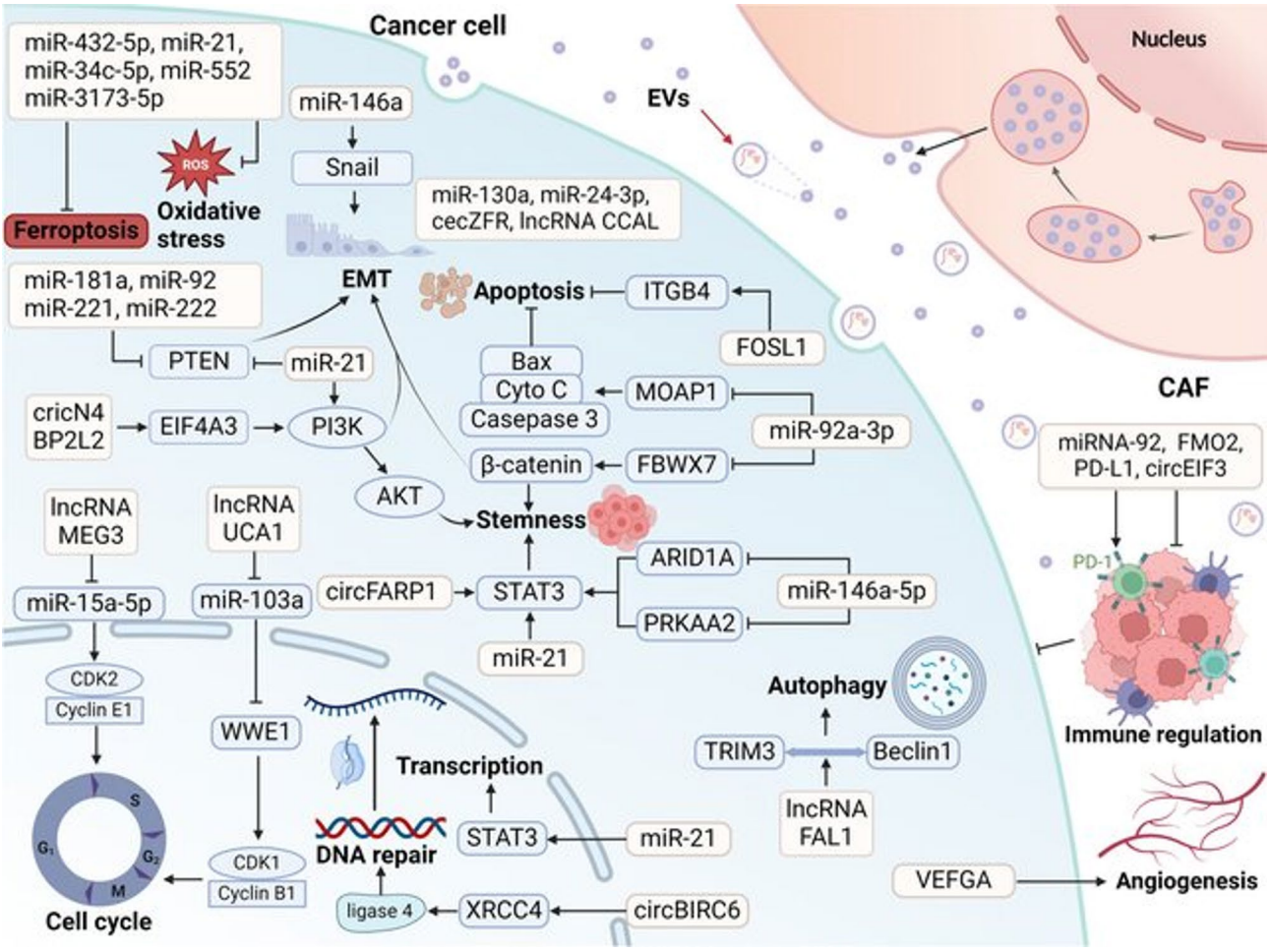

Fig. 9 CAF-derived EVs in cancer chemotherapeutic resistance. The detailed molecular pathways of CAF-derived EVs involved in cancer chemoresistance are summarized. CAF-derived EVs can deliver miRNAs, lncRNAs, circRNAs, and proteins to mediate cancer chemotherapy resistance by regulating the cell cycle, DNA repair, oxidative stress, EMT, apoptosis, autophagy, cancer cell stemness, angiogenesis, immune cell infiltration, and signalling pathways, including the PI3K/AKT, Wnt/β-catenin, and PTEN/mTOR pathways [137]

tumor relapse and metastasis after standard treatments. Importantly, both CSCs and NSCs depend on their specific niches—specialized microenvironments that provide vital signals for their survival and function. However, a significant difference is that CSCs often take over the niches of NSCs, using these supportive environments to foster tumor growth [141]. By mimicking the behavior of NSCs and hijacking their regulatory mechanisms, CSCs gain access to essential resources and signaling pathways that enhance their survival and proliferation. The TME is crucial for maintaining CSCs through complex interactions and communication between cells. CSCs modify the TME, fostering an immunosuppressive setting that aids their survival and significantly contributes to resistance against standard treatments [66]. This immunosuppressive state is established by the release of various cytokines and growth factors that effectively dampen immune responses and encourage tumor growth. Additionally, CSCs interact dynamically with other cells in the TME, including tumor-associated macrophages (TAMs) and endothelial cells. These interactions greatly influence CSC characteristics, boosting their stemness, ability to migrate, and invasive capabilities [142]. For instance, TAMs produce factors that enhance CSC plasticity, while endothelial cells help create a supportive blood supply that promotes tumor development. This complex interplay highlights the vital role of the TME in driving CSC-mediated tumor progression, as depicted in Table 1; Fig. 10.

The similarities between CSCs and normal stem cells (NSCs) create both exciting possibilities and significant challenges for developing therapies. It's crucial to understand the subtle differences and similarities between these cell types to design treatments that effectively target CSCs while preserving the functions of normal stem cells [143]. For instance, discovering unique molecular markers or pathways that are specific to CSCs could allow for selective targeting, protecting NSCs, and minimizing

Table 1 Relationships between stem cells and cancer cells: the important similarities and differences between normal stem cells and cscs, including their origin, self-renewal capacity, differentiation potential, growth regulation, therapeutic response, tumorigenicity, disease role, and therapeutic implications, are highlighted

Criteria	Stem Cells	CSCs	References
Origin	Derived from early developmental stages or reside in adult tissues	Arise from normal stem cells due to genetic and epigenetic alterations	[145–148]
Self-Renewal	Capable of extensive self-renewal to maintain tissue homeostasis	Exhibit unlimited self-renewal capacity, driving tumor growth and maintenance	[148, 149]
Differentiation	Differentiate into specialized cell types to replenish tissues	Can differentiate into various cell types found within the tumor, contributing to heterogeneity	[150–152]
Growth Control	Tightly regulated by intrinsic and extrinsic signals	Exhibit uncontrolled growth and proliferation, evading normal regulatory mechanisms	[152, 153]
Resistance to Therapy	Generally, resistant to cytotoxic agents	Highly resistant to conventional therapies (chemotherapy, radiation), contributing to treatment failure and recurrence	[149, 154, 155]
Tumorigenicity	Not tumorigenic	Possess the ability to initiate and sustain tumor growth in vivo	[156, 157]
Role in Disease	Tissue repair, regeneration	Tumor initiation, growth, metastasis, and therapy resistance	[146, 147]
Therapeutic Implications	Regenerative medicine, tissue engineering	Novel cancer therapies targeting CSCs, such as immunotherapy and small molecule inhibitors	[151, 155, 158, 159]

the risk of negative side effects. However, the malignant transformation of CSCs and their impressive ability to influence the TME present considerable obstacles. The overlapping characteristics of CSCs and NSCs complicate the development of treatments that can accurately differentiate between these cell types. Furthermore, the adaptability of CSCs within the TME underscores the urgent need for innovative therapeutic strategies that tackle both the inherent features of CSCs and their complex micro-environmental support systems. By integrating targeted therapies with methods that effectively modify the TME, it may be possible to address the challenges posed by CSC-driven tumor progression and resistance, as illustrated in Table 1.

CSCs and normal stem/progenitor cells utilize similar signaling pathways that influence their behavior and fate, including the Wnt/β-catenin, Notch, Hedgehog, TGF-β, and NF-κB pathways [132, 134, 162–164]. These pathways are essential for maintaining stemness and facilitating differentiation, but when they become dysregulated, they can lead to tumorigenesis by encouraging uncontrolled proliferation and hindering proper differentiation [132, 161]. The Wnt/β-catenin pathway is particularly important for sustaining the self-renewal of both CSCs and normal stem cells [132, 161, 162]. Increased activity of Wnt/β-catenin is frequently seen in cancers and is closely associated with the aggressiveness and survival of CSCs. Notch signaling plays a vital role in determining cell fate and helps maintain CSCs by allowing them to self-renew while inhibiting differentiation [134, 163]. When this pathway is disrupted, it can contribute to tumor progression and metastasis. The Hedgehog pathway, primarily recognized for its role in embryonic development, also plays a part in maintaining CSCs and is involved in tumor progression and resistance to treatments [39, 132, 163]. The TGF-β pathway enhances stemness by promoting self-renewal and can elevate the metastatic potential of CSCs. Meanwhile, the NF-κB pathway, known for its involvement in inflammatory responses, aids in the survival and self-renewal of CSCs by enhancing their resistance to stress and apoptosis [132, 161]. Targeting these signaling pathways presents significant potential for cancer therapy. However, given their critical roles in normal tissue homeostasis and regeneration, a careful approach to therapeutic intervention is essential [161, 164], and is dictated in Table 1. Indeed, an essential part of the approach is precision targeting, which could be achieved through inhibitors targeting specific pathway intermediates or combinations of pathway modulators, allowing selective modulation of only those pathway elements that are dysregulated in CSCs, without affecting normal stem cells. Some key signaling pathways mediating CSC and normal stem cell cross-talk as they pertain to current cancer treatments. Precision therapies targeting CSCs also have the potential to develop more effective and safe cancer treatments as they do not act upon normal stem cells, which gives them regenerative capacity.

Advances in identifying CSC-Specific markers and signaling pathways

Recent advances in cancer stem cell (CSC) research have significantly deepened our understanding of the molecular features that distinguish CSCs from bulk tumor cells

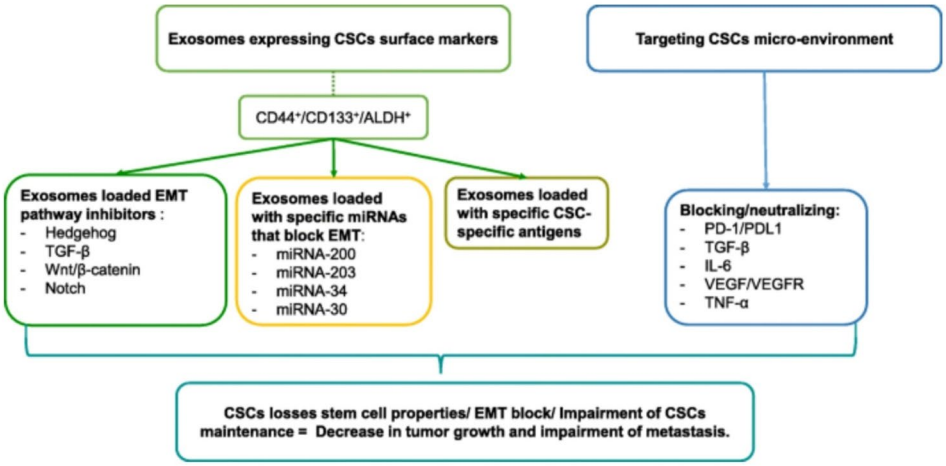

Fig. 10 Potential therapeutic strategies to target CSCs. Exosomes that exhibit CSC markers can be exploited to deliver miRNAs that disrupt the EMT pathway or critical CSC pathway inhibitors, hence achieving specific CSC targeting. Another way to target CSCs is to restrict their microenvironment, which stops new CSCs from forming [160]

and normal stem cells. Canonical surface markers such as CD133, CD44, EpCAM, and ALDH continue to serve as foundational identifiers of CSC populations across various malignancies. For instance, CD133 is widely expressed in brain, colon, and liver CSCs and is associated with tumor initiation and self-renewal capabilities [45, 108]. CD44 and its variant isoform CD44v6 are implicated in cell adhesion, migration, and epithelial-mesenchymal transition (EMT), particularly in breast, prostate, and gastric cancers [165, 166]. EpCAM, a cell adhesion molecule, is overexpressed in colorectal and breast CSCs and contributes to proliferation and metastasis [45, 108]. Meanwhile, ALDH activity is a functional marker of stemness and detoxification, especially in ovarian, lung, and breast cancers [165, 167]. Emerging markers such as CD123, primarily found in leukemia and acute myeloid leukemia (AML), offer new avenues for targeted immunotherapy due to their restricted expression profiles [21, 108, 118]. In addition to surface markers, several conserved signaling pathways have been identified as central regulators of CSC biology. The Wnt/β-catenin pathway plays a critical role in maintaining stemness and promoting proliferation in colon, breast, and liver CSCs. Inhibitors such as LGK974 and PRI-724 have shown promise in selectively disrupting this pathway [166, 167]. The Notch pathway, essential for cell fate determination and survival, is hyperactivated in glioblastoma and breast CSCs and can be targeted using γ-secretase inhibitors [45, 165]. Similarly, the Hedgehog (Shh) pathway supports CSC expansion and EMT, particularly in pancreatic and brain tumors, with agents like vismodegib under clinical evaluation [108, 168]. The TGF-β/SMAD axis facilitates EMT and immune evasion in lung and breast cancers, and its inhibition may reduce CSC-mediated metastasis [166, 169]. Lastly, the JAK-STAT3 pathway contributes to CSC proliferation and resistance to apoptosis, making it a viable target in liver and breast cancers [165, 166].

Therapeutic Selectivity and Targeting Strategies: The therapeutic selectivity of CSC-targeted treatments relies on exploiting molecular differences between CSCs and normal stem cells. One promising approach involves antibody-drug conjugates (ADCs) that bind to CSC-specific markers such as CD133 or EpCAM, delivering cytotoxic agents directly to the CSC population [45]. Small molecule inhibitors targeting dysregulated pathways like Wnt and Notch offer another layer of selectivity, as these pathways are often more active in CSCs than in normal stem cells [166]. Additionally, CAR-T and CAR-NK cell therapies are being engineered to recognize antigens like CD123 and CD44v6, enabling precise immunologic targeting of CSCs [21, 108, 118]. Despite these innovations, achieving true selectivity remains challenging. Many CSC markers are also expressed in normal stem cells, raising concerns about off-target toxicity. To address this, researchers are exploring context-dependent targeting, which leverages differences in expression levels and activation states, and microenvironment modulation, which alters the CSC niche to reduce stemness without affecting normal stem cells [165, 166].

Translational Potential across Cancer Types: CSC-targeted therapies are being actively investigated across a range of cancer types, with varying degrees of translational success. In breast cancer, inhibitors of Wnt and Notch pathways have demonstrated efficacy in reducing recurrence and metastasis [167]. In glioblastoma, targeting metabolic dependencies and transcriptional regulators of CSCs has shown promise in preclinical models [166]. In pancreatic cancer, glutamine metabolism inhibitors have been found to sensitize CSCs to radiation therapy, potentially improving treatment outcomes (Cancer

Biology & Medicine, 2024). In hematologic malignancies such as leukemia, CD123-targeted therapies are advancing through clinical trials, offering a model for CSC-specific immunotherapy [108]. However, the translational potential of these therapies is often limited by tumor heterogeneity, CSC plasticity, and immune evasion mechanisms. CSCs can dynamically shift between stem-like and differentiated states, complicating efforts to eradicate them. Personalized medicine approaches, including single-cell sequencing and multi-omics profiling, are being developed to overcome these barriers and tailor therapies to individual tumor profiles [166, 169, 170].

Challenges in Targeting CSCs without Harming Normal Stem Cells: One of the most significant challenges in CSC-targeted therapy is the biological overlap between CSCs and normal stem cells. Markers such as CD44 and CD133 are not exclusive to CSCs, and their expression in normal tissues can lead to unintended toxicity [165, 166]. Moreover, CSC plasticity allows non-CSCs to acquire stem-like traits under therapeutic pressure, enabling tumor regeneration even after CSC-targeted treatment [21, 118]. The TME further complicates therapy by providing a protective niche that sustains CSC survival and shields them from immune responses. Components of the TME, such as stromal cells and cytokines, actively support CSC stemness and resistance [165, 166, 170]. Additionally, the lack of universal CSC markers across cancer types necessitates the development of cancer-specific targeting strategies and functional assays to accurately identify and eliminate CSCs [166, 168], and summarised in Table 2.

Table 2 CSC-Specific markers and signaling pathways

Marker/Pathway	Cancer Types	Function in CSCs	Therapeutic Strategy	References
CD133	Brain, colon, liver	Self-renewal, tumor initiation	ADCs, CAR-T cells	[45, 108]
CD44/CD44v6	Breast, prostate, gastric	Adhesion, migration, EMT	Monoclonal antibodies	[165, 166]
EpCAM	Colorectal, breast	Cell-cell adhesion, proliferation	Photoimmunotherapy	[45, 108]
ALDH	Ovarian, lung, breast	Detoxification, stemness	ALDH inhibitors	[165, 167]
CD123	Leukemia, AML	IL-3 receptor subunit	Targeted immunotherapy	[21, 108, 118]
Wnt/β-catenin	Colon, breast, liver	Stemness, proliferation	LGK974, PRI-724	[166, 167]
Notch	Glioblastoma, breast	Cell fate, survival	γ-secretase inhibitors	[45, 165]
Hedgehog (Shh)	Pancreatic, brain	CSC expansion, EMT	Vismodegib	[108, 168]
TGF-β/SMAD	Lung, breast	EMT, immune evasion	TGF-β inhibitors	[166, 169, 170]
JAK-STAT3	Liver, breast	Proliferation, resistance	STAT3 inhibitors	[165, 166]

CSC modulation of normal stem/progenitor cells: CSCs play a crucial role in shaping the behavior of normal stem and progenitor cells in the TME, creating a complex interaction that fuels tumor growth, metastasis, and resistance to treatment. This relationship involves the recruitment and transformation of normal cells, adjustments in stemness and plasticity, and significant communication with immune cells. Together, these processes allow CSCs to flourish within the TME, positioning them as key players in tumor biology and important targets for therapy. CSCs actively draw in normal stem and progenitor cells into the TME, converting them into elements that aid the proliferation of tumor cells [128]. This recruitment is facilitated by signaling molecules and exosomes released by CSCs, which alter the behavior of surrounding normal cells, as demonstrated in Fig. 10. A major consequence of this transformation is the creation of an immunosuppressive environment. By utilizing normal cells, CSCs create conditions that protect them from immune detection [66]. For instance, immune cells that usually target and eliminate cancer cells are reprogrammed to take on roles that suppress immune responses, further safeguarding CSCs and promoting their survival. The TME provides essential signals, including cytokines, growth factors, and interactions with extracellular matrix components, that preserve the stemness characteristics of CSCs, enabling them to self-renew and differentiate as necessary [171]. Additionally, normal progenitor cells in the TME can develop CSC-like traits after prolonged exposure to the tumor environment. Factors released by CSCs and the surrounding TME can reprogram these cells, giving them improved self-renewal and survival abilities [172].

This phenomenon contributes to tumor heterogeneity, making treatment more challenging by creating diverse cell populations with different therapeutic vulnerabilities. The interaction between CSCs and immune cells within the TME is crucial in influencing tumor progression and resistance to therapies. Tumor-associated macrophages (TAMs) and endothelial cells play significant roles in this interaction. TAMs release cytokines and growth factors that enhance CSC stemness and promote angiogenesis, thereby providing a supportive environment for tumor growth [142]. Through their interactions with TAMs and other immune cells, CSCs help create an immunosuppressive TME that diminishes the effectiveness of cancer treatments [66, 171]. Although CSCs often exploit normal stem and progenitor cells to drive tumour

progression, not all are affected; some retain their regenerative capacity. This duality underscores the complexity of stem cell behaviour within the TME and the need for targeted therapies that disrupt tumour-supportive interactions without impairing tissue repair. CSCs foster a supportive niche through recruitment, transformation, modulation of stemness, and immune crosstalk, contributing to tumour progression and therapy resistance. Understanding these dynamics is crucial for developing effective treatments that eliminate CSCs while preserving normal stem cell function.

Targeting stem cell pathways in cancer: CSCs have become a crucial element in the development and progression of various cancers. These cells possess the ability to self-renew, differentiate, and promote tumor growth, making them vital targets for new cancer therapies. While there are similarities between normal progenitor and stem cells and cancer stem cells, targeting these normal cells may also offer potential for cancer treatment. CSCs represent a small subset of cancer cells that display stem-like characteristics, such as self-renewal and the ability to differentiate into various tumor cell types [173]. They are notably resistant to standard chemotherapy and radiotherapy, which contributes to tumor recurrence and metastasis [174]. Understanding the unique traits of cancer stem cells, including their specific surface markers and regulatory pathways, is essential for creating targeted therapies [175]. A major challenge in targeting CSCs is the heterogeneity found within tumors. Tumors typically consist of a diverse array of cells, including both CSCs and non-stem-like cancer cells, with varying proportions of these groups both within and among patients [176]. This diversity poses a significant challenge in developing effective therapies that can eradicate the entire tumor population [177]. However, focusing on the distinct characteristics of CSCs and normal progenitor/stem cells offers great potential for innovative cancer treatments, as it may improve treatment effectiveness while reducing harm to healthy tissues [16]. By concentrating on the specific markers and pathways that set CSCs apart from their normal counterparts, researchers can create targeted therapies that selectively destroy malignant cells without affecting healthy cells. This approach not only aims to improve patient outcomes but also addresses the challenge of cancer recurrence, as it targets the root cause of tumor growth and resistance to conventional therapies.

These innovative strategies are paving the way for personalized medicine, enabling treatments to be customized based on the unique characteristics of a patient's tumor and its surrounding environment. This shift in cancer treatment is anticipated to result in more effective interventions, decreasing the dependence on traditional chemotherapy and radiation, which often come with significant side effects. Consequently, ongoing clinical trials are investigating the effectiveness of these targeted therapies, offering hope for more sustainable and less toxic treatment options that enhance the quality of life for cancer patients [178]. Such advancements not only aim to boost survival rates but also prioritize the overall well-being of patients, making cancer care more compassionate and centred on the individual. This approach highlights the significance of personalized medicine, where treatments are crafted based on genetic profiling and specific tumor traits, ultimately allowing for a more precise attack on cancer cells while protecting healthy tissue. This transformation in cancer treatment emphasizes the necessity for ongoing research and collaboration among scientists, clinicians, and patients to create innovative strategies that can adapt to the changing landscape of cancer biology. As new therapies are developed, incorporating technology like artificial intelligence and machine learning into treatment plans can further improve the ability to predict patient responses and customize interventions accordingly.

CSCs and therapeutic implications

Targeting cancer stem cells: strategies and challenges. CSCs are essential for enhancing cancer treatment outcomes due to their critical role in tumor initiation, metastasis, and resistance to standard therapies. Recent studies have introduced promising strategies that leverage the distinct characteristics of CSCs. Immunotherapy methods, such as monoclonal antibodies aimed at specific CSC markers, CAR-T-cell therapy that modifies T cells to attack CSCs, and tumor vaccines designed to activate the immune system against CSCs, have demonstrated significant potential [44]. Additionally, nanotechnology applications, including the use of customized nano-materials for direct drug delivery to CSCs and the integration of nanotechnology with immunotherapy and targeted therapies, provide improved specificity and fewer side effects [179]. Moreover, targeting signaling pathways vital for CSC maintenance, like the Wnt/β-catenin and Notch pathways, through molecular inhibitors and gene editing techniques such as CRISPR/Cas9 can hinder the self-renewal capacity of CSCs [45].

Despite those advancements, challenges such as immune rejection and the ability of CSCs to adapt and develop resistance persist. Immune rejection and cellular plasticity are major barriers to effective cancer therapy, especially for immunotherapies and cell-based treatments. The immune system can recognize and eliminate therapeutic agents, reducing their durability and impact. Tumors can also reshape the immune environment, weakening key responses from T cells and antigen-presenting cells [180]. At the same time, cancer cells show high plasticity through processes like epithelial–mesenchymal transition (EMT), allowing them to

evade detection, resist treatment, and increase heterogeneity [92, 181]. Immune cells, such as natural killer (NK) cells, can also become dysfunctional through plasticity, leading to reduced tumor-killing ability [182]. Some strategies—like boosting antigen presentation or combining immunotherapies with anti-plasticity drugs—have shown promise [183, 184]. However, the reversible nature of plasticity often allows resistance to develop quickly. In addition, the tumor microenvironment, especially under hypoxia, promotes immune tolerance and further drives plasticity [93, 185]. While new therapies continue to emerge, the adaptability of tumor and immune cells remains a key challenge to lasting treatment success. Ongoing research is essential to refine these strategies and improve their clinical effectiveness in targeting these elusive cells.

Table 3 Summary of main findings in cancer therapy with stem cell biology as a target: A concise summary of significant facts, therapeutic breakthroughs, and future views in cancer therapy targeting CSCs

Category	Key Findings	References
Mechanisms Targeted	Critical pathways such as Notch, Hedgehog, and Wnt are essential for CSC maintenance and tumor growth.	[189, 190]
	Surface markers like CD44, CD133, and ALDH1 are identified as CSC-specific targets.	[191, 192]
Therapeutic Approaches	Agents targeting CSC-specific signalling pathways are under investigation in clinical trials.	[189]
	Nanotechnology enhances drug delivery to CSCs, improving efficacy and minimizing side effects.	[193]
Combination Therapies	Combining CSC-targeted therapies with chemotherapy, radiotherapy, or immunotherapy improves outcomes.	[191]
	Preclinical studies show synergy between CSC inhibitors and existing therapies.	[194]
Challenges	Tumor heterogeneity complicates CSC targeting.	[189]
	CSCs exhibit resistance mechanisms, including immune evasion and metabolic adaptation.	[195, 196]
Current Clinical Trials	Breast cancer: Trials on Notch and Hedgehog inhibitors in combination with chemotherapy.	[189]
	Pancreatic cancer: Early-phase trials targeting CSC-enabling microenvironments.	[198–200]
	Multiple myeloma: CSC-specific surface marker targeting is being assessed.	
Future Perspectives	Personalized medicine with genomic profiling enables precise CSC targeting.	[201–203]
	Development of advanced nano-carriers to selectively target CSCs.	[193]
	Multidisciplinary approaches involving clinical decision support systems are essential for integration into care.	[192]

The mechanism by which CSCs contribute to chemotherapy resistance and recurrence: CSCs are pivotal in chemotherapy resistance and cancer recurrence. Their unique traits, including quiescence, self-renewal, and pluripotency, allow them to evade traditional therapies [187–189]. Quiescence enables CSCs to stay dormant and avoid drugs that target actively dividing cells. Additionally, CSCs have high levels of drug efflux pumps that actively remove chemotherapeutic agents from the cell and possess advanced DNA repair mechanisms, which help them recover from treatment-induced damage [114, 122]. These mechanisms are helping them to survive and eventually reenter the cell cycle and drive tumor regrowth [187–189]. Additionally, interactions with the TME promote CSC survival and proliferation complicating the treatment outcomes [118] (See Table 3). Although targeting CSCs is a promising strategy to enhance treatments, the difficulty of selectively targeting and eradicating these resistant cells without affecting normal stem cells or creating additional heterogeneous niches poses a formidable challenge.

Major challenges in developing therapies that specifically target cancer stem cells: Developing therapies that specifically target CSCs poses significant challenges. The inherent diversity of CSCs, marked by their various phenotypes and self-renewal abilities, allows them to evade standard treatments and contribute to tumor recurrence [14, 43]. Additionally, CSCs interact with the TME, creating an immunosuppressive setting that complicates effective treatment. This complex relationship includes interactions with immune cells and the release of exosomes that further alter the TME, shielding CSCs from immune detection [43, 203]. Moreover, targeting CSCs raises concerns about immune rejection and the risk of disease relapse, as treatments might unintentionally create new CSCs [38]. The intricate nature of CSC biology calls for the development of specialized immunotherapies that can navigate these challenges [46], as illustrated in Fig. 11. Despite these hurdles, ongoing research into CSC-targeted therapies shows promise for enhancing cancer treatment outcomes. However, a thorough understanding of CSC biology and their interactions within the TME is essential for creating effective and lasting treatment strategies.

CSCs and their role in oncogenesis across cancer subtypes

CSCs represent a unique subpopulation within neoplasms, distinguished by their capabilities for self-renewal, differentiation, and the initiation of tumorigenesis. These cells are increasingly acknowledged as pivotal contributors to oncogenesis, metastatic spread, therapeutic resistance, and disease recurrence across

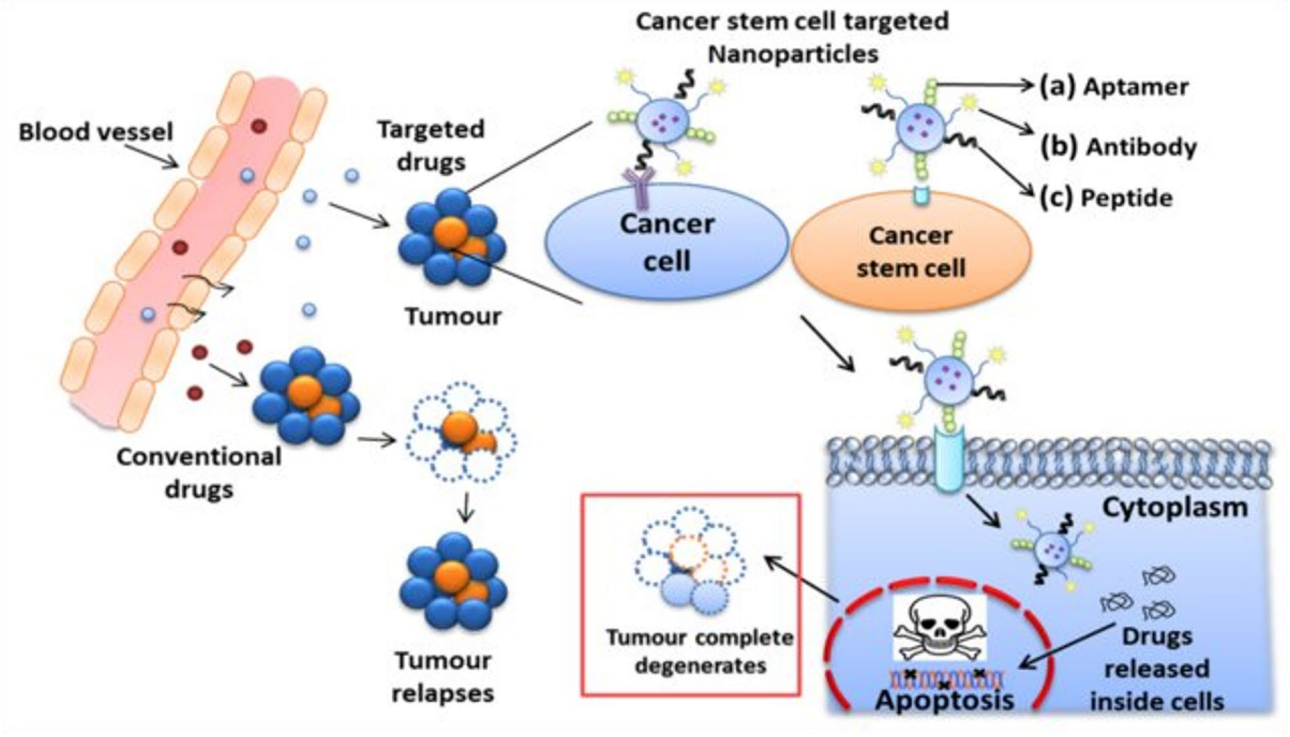

Fig. 11 NPs use several methods to target cancer cells. Anticancer drug-encapsulated nanoparticles are coupled to a variety of targeting molecules, including (**a**) aptamers, (**b**) antibodies, and (**c**) peptides specific to cancer stem cell markers. Once delivered, these targeted NPs can penetrate the tumor through increased permeability and retention (EPR) at tumor locations and bind to specific ligands expressed in cancer cells. NPs can be coupled with cancer cell- and cancer stem cell-specific targeting elements, ensuring that medications reach all types of cells in the tumor environment. When encapsulated medications are released into cells, they can activate signalling processes that lead to the total eradication of cancer cells. Compared with traditional medications, which target only cancer cells while leaving stem cells intact, this technique has shown encouraging results, hence stimulating the development of multidrug-resistant cells [204]

a diverse array of malignancies [88, 205]. In contrast to the bulk of tumor cells, CSCs demonstrate an ability to withstand standard therapeutic interventions and regenerate tumors, thereby constituting essential targets for efficacious cancer therapies. CSCs frequently display dysregulation in key signaling pathways, including Wnt/β-catenin, Notch, and Hedgehog, which regulate stemness and cellular survival, thereby directly influencing tumorigenesis [206, 207].

In the context of breast cancer, CSCs are frequently characterized by the $CD44^{+}/CD24^{-}$/low phenotype in conjunction with elevated ALDH1 activity. Empirical evidence has substantiated that these cells possess augmented tumorigenic capabilities, with the potential to replicate the heterogeneity of the original tumor in vivo [85, 208, 209]. Furthermore, breast CSCs exhibit a pronounced resistance to conventional therapeutic modalities, attributed to heightened expression of drug efflux transporters and proficient DNA repair mechanisms(211). Recent investigations also underscore the significance of the epithelial-mesenchymal transition (EMT) in preserving the plasticity and metastatic capacity of breast CSCs, positing that these cells can dynamically transition between epithelial and mesenchymal phenotypes in response to environmental stressors [38, 211, 212].

In glioblastoma, glioma stem-like cells (GSCs) play a crucial role in the aggressive behavior and recurrence associated with this malignant brain tumor. These GSCs are typically localized within hypoxic microenvironments and express markers such as CD133 and Nestin, which reinforce their stem-like characteristics and radioresistance [214–217]. GSCs leverage molecular signaling pathways, including PI3K/Akt and STAT3, to promote cellular proliferation and evade apoptosis, while their interactions with the tumor microenvironment—particularly via perivascular niches—facilitate immune evasion and enhance angiogenic processes [217, 218]. Recent single-cell RNA sequencing analyses have unveiled significant intratumoral heterogeneity and plasticity within the GSC populace, suggesting that targeting a solitary marker may prove inadequate for the comprehensive eradication of these cells [219].

Colorectal cancer (CRC) similarly reveals a well-characterized CSC population defined by the expression of CD133, CD44, and LGR5. These cells possess

the capacity to initiate tumor formation and are notably resilient to chemotherapeutic agents, including 5-fluorouracil and oxaliplatin [221–223]. The activation of the Wnt/β-catenin signaling pathway is essential for the sustenance of colorectal CSCs, with recent findings indicating that mutations in the APC and β-catenin genes drive unchecked expansion of these stem cells [205, 223]. Moreover, colorectal CSCs engage closely with both immune and stromal constituents to establish a supportive microenvironment that shields them from immune surveillance and augments their metastatic potential [224].

In pancreatic ductal adenocarcinoma (PDAC), recognized as one of the most fatal malignancies, CSCs have been characterized by the expression of $CD44^+$, $CD24^+$, ESA^+, and $ALDH1^+$ phenotypes. These cellular entities display augmented tumorigenic potential and facilitate chemoresistance via the activation of Hedgehog and Notch signaling cascades [226–229]. Importantly, pancreatic CSCs are associated with early dissemination and metastatic processes, partially attributable to epithelial-mesenchymal transition (EMT) activation and the release of immunosuppressive cytokines that modify the TME [230–232]. Recent investigations indicate that CSCs in PDAC utilize KRAS-mediated metabolic reprogramming to endure in hypoxic and nutrient-scarce environments, thereby further enhancing their resilience and aggressive phenotype [232, 233].

Table 4 Role of CSCs in Different Cancer Subtypes

Cancer Type	CSC Markers	CSC Role in Oncogenesis	Signaling Pathways	References
Breast Cancer	CD44, CD24, ALDH1	Tumor initiation, EMT, metastasis, recurrence, chemoresistance	Notch, Wnt/β-catenin, PI3K/Akt, IL-6/JAK2/STAT3	[217, 245–248]
Glioblastoma	CD133, Nestin, SOX2, OLIG2	Therapy resistance, recurrence, hypoxia adaptation, angiogenesis	HIF-1α, STAT3, Notch, TGF-β	[249–251]
Colorectal Cancer	CD133, CD44, Lgr5, EpCAM	Tumorigenesis, therapy resistance, metastasis, and tumor heterogeneity	Wnt/β-catenin, Notch, Hedgehog, TGF-β	[222, 252–254]
Pancreatic Cancer	CD44, CD24, ESA, ALDH1, CXCR4	Tumor initiation, metastasis, immunosuppression, and therapy resistance	KRAS, Hedgehog, Notch, CXCL12/CXCR4	[255–260]
AML (Leukemia)	CD34, CD38, TIM3, CD123	Dormancy, relapse, chemotherapy resistance, and immune evasion	Wnt, BCL-2, Notch, FLT3, NF-κB	[261–263]

In the realm of hematologic malignancies such as acute myeloid leukemia (AML), leukemic stem cells (LSCs) demonstrate the expression of markers, notably $CD34^+/CD38^-$, and possess the capability to reinitiate the disease even after apparent remission. LSCs circumvent chemotherapy by remaining in a state of quiescence and residing within protective bone marrow microenvironments [234, 235]. The targeting of specific LSC signaling pathways, such as BCL-2 (for instance, via venetoclax) and FLT3, has shown promising clinical outcomes; however, relapse is frequently observed due to the inherent heterogeneity of LSCs and clonal evolution [236–238]. Contemporary research highlights the critical need to address both LSC-intrinsic survival mechanisms and their interactions with the surrounding microenvironment to mitigate the risk of relapse [239].

Across various cancer types, CSCs contribute to therapeutic resistance and disease recurrence through an array of mechanisms: the upregulation of ATP-binding cassette (ABC) transporters such as ABCG2, the overactivation of DNA damage response pathways, and the preservation of a low reactive oxygen species (ROS) milieu [88, 206]. Furthermore, CSCs alter the TME by secreting cytokines (e.g., IL-6, TGF-β) that facilitate inflammation, angiogenesis, and immune evasion [199, 241–243]. Such interactions engender a niche that not only sustains CSC survival but also promotes metastatic progression.

Given their pivotal involvement in oncogenesis, CSCs have emerged as compelling therapeutic targets. Current investigative strategies encompass the targeting of surface markers (e.g., CD44 antibodies), the disruption of self-renewal pathways (e.g., Wnt and Notch inhibitors), and the modification of the TME to enhance the susceptibility of CSCs to conventional therapeutic modalities [233, 243, 244]. Nevertheless, the clinical translation of these strategies has proven challenging due to the plasticity of CSCs, their phenotypic diversity, and the potential risk of compromising normal stem cells. Consequently, future therapeutic approaches may necessitate combinatorial strategies that simultaneously target CSCs and their supportive niches while preserving the homeostasis of normal tissues, as summarized in Table 4.

Cancer stem cell heterogeneity based on single-cell data across various cancers in the tumor microenvironment

CSCs constitute a diminutive yet pivotal subpopulation of neoplastic cells characterized by their capacity for self-renewal, multi-lineage differentiation potential, and their role in tumor initiation and propagation. Furthermore, they exhibit a significant correlation with metastasis, mechanisms of immune evasion, and the development of resistance to conventional therapeutic modalities. Nevertheless, in contrast to prior perceptions of CSCs as a uniform cohort, a growing body of evidence substantiates

the notion that CSCs exhibit pronounced heterogeneity, both intra- and inter-cancer types. This heterogeneity is influenced by intrinsic genetic and epigenetic determinants, in addition to extrinsic signals emanating from the TME. Recent advancements in single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics have elucidated the comprehensive nature of this heterogeneity and have transformed our comprehension of CSC dynamics [265–268].

Traditionally, bulk RNA sequencing has obscured the existence of rare yet functionally distinct cellular entities, such as CSCs, by averaging gene expression across entire tumor populations. In contrast, scRNA-seq offers transcriptomic insights at the resolution of individual cells, thereby uncovering distinct CSC phenotypes, plasticity, and lineage trajectories within individual tumors [96]. For instance, in glioblastoma, Neftel et al. [268] delineated four dynamic CSC states—neural progenitor-like, oligodendrocyte progenitor-like, astrocyte-like, and mesenchymal-like—each possessing the capacity for interconversion in response to micro-environmental pressures. These transitions exemplify CSC plasticity and underscore the influence of micro-environmental signals on the modulation of stem-like behavior and therapeutic resistance [266].

In a similar vein, within triple-negative breast cancer (TNBC), Wu et al. [269] employed scRNA-seq to identify multiple CSC subpopulations, some of which express epithelial markers such as ALDH1A3, while others exhibit a mesenchymal phenotype, characterized by CD44 and SOX2 expression [270]. These distinctions not only highlight transcriptional diversity but also signify functional specialization—certain CSC subsets demonstrate heightened invasiveness, whereas others exhibit greater resistance to chemotherapeutic agents. The interactions of these subpopulations with immune cells and fibroblasts within the TME further exacerbate this heterogeneity.

The TME is instrumental in sustaining CSC heterogeneity and plasticity. It comprises non-malignant stromal cells, immune cell infiltrates, vascular structures, and extracellular matrix components that provide both physical and biochemical support to tumor cells [88]. Single-cell methodologies have revealed that the spatial arrangement of CSCs within the TME frequently mirrors the availability of niche-specific factors [271]. For example, in colorectal cancer, Li et al. [272]illustrated that CSCs preferentially inhabit hypoxic niches where hypoxia-inducible factors (HIFs) enhance the expression of stemness-associated genes such as LGR5 and CD44. Moreover, cancer-associated fibroblasts (CAFs) partake in paracrine signaling via WNT and TGF-β pathways to maintain CSC populations and facilitate phenotypic diversity.

Pancreatic ductal adenocarcinoma (PDAC) serves as another compelling illustration. Elyada et al. [273] employed cross-species scRNA-seq to elucidate how distinct fibroblast subsets, immunosuppressive myeloid cells, and regulatory T cells develop specialized niches that bolster CSC maintenance and influence their differentiation. These findings reinforce the concept that the TME functions not merely as a passive scaffold but as an active participant in the evolution and resilience of CSCs.

Functionally, the heterogeneity of CSCs is intricately associated with clinical outcomes [266]. In the context of non-small cell lung cancer (NSCLC), Kim et al. [274] delineated CSC subsets that exhibit elevated expression levels of drug resistance genes, including ABCG2 and ALDH1A1, which correspond to unfavorable prognostic indicators and instances of relapse. Moreover, lineage-tracing methodologies and pseudotime analyses have elucidated the reversible characteristics of CSC differentiation, a phenomenon characterized as phenotypic plasticity. This capability to transition between stem-like and differentiated phenotypes facilitates the survival of CSCs in the face of therapeutic interventions and enables tumor repopulation, thereby underscoring the imperative for therapeutic modalities that simultaneously target both CSCs and their associated microenvironments [207, 265].

In light of the intricate nature and adaptability of CSCs, therapeutic strategies must evolve in parallel. Focusing on a singular CSC marker or pathway is unlikely to yield adequate efficacy. Rather, a comprehensive multi-targeted strategy that concurrently disrupts critical stemness signaling pathways (such as Notch, WNT, and Hedgehog), modifies the TME, and constrains CSC plasticity is imperative. The advent of innovative technologies that amalgamate single-cell transcriptomics with spatial proteomics and epigenomics presents promising pathways to enhance such therapeutic interventions and tailor treatment to individual patients [264, 267]. In a nutshell, single-cell and spatial transcriptomic technologies have significantly propelled our comprehension of CSC heterogeneity and its complex interplay with the tumor microenvironment. By elucidating the dynamic interactions and functional diversifications of CSCs, these methodologies lay the groundwork for the development of next-generation precision oncology strategies aimed at achieving sustained tumor eradication and minimizing recurrence.

CSC paradoxes: identification and targeting issues

Identifying and effectively targeting CSCs remains a major challenge in cancer research and therapy. One key issue is the lack of specific biomarkers that can reliably distinguish CSCs from other tumor cells or normal stem cells. Many markers proposed for CSC identification are

also found in non-stem cancer cells or healthy tissues, making it difficult to isolate CSCs accurately and leading to inconsistent findings across different studies [88, 96]. This ambiguity has slowed the development of CSC-specific therapies. Additionally, CSCs are highly dynamic and can switch between stem-like and non-stem states in response to environmental signals or cancer treatment. This plasticity means that even if a therapy eliminates CSCs, non-CSCs can potentially take on stem-like properties, allowing the tumor to regrow and become resistant to treatment [174, 275]. Furthermore, CSCs are not uniform; they differ significantly both within the same tumor and across different patients. These variations, or heterogeneity, make it difficult to develop a universal therapy that works for all CSC types [88, 276].

Even when drugs targeting CSC pathways show success in laboratory settings, they often fail in real patients. This is because CSCs have strong resistance mechanisms, such as the ability to repair DNA damage, avoid immune detection, and activate survival signaling pathways. These traits enable them to survive conventional treatments like chemotherapy or radiation, leading to relapse and spread of cancer [243, 277].

The clinical relevance of CSCs is also still debated. Although experimental models suggest that eliminating CSCs can reduce tumor growth, translating these results into effective treatments for patients has been difficult. Many therapies that perform well in preclinical trials do not show the same benefits in clinical settings, possibly because of differences in how CSCs are organized and behave in human tumors [156, 275]. This hierarchical and adaptable nature means that targeting one group of CSCs might not be enough, as other cells can replace them.

Moreover, ethical and technical challenges remain in developing CSC-targeted therapies. There is a risk that treatments could also harm normal stem cells, which are essential for healthy tissue maintenance. In addition, tracking and targeting CSCs inside the human body requires advanced technology that is still being developed [88, 96]. The surrounding tumor environment and the interaction between CSCs and immune cells also complicate treatment efforts [174, 243]. Despite these challenges, targeting CSCs is still considered a promising strategy for long-term cancer control. Success will depend on improving our understanding of CSC biology, refining techniques for their identification, and developing combination therapies that can adapt to the complex and evolving nature of tumors, as depicted in Table 5.

Table 5 Main challenges in CSC identification and targeting

Challenge	Description	References
Marker Specificity	CSCs lack distinct markers, making it hard to differentiate them from non-CSCs and normal stem cells.	[88, 96]
Plasticity & Heterogeneity	CSCs can shift between stem-like and non-stem-like states, and show variation within and across tumors.	[174, 275, 276]
Therapy Resistance	CSCs survive conventional and targeted therapies through DNA repair, survival signaling, and immune evasion.	[174, 243, 277]
Clinical Translation	Promising preclinical strategies often fail in clinical trials due to tumor complexity and CSC dynamics.	[156, 174, 275]
Ethical/Technical Issues	The risk of harming normal stem cells and the need for advanced tools to track and target CSCs effectively.	[88, 96, 243]

Controversies and limitations of CSCs in the realm of cancer therapy

CSCs are increasingly recognized for their role in cancer progression, particularly in therapy resistance, tumor recurrence, and metastasis. Despite their significance, the application of CSCs in cancer therapy is fraught with controversy and practical limitations that complicate their clinical utility. One major controversy centers on the very existence and definition of CSCs. While some researchers argue that CSCs represent a distinct and stable subpopulation within tumors, others believe that stem-like properties may be a transient state that any cancer cell can acquire under specific environmental or therapeutic pressures [275]. This lack of consensus raises fundamental questions about how CSCs should be identified and targeted.

Another unresolved issue is the origin of CSCs. Some studies suggest that CSCs arise from normal tissue stem cells, while others propose that differentiated cancer cells can be reprogrammed into CSCs through processes like dedifferentiation and plasticity [88, 96]. This ambiguity in origin further complicates therapeutic strategies, as it implies that eliminating CSCs may not be sufficient if other tumor cells can regenerate them.

A significant limitation in CSC-targeted therapy is their heterogeneity and plasticity. CSCs are not a uniform population; they vary not only between different cancer types but also within individual tumors. This intra- and inter-tumor diversity hinders the development of universal CSC-targeting strategies [174]. Moreover, CSCs demonstrate remarkable plasticity, with the ability to switch between stem-like and non-stem-like states in response to environmental conditions or treatments. This dynamic behavior undermines therapies aimed at a fixed CSC phenotype and contributes to treatment failure and disease relapse [243].

Identification and targeting of CSCs are further challenged by the lack of specific markers that can clearly distinguish them from normal stem cells or other tumor cells. Many markers proposed for CSC identification are also found in non-stem cancer cells or even healthy tissue stem cells, increasing the risk of off-target effects and

reducing the precision of therapeutic interventions [96]. Additionally, CSCs are highly resistant to conventional therapies due to their efficient DNA repair mechanisms, high expression of drug efflux pumps, and resistance to apoptosis. These survival strategies allow them to endure treatments that eliminate most other cancer cells, contributing to tumor regrowth and metastasis [174, 277].

On the clinical side, many CSC-targeted therapies that have shown promise in laboratory settings have failed to achieve similar success in clinical trials. These translational gaps stem from the complexity of CSC biology and the supportive role of the tumor microenvironment, which shields CSCs from immune attacks and enhances their resistance to therapies [156, 243]. The tumor microenvironment, including factors like hypoxia and immune suppression, plays a critical role in maintaining CSC traits and further complicates therapeutic efforts. In summary, although CSCs present an attractive target for more durable cancer therapies, ongoing debates about their definition and origin, along with their heterogeneity, plasticity, and resistance mechanisms, pose substantial challenges. To overcome these hurdles, future research must focus on refining CSC identification, understanding their dynamic behavior, and developing combination therapies that target both CSCs and their supportive microenvironments.

The future of medicine: immunotherapy, nanotechnology, and personalized treatment

Overcoming challenges in cancer immunotherapy: Cancer immunotherapy has caused a revolution in oncology by introducing new treatment methods. Yet, its widespread use faces big hurdles. One main obstacle to its success is that tumors can become resistant, which can reduce how well it works over time. Tumors use different ways to do this, like changing their surface markers to hide from the immune system and using tactics to shut down the immune response. These changes make it harder for immune cells to find and kill cancer cells [278, 279]. To address this, scientists are working to find biomarkers that predict how well a patient will respond. This helps doctors choose the right patients for immunotherapy and tailor treatments to each person. These efforts aim to make immunotherapy work better and longer for more types of patients [278], as depicted in Figs. 12 and 13, and 14.

Another major concern is the occurrence of immune-related adverse events (irAEs), which often arise with therapies like immune checkpoint inhibitors. These adverse effects can range widely, from mild symptoms to severe complications that significantly impact a patient's quality of life [278]. While the ability of immunotherapy to activate the immune system is its key advantage, it can also unintentionally provoke immune responses against healthy tissues. Effectively managing irAEs is a crucial priority for clinicians, requiring a careful balance between alleviating these side effects and maintaining the treatment's therapeutic efficacy. Strategies such as using immunosuppressive agents are often employed to handle severe adverse reactions; however, these interventions can sometimes undermine therapeutic effectiveness. Ongoing research into the mechanisms behind irAEs is vital for developing safer and more refined immunotherapeutic protocols [280]. The variability and complexity of individual tumors also present a significant challenge to standardizing immunotherapy. Tumors display diverse genetic and molecular profiles, making it difficult to design universal treatment approaches. Additionally, many tumors create immunosuppressive microenvironments that can hinder the effects of immune therapies, reducing their efficacy [279, 280], as displayed in Figs. 12 and 13, and 14. Addressing tumor heterogeneity requires a deep understanding of the unique characteristics of each tumor and its interaction with the immune system. Recent advancements in technologies, such as single-cell analysis and comprehensive mapping of tumor microenvironments, are enabling researchers to create more personalized and effective treatment strategies. These new ideas show promise to overcome the obstacles linked to tumor diversity, which could widen the use of immunotherapy. To sum up, even with its game-changing potential, cancer immunotherapy faces big challenges that we need to beat to unlock all it can do. Treatment that stops working, unwanted side effects, and the complex nature of tumors are major roadblocks that call for ongoing study and fresh thinking. By tackling these limits, the field of immunotherapy can keep moving forward, leading to safer and better treatments for more patients. This work is key to helping immunotherapy grow into a tool that works for everyone in the fight against cancer.

Nanotechnology-based approaches for enhancing the delivery and efficacy of cancer therapies

Nanotechnology has significantly changed cancer therapy by introducing new methods for drug delivery, which greatly enhance treatment effectiveness. By using various nanocarriers like liposomes, polymeric nanoparticles, and gold nanoparticles, researchers can overcome biological barriers that usually impede the effective distribution of chemotherapy drugs. This progress has improved the pharmacokinetics of these medications, making them more effective at targeting cancer cells [281, 282]. Importantly, these nano-carriers enable the delivery of water-insoluble drugs, allow for the simultaneous use of multiple drugs in combination therapies, and can even be combined with imaging technologies to enhance diagnostic accuracy [283] (Fig. 13).

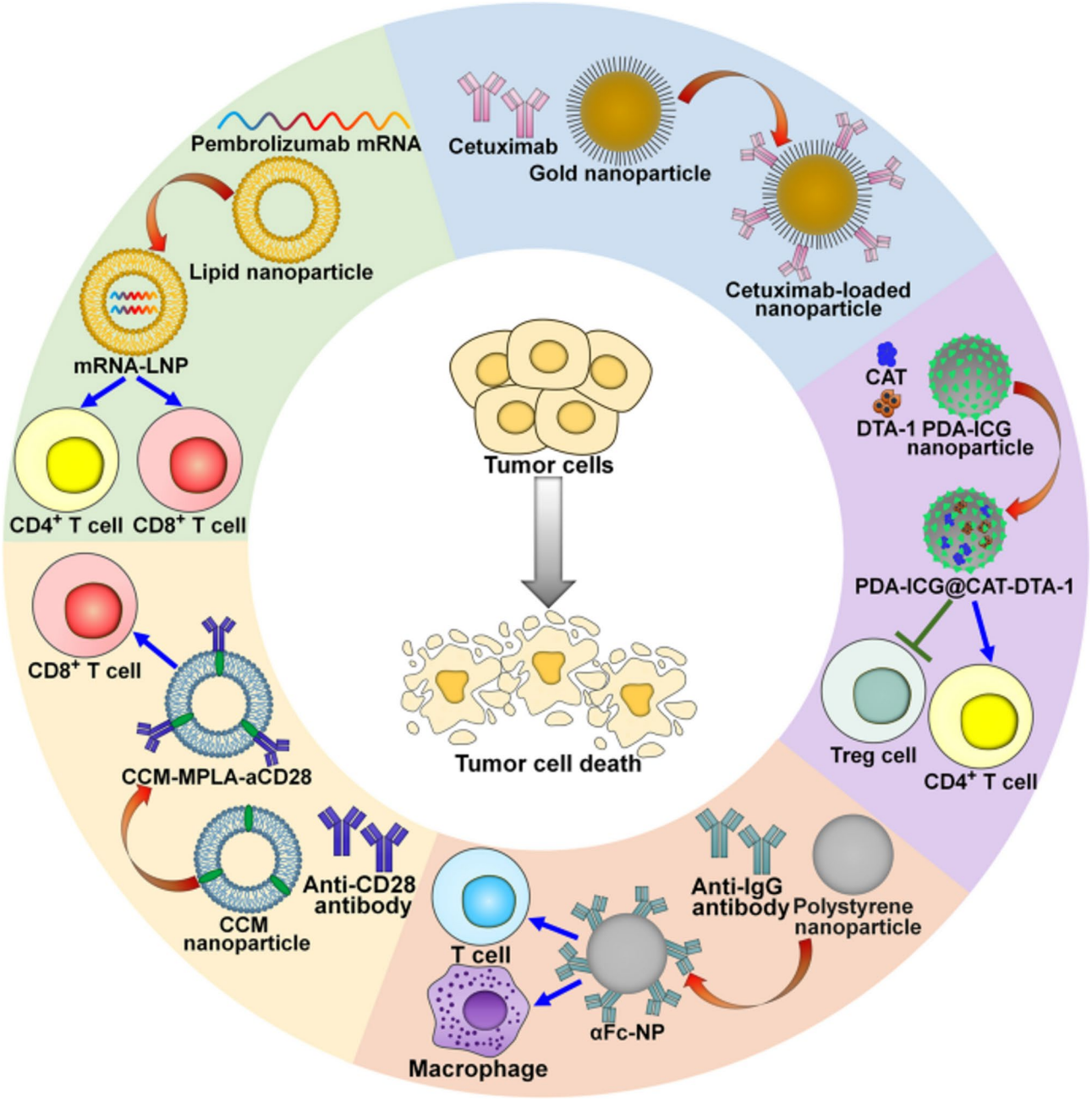

Fig. 12 Nanotechnology for therapeutic antibody delivery in cancer treatment. Tumor-targeting antibodies have been delivered via nanoplatforms with specialized functions. Cetuximab, for example, can enter tumor cells via radiosensitive gold nanoparticles. PDA-ICG nanoparticles are loaded with CAT and DTA-1 to create a flexible nanoplatform (PDA-ICG@CAT-DTA-1). By boosting the number of CD4 + effector T cells and reducing the number of Treg cells, PDA-ICG@CAT-DTA-1 reverses tumor immunosuppression. The anti-IgG antibody-conjugated nanoparticle (αFc-NP) effectively stimulates T cell proliferation and activates macrophages. CD8 + T cells can be activated by CCM nanoparticles linked with anti-CD28 antibodies (CCM-MPLA-aCD28). Furthermore, pembrolizumab mRNA-encapsulated lipid nanoparticles can stimulate CD4 + and CD8 + T cells. MPLA stands for monophosphoryl lipid A; LNP for lipid nanoparticles; Treg cell for regulatory T cells; CCM for cancer cell membranes; CAT for catalase; PDA for polydopamine; and ICG for indocyanine green [285]

Furthermore, nanotechnology takes advantage of the unique features of the tumor microenvironment, such as increased permeability and retention effects, the lower extracellular pH often found in tumors, and the presence of tumor-specific antigens. These characteristics facilitate the creation of targeted drug delivery systems aimed at improving the localization and effectiveness of treatments [282]. This targeted strategy addresses significant challenges in oncology, including the widespread problem of multidrug resistance and the need for early disease

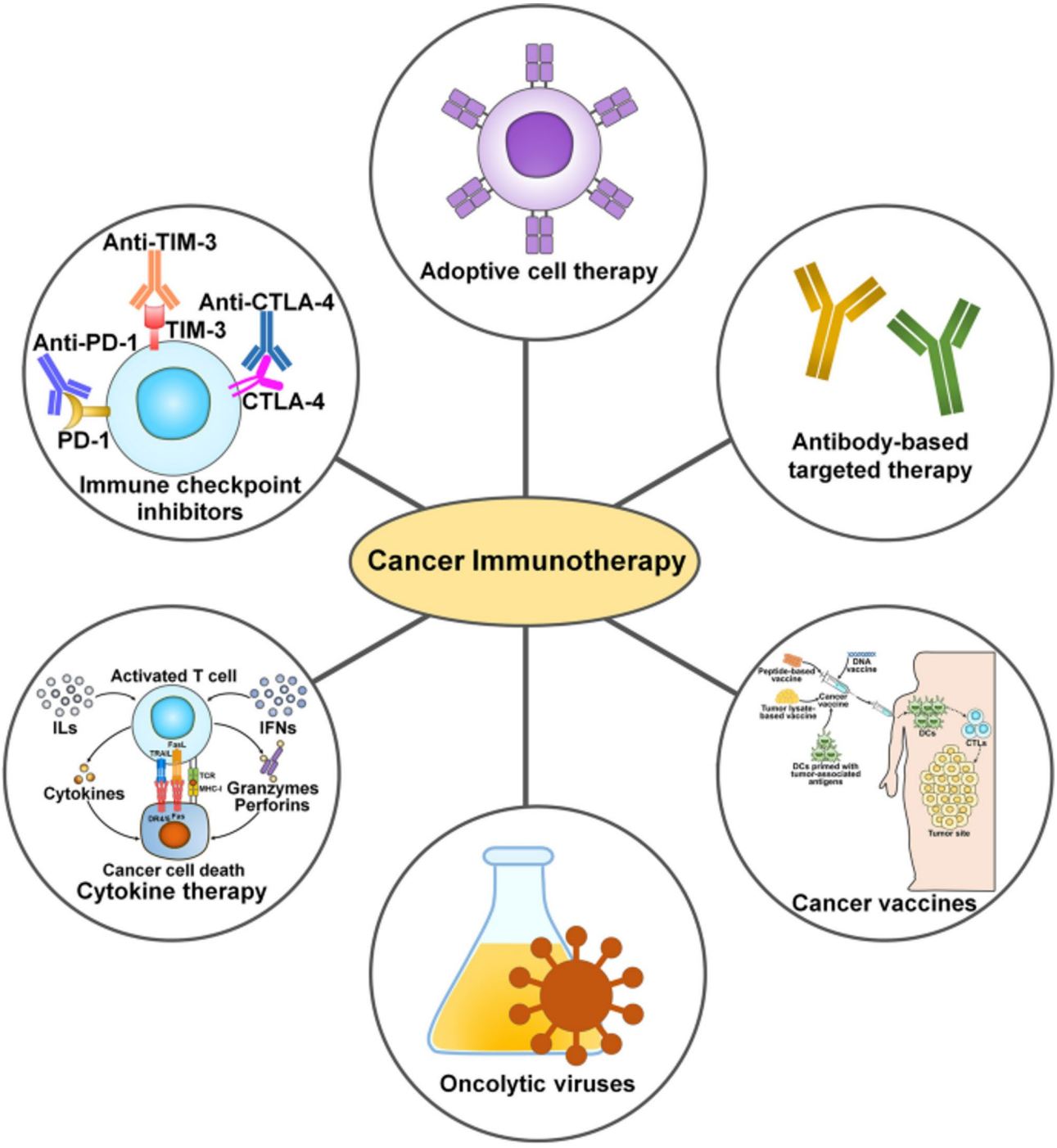

Fig. 13 Types of cancer immunotherapy treatments. Immunocheckpoint blockage, adoptive cell treatment, antibody-based targeted therapy, cancer vaccines, oncolytic viruses, and cytokine therapy are the primary immunotherapeutic approaches now employed in clinical cancer care. CTLA-4, or cytotoxic T lymphocyte-associated antigen; TIM-3, or T cell immunoglobulin and mucin domain-containing protein; and PD-1, or programmed cell death-1. Interleukins (ILs); interferons (IFNs); DR4/5, death receptor 4/5; FasL, Fas ligand; TCR, T cell receptor; MHC-I, major histocompatibility complex class I; DC, dendritic cell; and CTL, cytotoxic T lymphocyte; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand [285]

detection. By optimizing drug delivery, this approach has the potential to greatly enhance treatment outcomes, providing patients with more effective therapeutic options [284]. Additionally, incorporating nanotechnology in combination drug delivery offers the possibility of synergistic anticancer effects while reducing the toxicity commonly associated with individual drugs. This method not only improves the therapeutic index of the treatment but can also enhance patient compliance due to fewer side effects. This approach not only enhances the

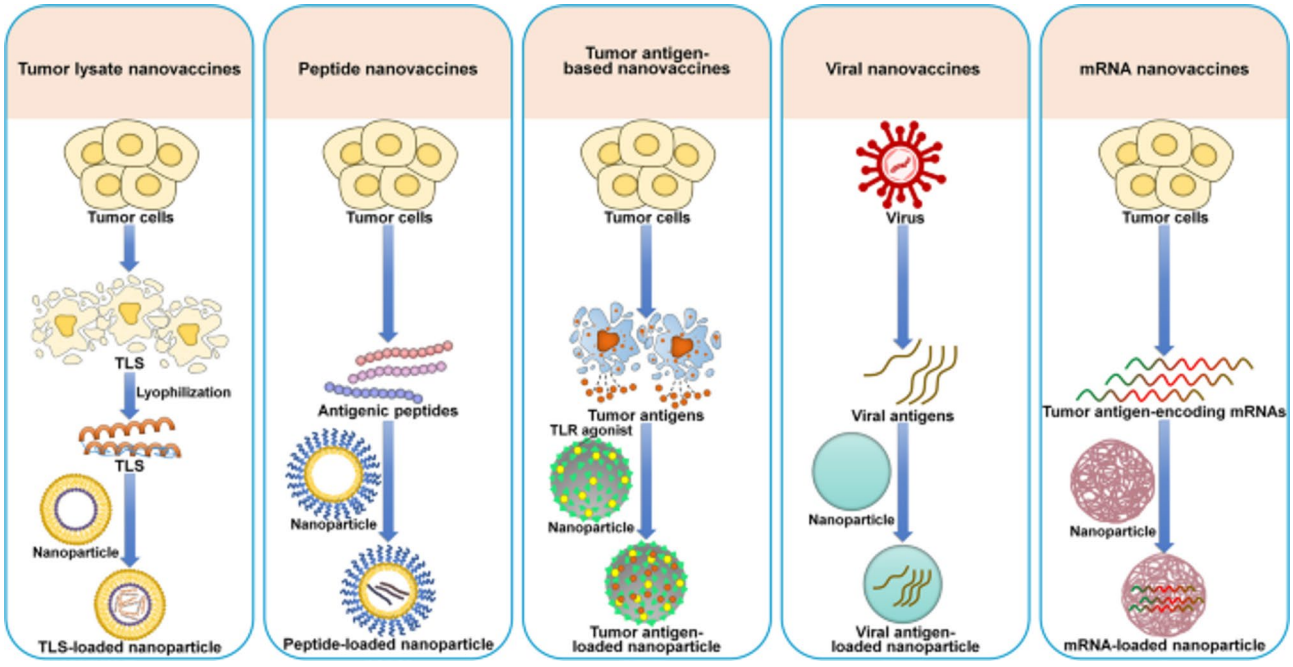

Fig. 14 A Plethora of cancer vaccines based on nanotechnology. Tumor lysate nanovaccines, peptide nanovaccines, tumor antigen-based nanovaccines, viral nanovaccines, and mRNA nanovaccines are major categories of nanotechnology-based cancer vaccinations. Tumor lysate (TLS); Toll-like receptor (TLR) [285]

therapeutic index of the treatment but can also lead to improved patient compliance due to reduced side effects [281]. As research in this field continues to advance, the promise of nanotechnology in enhancing both cancer diagnostics and therapeutics remains substantial, leading to innovative strategies that could redefine the standards of cancer care [284], as displayed in Figs. 12 and 13, and 14.

Advancing personalized medicine in cancer treatment: Personalized medicine is revolutionizing cancer treatment by focusing on tailoring therapies to the individual traits of patients, particularly their distinct genetic, proteomic, and metabolic profiles [286, 287]. This approach aligns treatment plans with the specific characteristics of each tumor, which can greatly improve treatment effectiveness and patient outcomes. By enabling the careful selection of medications and dosages suited to each person, personalized medicine offers a more focused and efficient treatment strategy that reduces unnecessary side effects while enhancing therapeutic success [191]. In this context, nanotechnology is crucial in advancing personalized medicine, especially in cancer care. It allows for real-time monitoring of biomarkers, providing essential insights into tumor behavior and responses to treatment. Moreover, nanotechnology improves drug delivery systems, making therapies more effective and less invasive compared to traditional methods (190). Despite the significant potential of personalized medicine, several important challenges need to be overcome for its broader adoption. These challenges include the development of cost-effective techniques to analyze large datasets produced by various omics technologies, the integration of these complex analyses into everyday clinical practice, and addressing socioeconomic disparities that could restrict access to personalized treatments [189, 192]. The successful adoption of personalized medicine in oncology needs a multidisciplinary approach that encourages teamwork among healthcare professionals from different specialties. This teamwork is key to using advanced technologies, like clinical decision support systems, which can improve decision-making and boost the quality of care [192]. Also, to tackle barriers in education, access, regulations, and insurance policies is vital to bring personalized medicine into standard cancer care plans [191, 192]. By clearing these hurdles, personalized medicine can make a big difference in how we treat cancer, making it more effective and fair for all patients, and changing the future of cancer care.

Clinical trials, research needs, and future perspectives

CSCs are crucial in the initiation, progression, and recurrence of tumors, making them a key target for new cancer therapies. Current clinical trials are investigating various strategies to target CSCs, either on their own or in combination with established treatments, across different types of cancer [288, 289]. These approaches aim to disrupt CSCs by focusing on their unique surface markers, the signaling pathways that regulate their

behavior, the mechanisms they use to expel drugs, like drug efflux pumps, and the signals they receive from their surrounding environment [290]. Combining CSC-targeting agents with traditional cancer drugs has shown significant promise in overcoming treatment resistance and preventing tumor relapse, which are major challenges in effective cancer management [290, 291]. However, a significant hurdle in this area is accurately identifying and isolating CSCs, which requires the development of new technologies and methods that can reliably differentiate these cells from their non-stem counterparts(289). Looking ahead, a particularly promising avenue involves using tests that can specifically identify CSCs in individual patients, guiding treatment decisions tailored to the unique characteristics of each patient's tumor. This personalized approach could greatly enhance the effectiveness of cancer therapies by ensuring that treatments are optimized for the specific biological context of the tumor, ultimately leading to better outcomes for patients [289], as illustrated in Tables 6 and 7. By considering CSCs and integrating personalized medicine strategies, the future of cancer treatment may become more targeted and effective, addressing one of the most pressing challenges in oncology today.

In today's digital era, stem cell therapy has become a promising avenue in cancer treatment, leveraging the ability to deliver cytotoxic agents directly to tumors while also showing potential in regenerative medicine [372]. This cutting-edge approach seeks to harness the unique characteristics of stem cells to enhance the precision and effectiveness of cancer therapies, addressing some limitations of traditional treatments. Recent studies have increasingly spotlighted CSCs(CSCs) as crucial therapeutic targets due to their significant role in tumor progression and resistance to standard therapies [38]. These findings indicate that CSCs could play a vital role not only in the early detection of cancer but also as renewable sources for developing new treatment strategies. CSCs have been found in various cancers, including breast, pancreatic, and lung cancer, where they significantly contribute to treatment resistance and disease recurrence [38]. Their capacity for self-renewal and sustaining tumor growth poses major challenges.

As a result, researchers are exploring methods to disrupt embryonic signaling pathways, such as the Wnt, Hedgehog, and Notch pathways, which are vital for maintaining CSCs. Targeting these pathways shows promise in improving treatment effectiveness by potentially undermining the survival and proliferation of CSCs, thereby reducing the risk of relapse and enhancing patient outcomes [373]. However, despite these advancements, the use of stem cell therapy in cancer treatment encounters significant challenges. Concerns such as immune rejection of transplanted stem cells, the risk of disease

Table 6 Therapeutic strategies targeting CSCs with repurposed drugs

Agent/Strategy	Mechanism	Cancer Type	Status/Notes	References
Chloroquine	Inhibits autophagy; downregulates DNMT1 and Jak2/STAT3 pathway	Glioblastoma, breast	Repurposed anti-malarial; modulates epigenetic regulation and CSC plasticity.	[293–297]
Doxycycline	Blocks mitochondrial biogenesis, EMT, and metabolic reprogramming in CSCs	Breast, prostate, and melanoma	Suppresses CSC traits through metabolic targeting	[292, 297
Artemisinin derivatives	Induce ferroptosis and downregulate stemness markers	Glioma, pancreatic	Potent anti-CSC effects; induces oxidative stress	[302–306]
Ferumoxytol	Iron oxide nanoparticle inducing magnetic hyperthermia and ferroptosis	Breast, colorectal	Disrupts CSC viability via iron-mediated oxidative damage	[223, 307–309]
Notch inhibitors (e.g., RO4929097)	γ-secretase inhibition impairs CSC self-renewal	Pancreas, breast, renal	Clinical interest in Notch pathway suppression in CSCs	[243, 310–312]
Salinomycin	Induces apoptosis and inhibits Wnt/β-catenin, ALDH, and ABC transporters	Breast, glioblastoma, prostate	Selectively kills CSCs; tested in various preclinical and early clinical models.	[312–315]
Metformin	Inhibits mTOR, suppresses EMT and CSC markers	Breast, ovarian, and pancreatic	Observational and preclinical evidence support CSC suppression	[317–320]
Disulfiram (with copper)	Inhibits ALDH and proteasome activity; induces ROS	Glioblastoma, breast, lung	Synergistic CSC inhibition with copper: promising preclinical and early clinical findings	[321–323]
BGB324 (Bemcentinib)	AXL kinase inhibitor blocks EMT and immune evasion	AML, NSCLC, breast	Phase II/III trials are ongoing; targets CSC-like EMT and invasion	[311, 324–327]
Napabucasin (BBI608)	STAT3 inhibitor reduces CSC self-renewal and survival	Colorectal, pancreatic, and gastric	Mixed Phase III outcomes; active in specific molecular subgroups	[319, 327, 328]
Curcumin analogs (e.g., EF24)	NF-κB, STAT3, and Wnt/β-catenin inhibition	Breast, colon, glioma	Enhanced bioavailability; demonstrated CSC inhibition in vitro and in vivo	[329–333]
ONC201	DRD2 antagonist and ClpP agonist inducing mitochondrial stress	Glioblastoma, endometrial, prostate	Early clinical trials show selective CSC killing	[335–337]
CBL0137 (Curaxin)	FACT complex inhibition reactivates p53, suppresses NF-κB, and stemness genes	Neuroblastoma, colorectal, pancreatic	Preclinical CSC suppression; clinical evaluation in progress	[337–340]

Table 7 Ongoing clinical trials targeting cancer stem cells

Trial Name/ID	Target/Mechanism	Cancer Type	Phase	Status/Notes	References
CanStem303C (NCT02753127)	Napabucasin (STAT3 inhibitor) + FOLFIRI	Metastatic colorectal cancer	Phase III	Inhibits the STAT3 pathway to suppress CSC self-renewal and survival	[328, 341–343]
BRIGHTER Trial (NCT02178956)	Napabucasin + Paclitaxel	Gastric and gastro-esophageal junction	Phase III	Combines CSC-targeting agent with chemotherapy; subgroup analysis ongoing	[344–347]
NCT03724851	Vactosertib (TGF-β receptor I inhibitor) + Pembrolizumab	MSS metastatic colorectal cancer	Phase 1b/2a	Aims to enhance immunotherapy by modulating CSC-supportive TGF-β signaling	[340, 348–350]
NCT02776917	Cirmtuzumab (anti-ROR1 antibody) + Paclitaxel	Metastatic breast cancer	Phase I	Targets CSC surface marker ROR1; monitors ALDH1/CD133 expression	[340, 351–353]
NCT02915445	EpCAM-targeted CAR-T cells	Breast & nasopharyngeal carcinoma	Phase I	Immunotherapy targeting CSC-specific EpCAM; early-stage safety trial	[350, 351, 353]
NCT01849744	FAK inhibitor (VS-4718)	Metastatic solid tumors	Phase I	Inhibits focal adhesion kinase (FAK), a key player in the CSC niche and survival	[355–357]
NCT01991938	PI3K/mTOR dual inhibitor (VS-5584)	Advanced solid tumors	Phase I	Targets CSC-enriched subpopulations by inhibiting survival and proliferation	[358–362]
NCT02688712	Galunisertib (TGF-β inhibitor) + chemoradiotherapy	Locally advanced rectal cancer	Phase II	Reduces CSC-mediated radioresistance and recurrence potential	[347, 362, 363]
NCT02859415	Mithramycin (SP1 transcriptional inhibitor)	Thoracic cancers (mesothelioma, lung)	Phase I/II	Disrupts CSC-related gene expression and induces apoptosis	[362, 365–368]
Cedars-Sinai Vaccine Trial	Personalized dendritic cell vaccine targeting CD133 + glioma CSCs	Glioblastoma multiforme	Phase I	Vaccine aimed at CSC surface marker CD133; individualized patient-specific therapy	[369–372]

recurrence, and the unintended generation of CSCs during transplantation present notable obstacles [38]. These complications point out the pressing need to conduct thorough studies to develop safer and more effective treatment protocols. Yet, in the realm of skin cancer, therapies based on stem cells have demonstrated particular potential, offering ground-breaking approaches to manage the disease more effectively [66]. To wrap up, despite the various hurdles in stem cell therapy, ongoing progress in this area continues to give hope for better cancer treatments. By tackling the constraints related to immune responses, cancer recurrence, and the biology of CSCs, scientists are paving the way for treatments that could improve patient outcomes and extend human life [38]. With continued breakthroughs and teamwork across fields, stem cell therapy can change cancer care.

Conclusion and home take messages

CSCs constitute an essential frontier within the field of oncology, given their pivotal functions in tumor initiation, progression, therapeutic resistance, and recurrence. In contrast to the predominant tumor cell population, CSCs display distinctive characteristics of self-renewal, differentiation, and quiescence, which not only contribute to tumor heterogeneity but also facilitate evasion from conventional therapeutic modalities such as chemotherapy and radiotherapy. The TME is instrumental in sustaining CSC maintenance and plasticity, thereby influencing immune evasion, therapeutic resistance, and metastatic potential. Despite the elucidation of critical regulatory pathways, including Wnt/β-catenin, Notch, Hedgehog, and TGF-β, therapeutic interventions continue to be hindered by the inherent heterogeneity of CSCs, their dynamic plasticity, and their overlap with normal stem cell pathways.

The complex nature of CSCs necessitates a transformative shift towards integrated and precise therapeutic methodologies. Innovative technologies such as nanomedicine, CAR-T cell therapy, exosome-based delivery systems, and CRISPR/Cas9 gene editing are increasingly being explored to improve the targeting of CSCs while mitigating collateral damage to healthy tissues. Nevertheless, substantial challenges persist, particularly in the translation of preclinical insights into safe and effective clinical applications, due to issues such as immune rejection, tumor evolution, and the intricate nature of CSC niches. Future investigations should emphasize the identification of robust, CSC-specific biomarkers to enhance therapeutic selectivity; the exploration of combination therapies aimed at concurrently targeting CSCs and their microenvironment; the elucidation of mechanisms by which CSCs modulate normal stem cells; and the application of computational biology and machine learning for the personalization of treatment strategies. Furthermore, the establishment of standardized methodologies for the isolation and characterization of CSCs will be crucial for ensuring consistent clinical applicability.

In conclusion, the trajectory toward progress necessitates multidisciplinary collaboration that integrates molecular oncology, regenerative medicine, immunology, and computational sciences. By advancing our comprehension of CSC biology and its interactions with the TME, we can inch closer to realizing sustainable cancer remissions and personalized, curative treatment options.

Abbreviations

ESCs	Embryonic stem cells
iPSCs	Induced pluripotent stem cells
HSCs	Hematopoietic stem cells
MSCs	Mesenchymal stem cells
TICs	Tumor-initiating cells
HDACis	Histone deacetylase inhibitors
SdFFF	Sedimentation field-flow fractionation
UHF-DEP	ultrahigh-frequency-dielectrophoresis
ALDH	Aldehyde dehydrogenase
lncRNAs	long noncoding RNAs
HOTAIR	HOX transcript antisense RNA
MALAT1	Metastasis-Associated Lung Adenocarcinoma Transcript 1
BCSCs	Breast CSCs
Wnt	Wingless-related integration site
NFκB	Nuclear factor kappa B
Notch 1	Neurogenic locus notch homolog protein 1
Hh	Hedgehog signaling
STAT3	Signal transducer and activator of transcription 3
BMP-2	Bone morphogenetic protein 2
CD	Cluster of differentiation
CAFs	Cancer-associated fibroblasts
CSCs	CSCs
NSCs	Normal stem cells
NPs	Nanoparticles
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
COX2	Cyclooxygenase 2
TME	Tumor Microenvironment
HIFs	Hypoxia-inducible factors
PD	L1-programmed cell death: ligand 1
SMAD3	Suppressor of mothers against decapentaplegic homolog 3
JAK–STAT	The Janus kinase and signal transducer of activation pathway
TGF-β	Transforming growth factor-beta and Hepatocyte growth factor
TAMs	Tumor-associated macrophages
EMT	Epithelial–mesenchymal transition
P-gp	P-glycoprotein

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Declarations

Ethics declarations
The available literature is succinctly reviewed in this work. There was no need for ethical approval or participant informed consent because this review article is based on published data.

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The authors declare no competing interests.

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