Cancer Stem Cells: the elusive tumour population discussed

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Abstract. Cancer Stem Cells (CSCs) are specific tumour populations playing important roles in tumour initiation, progression, and resistance to conventional therapies. Their involvement in processes like epithelial-to-mesenchymal transition makes them crucial players in metastasis. Traditional therapies often fall short in targeting CSCs due to their quiescent nature during treatments and their adaptability, such as increased resistance post-radiotherapy. CSC antigen heterogeneity also poses challenges to targeted therapy. Examples of CSC-targeting approaches are CAR-T cells and oncolytic viruses, discussed in this paper. Stem cells, present promising avenues for CSC-targeted therapies due to their potential as immune progenitor cells and inherent attraction to CSCs. This review provides an overview of the roles of CSCs in cancer, some examples CSC-targeted cell therapies, the potential of stem cells in these therapies, and the challenges and future directions in this critical area of cancer research.

Keywords: Cancer; Stem cells; Cancer Stem Cells; tumour resistance; targeted therapies.

1. Introduction

In the early 19th century, scientists observed the similarities between tumour cells and embryonic stem cells in their morphology and mechanism. Julius Coheheim (1839-1884) suggested that tumours source from 'embryonic rests' [1], alluding to stem cells in modern perception. In 1997, Bonnet and Dick identified cancer stem cells (CSCs) in Acute Myeloid Leukemia (AML) for the first time. As a subpopulation of tumour cells, with similarities to normal stem cells, they can self-renew and differentiate into other cell types [2]. It is now understood that CSCs contribute greatly to tumour initiation, development, and post-therapy relapse CSCs also undergo epithelial-to-mesenchymal transition (EMT), which plays an important role in metastasis [3]. Therefore, targeting CSCs is a promising strategy for treating cancer. However, CSCs are also one of the reasons most traditional cancer therapies, such as radiotherapy and chemotherapies, fail to eliminate some malignant tumours [2]. CSCs enter a temporary quiescent phase and become dormant during chemotherapy. This effectively makes them undetectable and invulnerable to chemotherapy drugs, which target the more rapidly proliferating cells. In contrast, radiotherapy induces further invasiveness and resistance of CSCs by inducing EMT and inhibiting p53-mediated apoptosis [3]. Despite the urgent need for CSC-targeted therapies, targeting of CSCs is difficult because most markers present in CSCs are also processed by adult stem cells (ASCs) or tissue cells. The crosstalk of CSCs and their tumour microenvironment (TME) also decreases the efficacy of conventional therapies and immunotherapies [4, 5].

As research in CSC biomarkers and signaling pathways is progressing, new approaches to targeting CSCs emerge. Examples of these are new drugs, antibodies, vaccines, and Chimeric antigen receptor (CAR) T cells, as well as virotherapy involving oncolytic viruses [2, 6]. However, challenges to these therapies include the delivery to CSCs, non-specific uptake by normal cells causing off-tumour toxicity, and in the case of many cell-based immunotherapies such as CAR-T, the production of immune cells. To these problems, the use of stem cells, including adult mesenchymal stem cells (MSC) and induced pluripotent stem cells (iPSC) offer possible solutions [6]. Since stem cells are attracted to CSCs, they can be potentially very useful in developing CSC-targeted therapies [6, 7].

This review explores CSC's roles in cancer initiation and progression and the related opportunities and challenges to target CSCs, including an overview of the status of CSC-targeted cell therapies. In addition, this paper will discuss about the potential of stem cells in CSC elimination strategies.

2. CSC model and its development

The clonal nature of tumours suggests that all cancers arise from single cells, as first demonstrated in human lymphomas. The enhanced understanding of tumour heterogeneity led to questions about the clonal origin theory, which led to the development of the CSC model in the early 90s following CSCs' identification in AML [8].

The original CSC model outlines that only a small population of tumour cells are responsible for tumour formation, while others are differentiated, non-proliferating tumour cells [9]. Therefore, the role of CSCs in tumour development is reminiscent of the normal stem cells in organ development, and the resulting hierarchy in cells is also similar to those in normal tissue [8, 10]. In many tumours, a clear hierarchal organization has been observed where CSCs are identified for their high self-renewal capability and long-term clonal repopulation in contrast to differentiated tumour cells. However, this cannot be generalised to all tumours, as some are more homogenous with most of their cell processing CSC-like characteristics [9, 11]. The most widely accepted definitive characteristics of CSCs include the ability to self-renew and form an identical tumour when xenografted, which the rest of the tumour mass cannot [4].

Recent studies of both solid and haematopoietic tumours have shown that the unidirectional hierarchy outlined in the original model is an oversimplification. To account for this, a clonal evolution model, in which genetically unstable cells are thought to accumulate mutation over time contributing to tumour malignancy and heterogeneity, has been developed alongside the CSC model. These two models are not mutually exclusive, as cellular plasticity means that tumour cells can convert into and out of the CSC state [9]. It is also common for tumours to have multiple CSC cell lines derived from the original CSC. Therefore, it is now thought that tumour subpopulations maintain a phenotypic equilibrium in stemness, which can be described quantitively using a Markov model [12, 13]. According to a study on breast cancer cells, the real picture is likely to be the two models incorporated into each other [14]. Therefore, a combination of both models overcome the weakness of the original CSC model, explaining most tumour's heterogeneity and better describe the complex organisation of CSCs in tumours (fig 1 and fig 2). Importantly, this highlights the difficulty of tumour elimination via both conventional therapies and CSC-targeted therapies, as will be discussed below.

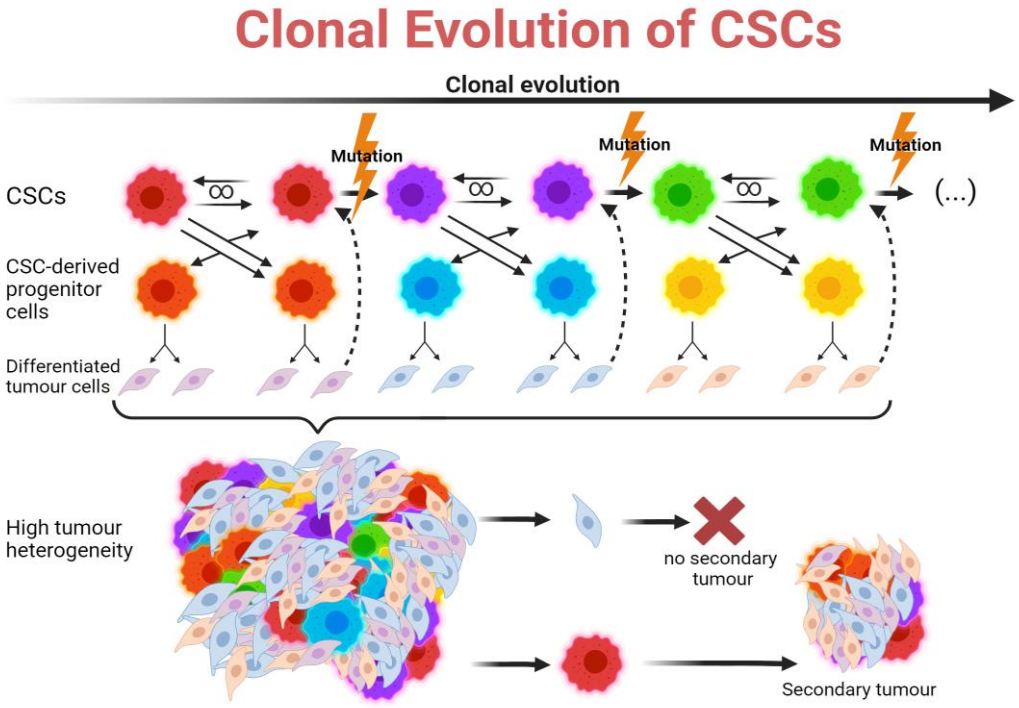

Fig 1. This diagram describes a hybrid model of CSCs and clonal evolution and their relation to tumour heterogeneity.

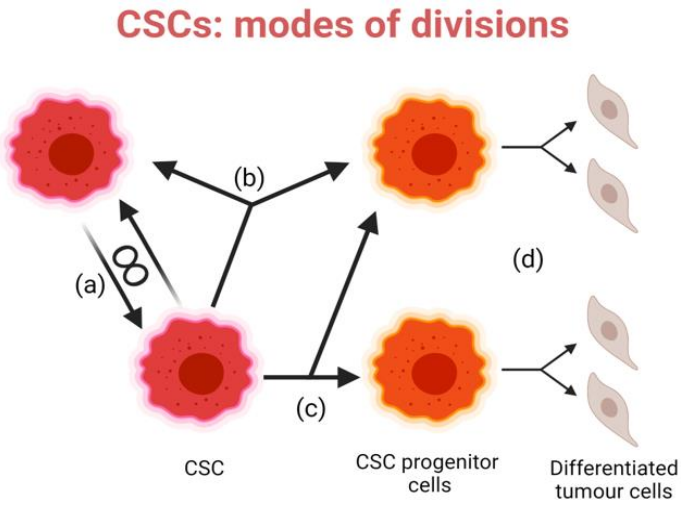

Fig 2. Modes of divisions.

3. Source & Generation of CSCs.

The transformation of adult stem cells and differentiated cells into CSCs can be induced by a wide range of external or modifiable factors [9]. In addition, stem cells are more likely to undergo this transformation as they already process replicative immortality. Although more mutations are required to accumulate, it is also possible for differentiated cells to undergo de-differentiation and acquire stemness through epigenetic plasticity during EMT. Both have been verified by clinical observations and lab experiments in vivo [9, 15]. During tumour development, CSCs divide symmetrically to give rise to new CSCs like normal stem cells. This is thought to be the main way CSCs maintain and increase their population in a tumour. The new CSCs can migrate to peripheral sites of the tumour leading to the formation of new growth foci and leave the tumour site completely to form metastases. However, cellular plasticity makes the transformation of differentiated tumour cells into CSCs possible [8, 9]. This has been demonstrated in-vivo by 3T3 cells, which are differentiated but process replicative immortality, after transplantation into immunodeficient animals [8]. As shown in fig 3.

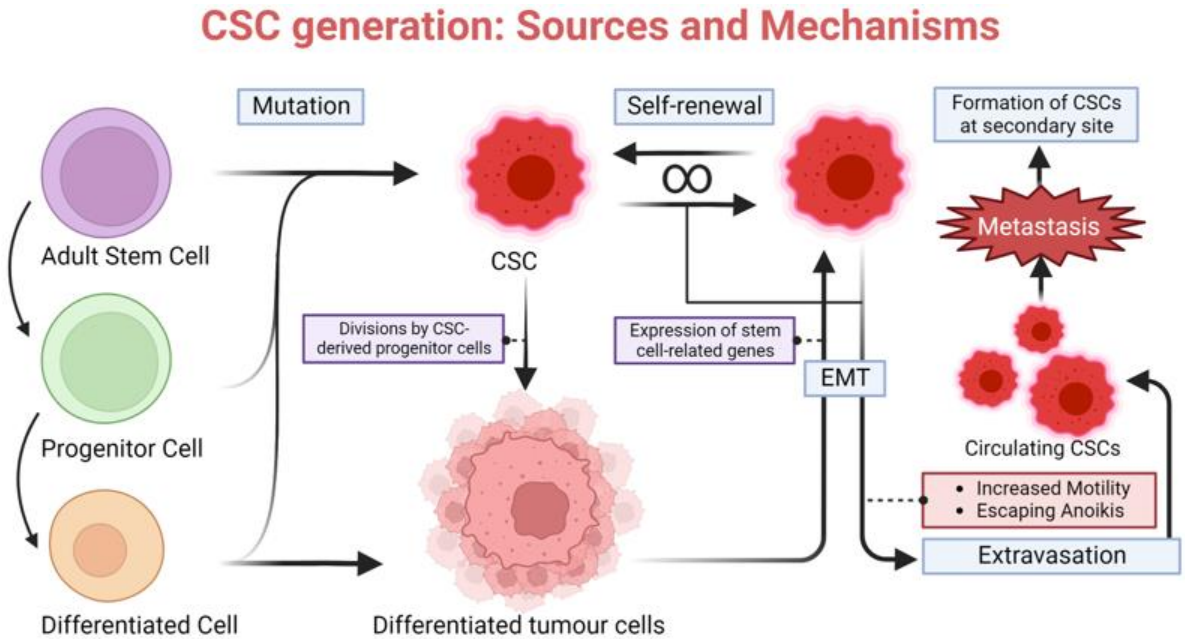

Fig 3. Possible sources of CSC include tissue resident adult stem cells, their progenitor cells and differentiated cells. CSC can be derived from differentiated tumour cells when they undergo EMT. Via EMT, CSCs are allowed to leave the tumour site and give rise to new lines of CSCs at the secondary tumour site.

4. CSCs in tumour progression

Observations in vivo and in vitro show that the content of CSCs in tumours is comparably rare, from 0.01-2%. They are diversely scattered throughout a tumour, possibly due to the migration of new CSCs to peripheral sites of a tumour to form multiple growth loci. Under regular conditions, each CSC sustains 100-1000 tumour cells [2, 8]. A team demonstrated that only a small population of CD44+CD24- breast cancer cells can generate new tumours when xenografted, while most of the other tumour masses cannot. Experiments as such show the importance of CSCs to tumour progression [3]. Notably, although defined by the ability to form a new tumour when xenografted into immunodeficient mice, CSCs are not strictly tumour-initiating cells, or cells in the patient to first acquire oncogenic mutations. As noted by the clonal evolution model, stemness can be acquired and lost as tumours evolve due to genetic and epigenetic instability. Therefore, CSCs populations present as moving targets, as those driving cell proliferation at one point might not be the ones involved in later stages of tumour development, for example, metastasis [4].

CSCs give rise to progenitor cells by asymmetrical division, which can rapidly proliferate several times before entering senescence. The progenitor cells are directly responsible for most of the tumour growth, as their proliferation rate is much faster than CSCs [15]. Interestingly, CSCs can also symmetrically divide into two identical progenitor cells. This leads to CSC depletion in tumours, so artificially promoting this type of division may be an alternative strategy to treat cancer [4].

Studies have shown that CSCs play an important role in metastasis, which can be significantly attributed to their involvement in EMT The EMT in CSCs is specifically “type-3 EMT” [2, 3]. By undergoing type-3 EMT, CSCs can metastasis by gaining invasiveness and entering the circulation. EMT enables circulating CSCs to avoid anoikis, the programmed cell death of anchorage-dependent cells in the bloodstream. Therefore, EMT plays a significant role in tumour invasion [3]. Only partial EMT may be required for CSCs to metastasis; as EMT is a highly flexible process, cells can be found between mesenchymal and epithelial types [3]. During EMT, differentiated tumour cells can become CSCs as they acquire stemness (fig 3). However, although it was thought that EMT share similar pathways with the emergence of stemness, recent studies suggest that they are parallel event. In extreme cases such as prostate and bladder cancer cells they can be mutually exclusive; full transformation into a mesenchymal state may result in loss of stemness [3, 16].

5. Opportunities and challenges of CSC targeting and elimination

Currently, CSCs can be identified through the expression of surface markers such as CD-133, CD44, CD44v6, CD-24, lgr5 and EpCAM [2, 17]. Alternatively, enzyme activities such as aldehyde dehydrogenase (ALDH) and expressions of surface proteins such as (ABC) can be used to identify CSCs [17]. However, many of these markers are not unique to CSCs, such as CD-24, CD-44, CD-133, and lgr5, are diversely expressed on a wide range of normal stem and non-stem cells [2, 4]. As a result, these surface markers are currently only effective in identifying CSCs in very heterogenous tissues [2]. Additionally, the similarities between CSCs and non-cancerous stem cells pose difficulties to therapeutic targeting as conventional targeting methods risk toxicity to normal stem cells [18]. As single biomarkers usually fail to distinguish CSCs from non-cancerous stem cells, multi-biomarker procedures have been developed to separate CSCs for improved accuracy and specificity [2].

CSCs can be identified by their ability to cause cancers in xenografted models. Common techniques used to separate CSC from tumour populations based on their biomarkers include fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS), and both are shown to be effective to isolate CSCs [2].

6. CSCs as challenges to conventional therapies & potential mitigations

6.1. Chemotherapy

Conventional chemotherapy drugs primarily target cells undergoing rapid proliferation, which is thought to be a common feature of cancer. However, unlike other tumour cell populations, CSCs tend to enter quiescence during chemotherapy, which commonly targets processes in dividing cells. Therefore, they can regenerate after the therapy, leading to tumour relapse and repopulation of tumour cells between treatment cycles [6, 19]. This mechanism of entering and exiting dormancy is related to many genes, and experiments show that transforming growth factor TGF-β plays a vital role in this process in breast cancer; its isoform TGF-β2 play a similar role in head and neck cancer [6]. Tumour relapse years after initial treatment shows that CSCs can reactivate after surviving long-term dormancy [19]. Two opposite methods were conceptualised to address this problem given initial tumour remission is achieved. First, therapeutics could be used to force CSCs re-enter the cell cycle. This makes it possible to eliminate remaining CSCs with currently available therapies, but risks generating novel mutations. Second, CSCs could be forced to maintain quiescence. This could be initially less risky, but the survival of quiescent CSCs means continued treatment for the rest of the patient's life, during which resistant clones could arise by mutation. Both approaches could affect somatic stem cells that share similar pathways for cell cycle regulation. Weighing their potential advantages and disadvantages, the decision should be made based on the patient's age and preferences [20].

In addition, CSCs also resist the DNA-damaging effect of chemotherapeutic drugs delivered through ROS, by a cellular system of anti-oxidative defence. The levels of ROS in CSCs are generally lower than in most cancer cells, which process high levels of ROS due to their active metabolism and abnormal proliferative signaling pathways [21]. ROS resistance in CSCs is achieved through the upregulation of free radical scavenging systems. The upregulation of ALDH in CSCs removes oxidative stress and removes free radicals by oxidizing intracellular aldehyde, which reduces CSC's vulnerability to chemotherapeutics such as oxazolidine, taxanes and platinum drugs [2, 19]. Because of this, ALDH is also regarded as a viable biomarker to identify CSCs in tumours and a viable therapeutic target, despite their role in cellular detoxification and stem cell regulation under normal circumstances [19]. Aside from this, ALDH1A1, a common isoform of ALDH, activates DNA-repair mechanisms in CSCs. Resistance to ROS-induced DNA damage, combined with the high activity of the DNA-repair mechanism, CSCs can overcome G1/S arrest or escape apoptosis which chemotherapy aims to achieve [19,21].

Another mechanism of CSC resistance to chemotherapy is drug export mediated by ABC transporters commonly expressed on the surface of CSCs [2, 17]. Some believe that it is the most powerful way CSCs resist chemotherapy, as ABC transporters effectively translocate chemotherapy drugs out of the cell into the extracellular space, leading to multi-drug resistance [19]. Inhibitors of ABC transporters can increase the efficacy of chemotherapeutic agents against CSCs, though their clinical application is challenged by potential side effects since the same ABC transporters are present in normal cells as well [17]. Since ABC transporters facilitates Hoechst 33342 dye efflux, CSCs will show as dye-negative side population (SP) when a tumour population is stained with Hoechst 33342. This helps to identify the location of CSCs in a tumour, and FACS can be used to isolate this SP from the rest of the tumour [15, 17, and 19].

6.2. Radiotherapy

Radiotherapy involves using ionising radiation to damage cell DNA directly through energy transfer or indirectly through the generation of ROS, which induces apoptosis causing tumour remission. However, like chemotherapy it faces difficulties to target CSCs, which have developed strong self-renewal and DNA repair mechanisms, leading to considerably high risks of tumour relapse [2].

Due to the similarity of radio- and some chemotherapy-induced damage in respects such as the generation of ROS, CSCs' resistance to radiotherapy shares many pathways to their chemotherapy resistance. One such pathway is the increased production of free radical scavenger molecules such as aldehyde dehydrogenase (ALDH) and glutathione (GSH), which lower the ROS level in CSCs, thus reducing radiotherapy-induced DNA damage [22]. In addition, the upregulation of DNA damage repair mechanisms further reduces the resulting DNA damage [19].

Apart from intrinsic mechanisms, the location of CSCs also contributes to radio resistance; lowered ROS generation due to low concentration of oxygen may partly protect CSCs in hypoxic niches [22]. It has been thought and shown in some cancer that hyperbaric oxygenation therapy (HBOT) may improve resistant hypoxic cells' response to radiotherapy, which could include CSCs in hypoxic niches, by temporally oxygenating the tumour. However, these data should be interpreted with caution and further research is required to confirm whether HBOT directly caused these improvements [23, 24].

Radiotherapy damages DNA to induce cell death in cancer cells, but it can also lead to increased malignancy and resistance. This is because it can activate genes related to epithelial-to-mesenchymal transition (EMT) like TGF-β, SNAIL, and SLUG, leading to the suppression of p53-mediated cell death and increased resistance to therapy. This also increases the risk of metastasis due to CSCs gaining invasive behavior and the ability to avoid anoikis 3. In addition, radiotherapy-induced EMT promotes the dedifferentiation of tumour cells into CSC, further contributing to tumour relapse and metastasis. Multiple EMT-related pathways are involved in radiation-induced generation of CSCs, including Notch and NF-κB [23]. The generation of CSC upon radiation and the difference in radio resistance of CSCs and other tumour cells leads to the enrichment of radioresistant CSCs after therapy, resulting in wide-spread radio resistance within a tumour. Multiple EMT-related pathways, such as NF-κB and Notch are involved in radiation-induced CSC generation [23]. It is thought that by targeting EMT-related signaling pathways the efficacy of radiotherapy can be improved in patients with radioresistant tumours, but further research is needed for clinical applications [24].

6.3. CSC-Targeted therapies

Currently, various CSC biomarkers have been explored in CAR-T therapy research, such as CD19, CD20, CD22, CD123, EpCAM, ALDH, and DNAJB8 [2, 25]. While CSCs have downregulated MHC expression, CAR-T therapy is MHC-independent, effectively bypassing a major mechanisms of CSC resistance to immunotherapies [26]. Emerging CAR-T cell therapy gives prospect to eliminate CSCs by precise recognition of TAAs while sparing normal cells, especially normal stem cells to avoid toxicity [18]. However, the heterogeneity of CSC-antigens poses great challenges to traditional CAR-T therapies that only targets one antigen, which may be co-expressed on normal stem cells and not all CSCs in a patient. Multi-biomarker targeting strategies can be adopted to further enhance the CSC-targeting of CAR-T therapy, and this can be achieved with dual-specific or universal CARs targeting two or more antigens [9, 18]. Targeting two of more antigens, it is possible to create logic-gated intracellular network (LINK) CAR to achieve Boolean-logic AND-gated, AND-NOT-gated cytotoxicity, broadening the opportunity to precisely target CSCs beyond single antigens and limit toxicity to normal tissues [27].

Depending on the method used, targeting multiple antigens can either mitigate or increase the risk of on-target-off-tumour toxicity. To further limit CAR-T action to CSCs and associated tumour tissue, methods to regulate CAR-T cell activity in vivo can be applied. For example, split-CARs may be utilized, which cannot carry out antigen recognition without single-chain variable fragments (scFv) being delivered separately into the patient, allowing regulation of CAR-T cell activity. Extra safety measures can be introduced through suicide genes, such as iCasp9, and inhibitory CAR (iCAR) constructs, which may target antigens only present on healthy cells to inhibit the cytotoxic effect on healthy tissue [9]. In addition, research demonstrate that emapalumab-mediated IFNγ-neutralisation mitigates severe toxicity without compromising the tumour-killing ability of CD-19 Targeted CAR-T cell against lymphoma [28].

6.4. Oncolytic viruses

Oncolytic viruses (OVs) are genetically modified viruses with replicative properties only in cancer cells. OV therapy aims to induce tumour cell lysis and the subsequent release of danger signals triggering a series of further immune responses [6, 29, and 30]. OV-induced cytotoxicity is often triggered by the activation of apoptotic pathways, and the induced cytolysis releases progenitor OVs, which then infect nearby tumour cells. Therefore, OV therapies can amplify their therapeutic payload over time to propagate through the tumour, potentially reaching the CSC population [31, 32]. To counteract the limited capability of viral replication in necrotic tumour masses, strategic sites for intratumoral injection can be chosen to increase the probability of CSC elimination induced by OV infection. OVs' therapeutic effect can also be furthered by the subsequent immune response targeting the tumour following the cytolysis of tumour cells, while larger OVs can be engineered to carry immunostimulant transgenes [31]. Experiments in mice models showed that the OV-triggered immune responses lead to sustained immunity against tumour generation [32].

Remarkably, CD133-retargeted oMV engineered to target glioma CSCs not only spared CD133-negative cells in proximity, but also somatic cells presenting CD133 such as the HSCs, which is possibly due to mutations leading to increased susceptibility to β-interferon responses that are impaired in most cancer cells [31,33]. The tumour-specific toxicity of OVs can also be achieved by the deletion of viral genes, such as γ34.5 in oncolytic Herpes Simplex Virus (oHSV). This means that oHSVs cannot bypass protein kinase RNA-activated response (PKR) preventing the replication of viral proteins but can still replicate in CSCs with PKR down-regulated by the activation of MEK [33].

One major challenge for the infusion of naked OVs is compatibility with a functioning immune system; due to the pathogenic origin of many viruses in nature, OV derived from them can be quickly recognised and eliminated/removed by natural immunity [6, 32, and 33]. This could be addressed by vectors or carrier cells for OVs. MSC serves as good candidate for this role, as will be discussed in the following section.

7. Potential uses of stem cells to enhance CSC targeting

7.1. Stem cells as immune cell progenitors

As CAR-T cells face the drawback of rapid differentiation into short-lived effector cells in-vivo, alternative sources of CAR-T cells are required especially in patients whose immune cell populations have been severely lowered by chemotherapy or old age. Multipotent stem cells, such as iPSCs and ESCs are good candidates for the generation of T lymphocytes and may even provide an unlimited supply for the manufacture of CAR-T cells [6]. A study on iPSC-derived CAR-T cell show efficacy against solid tumour comparable to primary CAR-T cells but persisted longer in vivo. While iPSC can be derived from patients autologously, they also have greater potential of being mass-produced as allogenic CAR-T products, with 'off-the-shelf' availability. While this increases the risk of adverse immune effect and tumorigenesis, such risks can be attenuated by suicide genes, antigen-specific induction systems, and replacing endogenous TCR with pleiomorphic TCRs [34].

7.2. Stem Cells as OV carriers

To enhance the targeted delivery of OVs and protect them from immune responses, carrier cells may be used [6, 31]. MSCs are good candidates for such use for their tumour-tropic properties and easy obtainment [35]. It was found that MSCs collected from ovarian cancer patients are potentially more capable as candidates for oncolytic measle virus (oMV) than those from healthy donors. This capability is maintained in frozen samples after thawing, creating possibilities of their uses in cancer treatment [6]. MSC can further enhance OV therapy by carrying other therapeutic agents simultaneously, such as Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL). TRAIL/oHSV loaded MSC is demonstrated to avoid tumour resistance, being able to induce apoptosis in both TRAIL- and oHSV- resistant glioblastoma in mice models [7].

8. Challenges to current CSC-targeted therapies and future directions

Currently, there are many challenges towards achieving effective CSC targeting. First, there is currently no specific markers for CSC found, making specific targeting and on-target-off-tumour toxicity avoidance difficult [36]. Second, CSC populations characterises both heterogenity and plasticity within a tumour, signifying that multiple markers might have to be used simultaneously to achieve efficient targeting, which also raises the risk of toxicity to normal tissues. Third, CSCs share not only markers but also signaling pathways with normal stem cells, further limiting the range of applicable therapeutic targets. Fourth, the interaction between CSC and their TME is not well understood, which is known to contribute to their drug resistance and immune escape [2]. Some of these points will be discussed in detail below. Possible ways to overcome these issues include intervention at more regulatory levels, such as RNA editing, metabolism and epigenetics, and explore natural products that target CSCs [2]. Interestingly, novel tumour-agnostic but precise method to eliminate tumour cells presents an alternative way of overcoming CSC heterogeneity and resistance, which will be discussed towards the end.

One of the biggest challenges to CSC-targeted therapy is the non-specific and diverse nature of CSC antigens due to clonal evolution [9]. Meanwhile, combination therapies involving CSC-targeted and conventional therapies have been explored to overcome this [9]. This suggests the advantage of implementing CSC-targeting approaches into combination therapies rather than serving as monotherapies, also the necessity of developing rapid biopsies, screening technique and a more comprehensive library of CSC biomarkers to allow for timely, personalized second-line therapy [9, 18].

As novel CSCs could arise from multiple origins, an ideal CSC-targeting strategy would also prevent the regeneration of CSCs and new, potentially untargeted, CSC-population from forming. This is one of the main aims of differentiation therapy which is to achieve tumour depletion by driving terminal differentiation [37]. Differentiation therapy has been extensively explored in acute myeloid leukemia (AML), where it has achieved notable success [38].

However, applying differentiation therapy to solid tumours is more challenging due to significant variation in their molecular mechanisms for stemness, even those with similar histology or grade. Additionally, few reagents shown capable to induce differentiation are applicable to clinic. The harder-to-acquire nature of solid tumours biopsies compared to haematological tumours hinder in-depth research in this field. Also, the assessment of effectiveness of differentiation therapy is rather obscure as their effectiveness alone might not relate to tumour eradication [37, 38]. Despite this, differentiation therapy offers an alternative p53-dependent treatment for malignant tumours, addressing the common therapeutic resistance of CSCs which commonly inhibits master apoptotic control genes via stemness-related gene expression [39]. So, although differentiation therapy likely cannot be effective monotherapies for solid tumours, they can be valuable components of combination therapies aimed to achieve CSC elimination. To optimize this approach, a more holistic pathophysiological model can be developed based on modern understanding of cellular mechanisms of tumour progression and differentiation. In addition, differentiation therapy may deliver efficient targeting of minimal residual disease [38, 39].

CSCs are in complex interactions with their TME. However, most studies have been conducted on isolated CSC population or in immunodeficient mice, lacking the complex interaction between TME and an adaptive immune system [2]. The crosstalk of CSCs and the TME are shown to enhance chemoresistance and support immune escape by inhibiting infiltration of CD8+ T cells and limited efficacy of immune checkpoint inhibitors [5]. TME generated through CSC differentiation also plays important roles in tumour progression, drug resistance, angiogenesis, and metastasis [40]. Understanding the interaction between CSCs and TME will elucidate current obstacles to efficient CSC-targeting techniques, thus making CSC-targeting a more viable strategy.

9. Conclusion

Although the original CSC concept is based off AML alone, cancer cell expressing stemness properties have been identified in various other concepts, evident to contribute to setbacks faced by most current therapies. The CSC theory, especially when integrated with clonal evolution, has clinical significance in a wide range of tumours and sheds light into the complex hierarchy and structure of TME. Their elimination or suppression is crucial in achieving tumour remission and preventing tumour relapse. While their targeting has been difficult due to the lack of available specific marker, thus potential toxicity to normal tissues, serval types of therapies such as CAR-T and oncolytic virus have been explored to target identified CSC markers for their elimination. Various application of stem cells could greatly enhance the efficacy of these therapies. While the classic CSC model seem to present CSCs as prime targets for cancer therapy, it is important to note that in most tumours, CSCs are shifting targets due to plasticity. This limits the clinical practicality of targeting single selected CSC markers, which some studies aim to achieve. Considering clonal evolution, methods to target different components of the CSC niche would be a better strategy than targeting single CSC cell lines. However, as CSCs play vastly different roles in many stages of tumour development including post-therapy relapse, it seems likely that challenges posed by this elusive tumour population will be overcome by exploring combination therapies involving both existing and novel therapies, supported by the development of more holistic pathophysiological models.

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