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Review Article

# Immunoresistance a Major Challenge in Cancer Therapy

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## Abstract

CSCs, or Cancer Stem Cells, are a specific group of cells that are present in the tumor mass and, unlike viable tumor cells, they do not need the initiation potential to induce tumor development. CSCs features might be responsible for failed treatment, metastases, recurrence, or even for propagation into more malignant tumors. In this review we analyze the role of CSCs in cancer immunoresistance and discuss the extensive overview of the dynamic interactions in the tumoral microenvironment that implicates CSCs and the host-immune system, which are determinants for successful therapy. Immunotherapies are a series of promising clinical approaches carried out to activate the immune system developed to target specific antigens to induce beneficial clinical responses. Given CSCs' properties, the difficulty of eliminating these cells increases as they induce poor immune-recognition and active immunosuppression during tumorigenesis. This finally generates immunoresistance that cannot be overcome by traditional cancer therapies and is one main reason for unsuccessful cancer therapy. This is one of the major challenges faced in solid tumors. Finally, we discuss new insights into anti-tumor immunity that focuses on CSCs and on the stemness phenotype and its bi-directional microenvironment interaction.

***Keywords:*** *Cancer Stem Cell (CSC); Quiescent CSC; Immunotherapy; Immunoresistance and Cellular Plasticity*

## Cancer stem cells

Cancer Stem Cells (CSCs) are a small subpopulation of cancer cells that possess the ability to self-renew, differentiate to form a tumor mass and proliferate after an prolonged quiescence period [1]. A tumor mass has CSCs, as well as viable tumor cells that lack the tumor initiation potential. Therefore, the malignancy of cancer cells lies mainly in CSCs, which are responsible for aggressiveness, metastases, relapse and immunoresistance [2]. Despite debate about the CSCs theory, substantial evidence supports their participation in cancer. The concept that cancer is supported by a cell population with stemness properties has led to cancer therapy approaches to be reconsidered. Numerous research projects have focused on identifying and understanding the main mechanisms that rule CSCs´ behavior which, in parallel, serve to develop specific drugs to target against CSCs and their intracellular signaling pathways with a significant clinical potential.

Tumors evolve progressively and acquire genetic and phenotypic differences that become unique among existent tumors and also inside tumor cell populations, which complicates diagnosis, prognosis and treatment. This tumor heterogeneity can be explained based on two important theories: the stochastic theory, or the clonal evolution model, and the cancer stem cell model (CSC model), or the hierarchical theory. The stochastic theory states that all tumor cells are biologically alike cells that possess a similar ability to self-renew and to produce new tumor cells. Furthermore, heterogeneity of cells appears from the subclonal differences that occur due to the epigenetic or genetic changes which happen during cancer development. Hence no variation exists inside tumor subpopulations according to their tumoral initial potential. On the contrary, the CSC model suggests that TICs, or Tumor-initiating cells, are responsible for cancer development initiation, and also for tumor progression and recurrence after chemotherapy [3]. The hierarchical model further elaborates that CSCs as small subpopulations encompass a variety of properties, for instance self-renewal and cellular plasticity, and also act as multi-

potent cells capable of generating several differentiated cell types to finally constitute the tumoral bulk where CSCs´ prevalence differs between tumor types and individuals [4]. However, no exclusionary models exist, but a unified model has been proposed. In this unified model, acquiring mutations may lead to the clonal expansion of TICs. In parallel, another cell acquires a different mutation which allows it to form a new subclone. Thus, with time mutations accumulate and different subclones might evolve in parallel. Since, CSCs are not considered static entities, genetic and epigenetic alterations can influence their incidence. Consequently, some subclones may contain only a few self-renewal CSCs among a large number of non-stem cells. Other subclones can contain a relatively large number of CSCs, and finally some tumorogenic subclones may have genetic and/or epigenetic alterations that confer a high renewal potential during cancer development. All these subclones finally constitute intratumoral genetic diversity. However, the most relevant tumor cells, which are capable of linking stemness, prognosis and therapy, are those responsible for long-term clonal growth and conserve self-renewal capacity as a hallmark to avoid clonal exhaustion [5].

Apart from being an isolated mass of cancer cells, tumors are complex structures. So, cancer biology must be analyzed to understand such a heterogeneous cellular niche. Indeed, during tumor development, cancer cells modify the cellular and molecular composition of stroma. In this complex tumor microenvironment, some crucial components are implicated, such as Cancer-Associated Fibroblast (CAF), to produce different proteins that modify the extracellular matrix (ECM) structure by increasing the invasiveness of CSCs´ phenotype. Moreover, endothelial cells and immune system cells, such as Tumor-Associated Macrophages (TAMs), Dendritic Cells (DC), and Myeloid-Derived Suppressor Cells (MDSC) are critical components in the microenvironment. Clearly inside the tumor microenvironment, cellular interactions encourage signals pathways, along with surrounding stromal cells that regulate the secretion of paracrine and autocrine growth factors and cytokines, which determines tumorigenesis and metastasis. These interactions are bidirectional between tumor cells and their dynamic microenvironment, which is influenced by tumor metabolic shifts, active tissue remodeling, immune cell recruitment, and finally by pharmacological selective pressure applied during treatment [6]. Tumors acquire mechanisms that regulate the immune microenvironment, which include the release of immunoregulatory factors [7], the modulation of costimulatory pathways (also known as immuno-checkpoints) [8] and the stimulation of suppressor cells like MDSC, TAMs, DC and regulatory T-cells (T-regs) [9,10].

Immunosuppressive molecules production leads to the recruitment of cells that are capable of suppressing the immune system and to loss of tumor antigen presentation/processing machinery, which make CSCs less immunogenic. In line with this, CSCs are responsible for presenting the tumor antigen to T-cells, which is essential to recognize the antigen or to produce a response. This CSCs capacity is predetermined by antigen-presenting molecules, such as MHC-I and MHC–II, CD80, CD86 (co-stimulatory) and co-inhibitory molecules such as cytotoxic T-lymphocyte antigen 4 (CTLA4), programmed death receptor-1 (PD-1), B7-H3 and B7-H2 [11]. CSCs are also able to down-regulate the action of MHC-I in the absence of the MHC–II molecule, which leads to the down-regulation of the Low-Molecular-weight Protein (LMP) antigen that presents machinery and beta macroglobulin [12]. This results in CSCs escaping attacks by the immune system. Thus low immunogenicity is mediated partly by PDL-1 (programmed death-ligand 1) expression, also known as B7-H1, which contributes to immunoresistance by depressing T-cell activation [13], along with other immune surveillance inhibitory molecules like PD-1, CTLA-4, and other B7 family members [14]. Therefore, low CSCs´ immunogenic is determinates by cellular recruitment with immunosuppressive capacity, molecules releasing that reduce the host-immune system response and finally, through lack of tumor antigen expression [15].

Low CSCs immunogenicity is also based on immunosuppressive molecule release, e.g. transforming growth factor-beta (TGF-β), which is a tumor immune evasion factor that promotes metastasis by increasing tumor cell invasion. It also inhibits the function of immune cells by suppressing or altering the activation, maturation and differentiation of innate and adaptive immune cells, including Natural Killer (NK) cells, DC, macrophages, neutrophils, and CD4 + and CD8 + cells [7]. It also plays an important role in the differentiation and induction of natural and induced T-regs, which contributes to the creation of an immunotolerant microenvironment [16]. Galectin-3, a β-galactoside-binding protein, is an immunosuppressive molecule expressed by tumor cells that modulates the immune and inflammatory response through the apoptosis of T-cells. Its effects are due to the molecule's interaction properties. Indeed, it binds specifically to NK by inhibiting the cytotoxicity of these cells, which are effector lymphocytes of the innate immune system and a first-line defense

against tumors. Thus Galectin-3, a soluble inhibitory ligand that targets the NK cells secreted by CSCs, helps tumors escape immune attack, in consequence might be consider as a novel therapeutic target [17].

CSCs produce other types of immunosuppressive molecules, which are cytokines such as IL-4, IL-10 and immunoregulatory cytokines, IL-6, IL-8 and IL-13 [14]. IL-10 is a cytokine that inhibits antigen presentation and MHC class II expression. IL10 acts on DC and macrophages and protects tumor cells from cytotoxic T lymphocytes [18]. In fact, during tumor progression the macrophages phenotype is modified. Macrophages are generally classified into two categories; M1, involved in pathogen removal, inflammatory response and antitumoral activity, and M2, which presents contrary features, including pro-tumoral activities; within the tumor tissue, macrophages are named TAMs, which are considered a main stromal component, show analogous functions to M2, and are correlated with tumor progression and poor clinical outcome. Several studies have demonstrated that TAMs are related with inflammation, IL-6 release and are involved in the regulation of tumor cell cycle progression, apoptosis suppression and angiogenesis, building a favorable tumor microenviroment. Hence, TAMs infiltration represents a negative prognostic factor for cancer patients [19-21]. IL-4 is an important negative regulator of apoptosis that confers resistance to chemotherapy-induced death. It has also been shown to be responsible for the inhibition of T lymphocyte proliferation and together with IL-13, it induces the polarization of macrophages into an M2 (anti-inflammatory) phenotype by facilitating tumor growth, invasion and angiogenesis [22] (Figure 1).

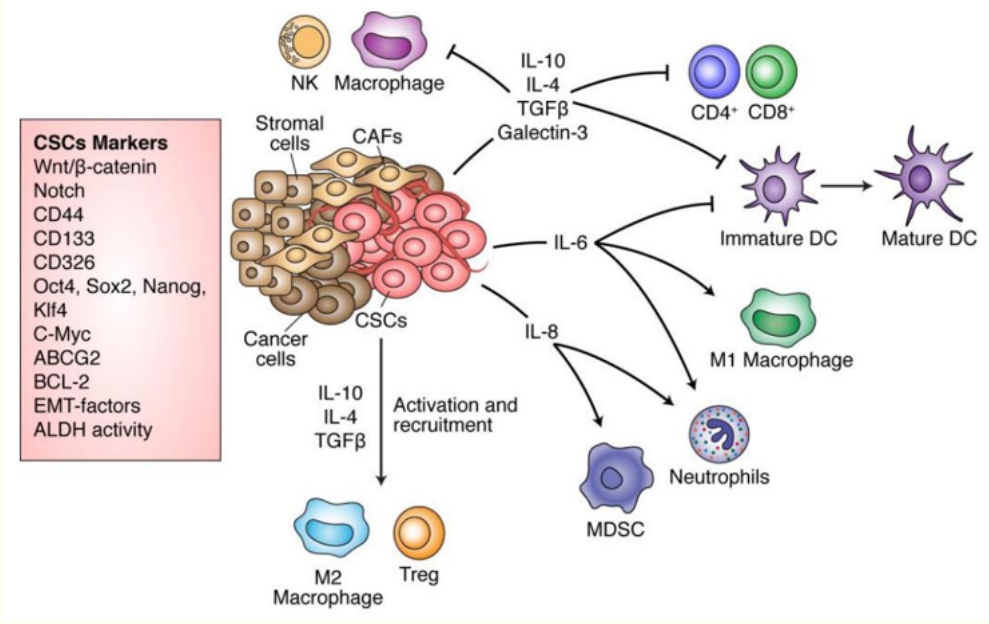


***Figure 1:*** *Cross-talk representation between Cancer Stem Cells (CSCs) niche and immunoregulatory factors in tumor microenvironment.*

Invasiveness and metastasis capabilities have been associated with epithelial-mesenchymal transition (EMT) in cancer. EMT program can be activated rapidly in epithelial cells in response to physiological signals. During gastrulation epithelial cells from epiblast layer undergo EMT become into mesenchymal cells. EMT is critical in morphogenesis during embryonic development. Indeed, it has been associated with the stemness profile in cancer. EMT has been reported as a fundamental driver in CSCs and features recovery, including dissemination, dedifferentiation and the invasion potential, as it is responsible for the cell motility driven by its "transcription factors" that control various signaling pathways, such as Wnt/β-catenin [23]. There is evidence to support the role of TGF-β, IL-6 and IL-8 in EMT induction

[24-26]. EMT and CSCs generation are closely related to the TGF-β signaling pathways. TGFß1 is released by tumor cells and the microenvironment and represents a main inducer of EMT during both embryonic development and tumor progression. This TGFβ1 signaling pathway is usually over-activated in several carcinomas capable of promoting invasion and metastasis. TGFß1 signaling pathway involves SMAD and GTPases and in epithelial cells, TGFß1 also activates AKT through PI3K, which entails mTOR activation which augment cell size and synthesis of protein, additionally increase motility and invasive capacity [27]. On the other hand, there are alternative pathways that participate in the of EMT induction, including WNT, NOTCH, cytokines such as IL-6, prostaglandin E2 (PGE2) and growth factors such as epidermal (EGF), FGF, HGF, PDGF or VEGF. Thus, EMT program could be activated in response to a wide variety of paracrine signals [28].

Certainly IL-6 is a pleiotropic cytokine, a well-known regulator of immune responses and a key contributor in the pathogenesis of many inflammatory diseases [25]. IL-6 induces EMT phenotype expression by enhancing invasiveness and promoting tumor cellular proliferation by inducing 'EMT transcription factors', e.g. *VIMENTIN*, *N-CADHERIN*, *SNAIL* and *TWIST* expression, and repressed E-cadherin. Such action establishes a link among EMT, IL-6 and poor patient survival outcome in human carcinomas [25,29]. The overexpression of EMT transcription factors up-regulates IL-6 secretion and activates STAT3 in parallel, which indicates the existence of a positive feedback loop that involves autocrine IL-6-mediated signaling [25]. Positive loop similarities exist among IL-8, tumoral EMT and stemness features. As a direct relationship links IL-8, *SNAIL* and master regulators of pluripotency stem cell-associated genes, such as *SOX2*, *OCT4* and *NANOG* [30], EMT induction yields cells with a stemness profile and also malignant cells with CSCs properties [31]. Indeed, EMT drivers such as SNAIL1 and SNAIL2 have been correlated with recurrence and poor overall survival in cancer. EMT is an important mechanism in the development of resistance to conventional antitumor therapies. Previous studies suggest that reversible epigenetic alterations observed in acquired resistance are regulated by EMT program. In breast carcinomas, characterized by mesenchymal genes expression, shown worse prognosis and poor overall survival, suggesting that there is a higher tendency to develop treatment-resistance in tumors where EMT is activated [32]. Besides, EMT has been correlated with histological grades, tumor sub-types and others clinico-pathological parameters, usually related with worse outcomes groups, including the resistance to EGF receptor treatment which is relevant in lung cancer patients. Therefore, EMT phenotype is a useful tool for stratify patients and as a predictor of clinical outcomes [28].

CSCs' secretion of multiple immune-suppressive cytokines and angiogenic factors drives tumor progression by recruiting cells with tumor-encouraging phenotypes. The stromal fibroblast of the tumor also takes part and plays a major role in CSCs production by facilitating CCL-2 release [33]. In fact in CAF, secreted CCL2 is capable of inducing the generation of CSCs through the activation of *NOTCH* signaling. *NOTCH* activation promotes cellular self-renewal by maintaining a undifferentiated cellular state. Thus, it is recognized as key regulator in stem cells [34]. Substantial evidence shows that *NOTCH* signaling is implicated in tumor progression and maintenance. Indeed, once *NOTCH* activity is blocked, CSCs proliferation decreases through the STAT3 de-phosphorylation status [34]. In the same line, a paracrine effect on tumor microenvironment fibroblasts is responsible for cytokines set, which promotes CCL2 secretion via STAT3 activation [33]. Thus, an interactive loop is established among STAT3, CCL2 and the *NOTCH* pathway in CSCs proliferation inside the tumoral niche, which is crosstalk signals-dependent. The STAT3 pathway is also known to promote the immune escape of these cells by preventing macrophage activation [35,36]. The STAT3 pathway also regulates the transcription of immunosuppressor factors, such as VEGF, IL-10 and TGF-β [37]; antitumor immunity capacity is repressed by reducing the cytotoxicity of T-cells in this way. Hence CSCs' self-renewing properties, tumor immunosuppression capabilities and spontaneous growth are governed by the STAT3 pathway [38]. Consequently, in order to impede the crosstalk that prompts CSC-mediated disease progression CCL2 seems as a potential target.

The soluble and membrane-bound molecules expressed by CSCs are also known to create an immunomodulating effect, which helps CSCs to escape immune attack. CD200 is a transmembrane glycoprotein expressed in CSCs that facilitates the immune escape of these cells [39]. In fact, CD200 interaction with its receptor CD200R (present on the surface of myeloid cells) carries an inhibitory signal to macrophages by promoting immune tolerance, which permits CSCs to modulate the macrophage function, and also neoplastic evolution [21,22]. Hence a blockage strategy is disclosed as a therapeutic opportunity in the receptor-ligand complex.

The resistance of many solid tumors to cancer therapy (including chemotherapy, immunotherapy and radiotherapy) is the result of CSCs. Diverse mechanisms can explain CSCs-driven resistance, including mainly the atypical activity of aldehyde dehydrogenase (ALDH), ABC transporters, epigenetic deregulations, improved DNA damage response and survival signaling activation, including proteins: e.g. B-cell lymphoma-2 (*BCL-2*). Thus, high levels of anti-apoptotic proteins, such as *BCL-2* and B-cell lymphoma extra-large (*BCL-XL*), become available and play a critical role in CSCs' survival by promoting apoptosis resistance.

These proteins protect CSCs from not only cancer therapies, but also from the immune cells responsible for apoptosis, such as T-cells or NK cells [42]. These findings support the utility of including inhibitors of anti-apoptotic *BCL-2* family members in therapeutic strategies that intend to eliminate quiescent CSCs by preventing cancer recurrence. Besides, CSCs express strong resistance to DNA damage and increased resistance to apoptosis due to induced levels of DNA damage repair mechanisms that allow CSCs to survive after treatment [43]. Besides, CSCs overexpress ABC drugs transporters, which are proteins expressed in cell membranes capable to pump molecules out of cells. Thus, radiation and chemotherapy resistance are considered an inherent property of CSCs explained by independent mechanism above mentioned such as drug-efflux pump (ABC transporters), higher DNA-repair capacity and improved ROS protection [44].

It has been described that the tumor immunoediting of CSCs involves three important phases: the elimination phase, the equilibrium phase and the escape phase (Figure 2). The elimination phase, or tumor immune surveillance, has two important types of immune response: innate immunity and adaptive immunity. The host's immune system has special cells, like NK cells, and these T-cells are activated in response to the cytokines secreted by CSCs. The innate immunity activation results in the secretion of tumor killing NK cells and in the secretion of IL-12 and interferon gamma. These activate immune cells and the secreted agents attack tumor cells and cause a cytotoxic effect for tumor cells via a different mechanism, such as perforin, TRIALs and ROS [45]. After the elimination phase, most tumor cells are eliminated; indeed, an effective elimination phase could generate a protective response, re-establish a normal tissue state. However, whether some tumor cells escape the next immunoediting phase, which is the equilibrium phase. The non-immunological-mediated phenotypes of tumor cells are selected, and these cells' growth is achieved at the end of this long phase. Tumor cells are substantially modified and become more resistant to immune system attack, due to a reduced the immunogenic capacity of the system. Those cells capable of growing in the equilibrium phase move to the last phase, or the so-called escape phase. Cells become more resistant to immune attack by undergoing genetic and epigenetic changes. These changes allow this special group of cells to escape immune attack. Most of these cells are usually CSCs which later challenge the effectiveness of cancer therapies [46].

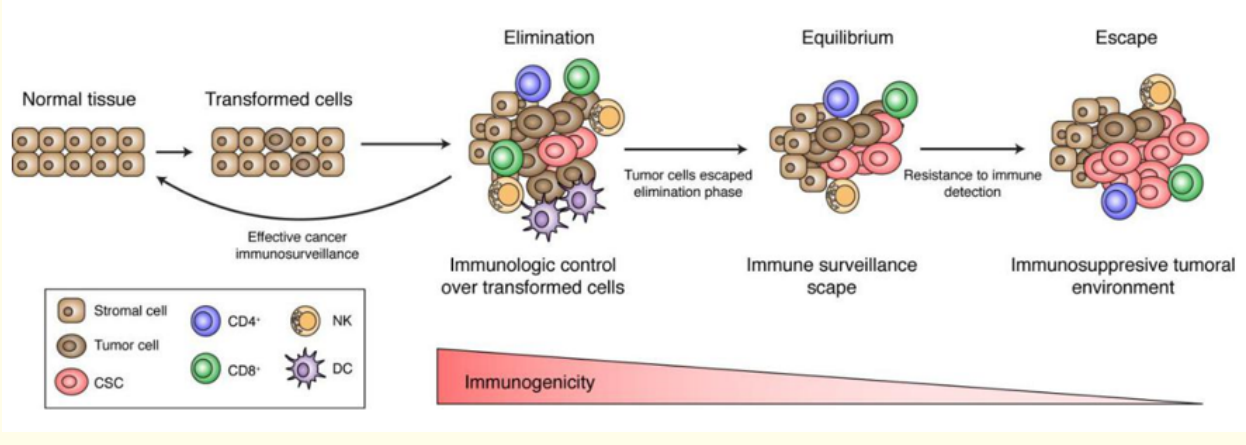


***Figure 2:*** *Schematic representation of immunoediting concept. Three phases are described from immune surveillance to immune escape. Transformed cells can be eliminated by immune efficient cells but this phase could result in a selection of transformed cells with a decreasing of immunogenicity and become resistant against immune attack generating escape of cancer cell and tumor progression.*

## Immunoresistance of CSCs

CSCs are clearly highlighted as being responsible mainly for conventional chemotherapeutic failure. Despite in-depth studies into characterization profiles, CSCs markers fluctuate during tumor formation and the majority of markers loses specificity and gain heterogeneity in the same tumor. All this supports the concept that phenotype evaluations should be complemented by functional CSCs validation.

As immunoresistance is one of most relevant problems caused by CSCs, most cancer therapies fail. The reversible G0 phase is where cells escape to enter the cell cycle due to the response created by physiological stimuli, which is otherwise known as cell quiescence. Recent results have shown that quiescence is an actively maintained state in which various signaling pathways, and epigenetic and transcriptional regulation, govern this reentry mechanism, which is involved in maintaining a state of equilibrium that allows quick activation [47]. Tumor suppressor p53, RB, cyclin-dependent protein kinase (e.g. p57, p21 and p27), the Notch pathway and micro-RNAs regulate stem cell quiescence [47]. Furthermore, the FoxOs family of transcription factors and NF1 are also involved in cell quiescence regulation by controlling cell cycle and differentiation processes [48]. Adaptive responses to the stress created by the environment allow the long-term survival of CSCs or quiescent CSCs. Adaptive responses include metabolic responses, and genomic integrity is also seen in these types of quiescent cells.

Tumor cells follow a specific phenomenon, during which DTC (disseminated tumor cells) can remain in either a non-proliferating (quiescent) state for longer durations due to cancer therapies or an early tumor development phase. However, the activation of these non-active cells leads to tumor proliferation, and also to relapses after several years [49]. Several research works have shown that CSCs with the quiescent property are also found in the tumor [44]. In addition, the quiescent nature of CSCs protects these cells from antiproliferative chemotherapy drugs or factors, which plays a major role in the immunoresistance to the majority of traditional cancer therapies. Therefore, targeting quiescent CSCs represents major challenge.

Tumor dormancy, a term that defines a group of cancer cells in the quiescence state, is arrested in the G0-G1 cell cycle phase. It can be broadly classified into three different situations; initially it is described as reversible cell cycle blocking because mitogenic signaling pathways are lacking. Angiogenic dormancy is a second situation in which a whole tumoral mass is preserved through a balanced equilibrium between dividing cells and those that undergo cell death due to poor vascularization. Third and finally, there is the dormancy-immunologic state, which is led by the immune system, and retains tumor mass through firm cytotoxic pressure. Consequently, and as previously mentioned, immune cells are a critical component in a tumoral microenvironment, and play a pro- and anti-tumoral role in which tumor mass expansion is in a latency state, surrounded by a non-permissive environment controlled by the immune system [49].

Slow-proliferating CSCs are targeted by two important strategies. The first one, in which CSCs are forced to enter the cell cycle, results in a locked-out situation, which includes most conventional cancer therapies, such as mitotic inhibitors, topoisomerase inhibitors and antimetabolite drugs, which all target only proliferative cells. However, a locked-out situation puts the process at risk if it is applied with no other specific therapeutic tool against CSCs. This often occurs with traditional anti-cancer therapies that are unable to eradicate all the CSCs in the tumor mass, when it finally results in recurrence, severe proliferation and disease progression. The activation of dormant CSCs leads to cellular proliferation, which undergoes serious epigenetic and genetic changes [49]. This scenario challenges anti-cancer treatments because the resistance of CSCs against conventional cancer therapies increases. Thus treatments must include novel strategies against CSCs, and must bear in mind the quiescence state of CSCs to eradicate them while they remain in this dormant stage, or through drugs capable of driving epigenetic mechanisms to induce dormancy, in order to simultaneously prevent and avoid aggressive phenotype changes and metastatic dissemination.

CSCs' plasticity also contributes to immunoresistance; indeed, cancer therapy partly fails due to this feature of CSCs. The static theory of CSCs has been disregarded and the transient theory is now used mainly due to the changes observed in CSCs following variations in microenvironments. Recent evidence in melanoma and breast cancer has demonstrated that small cancer cell populations can shift and

modify their differentiation (CSCs) state according to the microenvironment response, which has been reported as being a more complex CSCs model thorough transient plasticity which, in turn, permits cancer cells to acquire stem-like cell properties as part of an adaptive response [50]. The epigenetic changes that occur in CSCs result in these cells' adaptable plasticity. Indeed, independent studies have validated the dynamic model by identifying the potential transcription factors responsible for, introducing each one into different combinations. It has been found that glioblastoma cells and colorectal cancer are capable of recovering CSCs properties through different combinations of transcription factors. However, not only transcription factors are involved in plasticity modulation because tumor suppressors (PTEN) and micro-RNA (MiR-7) are able to modulate it [50]. Non genomic alterations and mutant cell selection also take part in CSCs' plasticity. Cancer recurrence can be clearly explained by Darwinian selection in many cases. An open inquiry still stands as to whether resistance mutations are essential and irreplaceable to promote cellular phenotype changes during cancer progression, which might be re-generated in attempts to eliminate cancer cells. Therefore, upcoming and advanced therapeutic strategies should be considered, including those that are targeted specifically to therapeutically employ differentiation pathways. Hence, these novel strategies should include robust epigenetic reprogramming lines as part of the new approach [51]. The microenvironment plays a major role in the regulation of CSCs by providing several environments to facilitate variations in CSCs production. These environments also regulate the metastatic potential of tumor cells, including the organization of extracellular matrix, hypoxia and several other factors. One study shows that glioblastoma and neuroblastoma have small leucine-rich proteoglycans, such as decorin and lumican. These cells form neurospheres and express severe immunoresistance [52]. The immune cells in the microenvironment of tumor cancer tissue also secrete special factors, including IL-22, which has been described as a protective immune molecule against infections that is produced by CD4 (T-cell). This interleukin activates STAT3 and leads to the expression of specific genes that encode *NANOG*, *SOX2* and *POU5F1*. These immune factors are thought to increase the potential of tumors, especially in colorectal cancer. The pro-inflammatory cytokines released by the immune cells that lie in the tumor microenvironment help tumor cells by defining and promoting the stemness profile [53]. Indeed, non-CSCs might substitute stem cells lost during treatment through cellular plasticity. Hence, this non stem cell-like could represent other source of chemoresistance cells responsible of recurrence.

Another factor that can influence CSCs' plasticity is hypoxia. Cancer cells with a high proliferative rate depend on aerobic glycolysis rather than CSCs, which prefer the oxidative phosphorylation-type metabolism (Warburg effect) that offers CSCs a resistance mechanism to cell damage. In solid tumors, CSCs have specific hypoxic regions inside the tumor [54]. This state is created by the high oxygen demand of tumor cells and by the low oxygen supply in the tumor due to inefficient vascularization [55], which leads to the activation of HIFs. These special proteins, which are activated by the tumor's hypoxic state, result in the regulation of the expression of various genes, epigenetic regulation, extracellular matrix re-organization, cell metabolism regulation and proliferation, migration, among many other factors, which lead to genomic alterations and epigenetic changes. Both collectively and individually, these changes take part in cellular plasticity, which is shown by the very marked expression of stem cell markers in solid tumors, e.g. in tumors of the lung, brain, kidney, colon, breast and liver. All these changes observed in solid tumors (hypoxic conditions) are the result of HIF protein activation, which probably results from adaptation to the low oxygen environments within tumors.

Furthermore, the hypoxic state in solid tumors also promotes metabolic cellular changes, and more specifically increases glycolysis and decreases mitochondrial function. This energy production pathway results in lactic acid formation, and the acidification of the cell microenvironment, further increases the CSCs-like properties as evidence has demonstrated that the relationship between EMT genes expression is directly influenced by low oxygen levels, and this established a link between stem cells plasticity, EMT, metabolic reprogramming and hypoxia [56]. This is seen in glioblastoma when exposed to low pH conditions, which show a more marked CSCs marker expression, including OCT4, SOX2 and NANOG. Hence, hypoxic tumoral levels correlate with the NOTCH, BMP and Akt/mTOR signaling pathways, and this demonstrates that hypoxia does not influence cellular metabolism alone as it is critical in the stemness profile, chemoresistance and tumor progression [57].

The metabolic changes mediated by HIF represent an adaptive advantage to promote metastasis and invasion in cancer. Another available energy source is used by CSCs to invade and survive at remote sites. Indeed CSCs with the CD36+ phenotype, obtained from human

oral carcinomas, overexpress the enzymes involved in lipid β-oxidation. CD36+ cells are aggressive CSCs capable of taking lipid acids from the microenvironment to generate energy through lipid β-oxidation. Therefore, a high-fat diet might independently increase the metastatic potential of CD36+ [58].

CSCs' cell plasticity confers CSCs a metastatic potential. Not every tumor cell can metastasize from its primary location because of the larger barrier formed around the tumor by the body's immune system [59]. Accordingly, each tumor's metastatic process is ineffective and very few cancer types can metastasize to distant locations. Indeed, during metastatic establishment metabolic adaptation of CSCs is critical. The metastatic potential defines a tumor's ability to spread to other organs. This potential depends mainly on the degree of differentiation of cancer cells, genetic alterations and the epigenetic changes that promote gene expression modification. Individually these factors are responsible for the metastatic tumor potential. Metastases of tumors result from the genetic and epigenetic changes that trigger gene modification at various levels, which lead to severe immunoresistance and resistance to cancer therapy. Thus, CSCs' quiescence, CSCs' plasticity and CSCs' metastatic potential contribute to immunoresistance, and are the sole reason why cancer therapies fail.

## Immunological targeting of CSCs

The immunological targeting of CSCs forms a rational choice as CSCs and, after differentiation series, their progeny expresses different genes and thus has distinct antigens. This leads to the notion of individually targeting for this small subpopulation. CSCs in tumors also exhibit heterogeneity because of the epigenetic and genetic changes that CSCs undergo to proliferate and spread to distant locations. Furthermore, multiple antigens targeting makes immunotherapy more successful for treating CSCs.

The multiple antigens expressed by CSCs are one of the major challenges of immunotherapies, but the introduction of multiple antigens targeting immunotherapy is hopeful for treating different tumors. Nevertheless, CSCs' plasticity generated by microenvironment variations and the signaling context still leave an open question about using immunotherapies as a definitive therapeutic option.

ALDH, CD44, CD133 and HER2 are the major CSCs antigens that are immunologically targeted. These antigens act as markers to identify and isolate specific types of CSCs. In particular, ALDH catalyses aldehydes oxidation, acts in retinoic acid signaling, plays a protective role against reactive oxygen species and acts in cellular detoxification. However, ALDH appears to be a good predictive marker for tumors, and ALDH activity is strong in lung cancer CSCs and is enriched in several other cancer types. Moreover, ALDH appears to be related with poor prognosis in lung cancer patients, has become an interesting prognostic marker to monitor recurrence and tumoral persistence, and simultaneously appears as an appealing therapeutic target [60]. Apart from this, the CSCs niche in a tumor is specifically localized, and is partly responsible for the properties and the phenotype of CSCs; for instance, self-renewing and differentiation. This CSCs niche promotes immunoresistance and severe resistance to traditional cancer therapy. Given CSCs´ properties analysis and its relationship with the intratumor plasticity, no doubt that it seems attractive pursue therapies/drugs to modulate CSCs-niche.

## Immune checkpoints as a method of battling immunoresistance

Immune checkpoints are specific cell surface molecules that endogenously regulate to the immune response. These cell surface molecules limit the possibility of the autoimmune action of these immune cells by activating co-inhibitory pathways (CTLA4, B7-H2, B7-H3, PD-1/PD-L1 axis) [61]. These pathways are thought to induce tumor immunoresistance. The major effect of this inhibitory pathway in the tumor is to suppress the microenvironment around the tumor and to prevent immune attack. The reciprocal modulation in the CSCs niche between immune cells and CSCs is carried out by the secretion of paracrine factors [62-64]. The PD-1 and PD-L1 antibodies are of much more clinical interest, especially in lung cancers [65,66]. The PD-L1 expression in tumors down-regulates T-cell response activation through the PD-1/PD-L1 axis. Blocking this pathway results in the activation of T-cells, thus the immune response is activated against tumor cells.

*MYC* is a well-known oncogene that is overexpressed in many cancers, and is involved in cellular proliferation, apoptosis and differentiation. c-MYC is a reprogramming factor (Yamanaka factors) [67] which, through Wnt/β-catenin signaling, leads to quiescent CSCs cell cycle progression, and is simultaneously capable of directly regulating the expression of immune checkpoints as a strategy to avoid immune surveillance. Indeed, the transgenic mouse model has demonstrated that through *MYC,* inhibition leads to CD47 and PD-L1 down-regulation by enhancing the immune anti-tumoral response [68]. Furthermore, total tumor removal is possible after oncogene inactivation, but a host -response is necessary for tumor regression as *MYC* inactivation is not enough as it requires CD4 (T-cell) and Thrombospondin-1. There is no doubt that further studies are required to clarify the other mechanisms involved in this process, including the reduction of cellular proliferation, apoptosis induction and other host-dependent processes, such as inhibition of tumoral angiogenesis and induction of tumor senescence, where *MYC* rules tumorigenesis by modulating immune molecules [68]. The immune response seems to be a focal point of the process which, through *MYC* inaction, is capable of restoring the anti-tumor response [68]. Therefore, this is further solid proof that the host-immune system acts a control barrier in tumor formation as part of immune resistance. Hence, the identification of mediators with immunomodulating capacity is required to design novel protocols that target against CSCs. These protocols should particularly include the tumor microenvironment, which plays a decisive role in immune response, and probably in successful immunotherapeutic approaches, and opens up another therapeutic approach based on the cellular bulk that maintains long-term growth.

## Conclusion

Cancer cells with a stemness phenotype, known as CSCs, constitute an exciting research field which, in immunology and its immune-regulator agents, has found a synergic partner that highlight promising guidelines in cancer therapeutics. Despite CSCs´ model is still being object of debate, considerable evidence has demonstrated that these cells release factors that have immunomodulatory effects. Hence, in this scenario, CSCs´ role in carcinogenesis demands specific therapeutics strategies to be addressed. This novel field with a clear and substantial translational potential should not only focus on the initial therapy, but should move further afield in an attempt to be more effective than conventional therapy in order to respond to an even more complicated question in cancer treatment: the eradication of minimal residual disease. Doubtlessly in-depth studies, and understanding CSCs' biology and its complex molecular interactions, are crucial insights into cancer development, clinical progression and finally, into successful treatment.

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