

Molecular Targeting of Breast Cancer Stem Cells: A Promising Strategy For Management And Eradication.

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ABSTRACT: In spite of developments in breast tumors management therapeutic approaches, relapsing, metastasis and resistant are highly observed among the patients, consequently, reduced overall survival. previously, considerable attention has been placed on the intrinsic subtyping depended in the existence or absence of traditional immunohistochemistry (IHC) markers for example estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-related protein (HER2). However, it is extensively understood that cancers are consisted of heterogeneous populations of cells with a hierarchical organization established by cancer stem cells (CSCs). In breast cancers, this CSCs displaying stem cell characteristics is identified as breast CSCs (BCSCs). This small population display a CD44+/CD24-/low phenotype with high ALDH activity (ALDH+), and owns advanced tolerability to chemotherapy, other cancer therapeutic approaches and has the capability of tumor bulk reproduction after reduction of cell populations sensitive to first-line therapy which leads to cancer recurrence. In this review, we present special consideration to BCSCs with future guidelines in the creation of a targeted therapy for this population.

Keywords: cancer stem cell, chemoresistance, epithelial–mesenchymal transition, radio resistance, therapeutic implication

1. INTRODUCTION

Breast cancer (BC) is a widespread human malignancy and a very frequent source of cancer-related deaths in women worldwide. It is currently thought to be a multifactorial disorder involving associations between environmental, hormonal, and genetic factors,

dietary variations, or nutrient exposures. Patients with BC may also have a wide variety of behavioural, pathological and molecular features [1].

BC has been categorised into three groups based on clinical and histopathological features as well as on oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor protein-related receptor expression (HER2). Hormone-positive BC comprises nearly 70% of BC patients and is characterised by overexpression of ER and/or PR. People with this type of BC have a greater diagnosis than other forms of cancer [2].

The additional subgroup is HER2+ characterized by overexpression and/or activation of the human epidermal growth factor receptor 2 (HER2, also known as ErbB-2, ERBB2, or HER2/neu)[3,4]; It signifies 20% of BC patients and is associated with a more aggressive phenotype and a poor prognosis[3]. Temporarily, the loss of expression of the above-mentioned receptors causes cancer known as the Triple-Negative BC (TNBC) subdivision. TNBC characterizes 15–20 percent of patients and is characterized as aggressive and low prognosis[5]. This histopathological organization is the commonest used. Presently, five phenotypically distinct subcategories that are associated with clinical results have been identified, including ER+/Luminal (luminal A and B) [6], basal-like, erb-B2+, normal-like, and claudin-low based on gene expression trends [7].

This molecular arrangement is supplementary knowledge for the traditional classification of patient tailoring and forecasting prognosis. Six distinct BC subtypes and the diversity of the tumours suggest the variability of BC. Tumor is a complex system of various cancer cell copies and other cell types, such as stromal, immune or endothelial cells [8]. Understanding this heterogeneity is also important to be able to establish tailored therapies. For several years, cancer was believed to have started due to an aggregation of genetic mutations in normal somatic cells, which offered a selective advantage that contributed to the transformation of normal human cells into highly malignant derivatives[9]. However, over the past few years, the hypothesis of Cancer Stem Cell (CSC) has modified that belief [10].

2. Cancer Stem Cells

Cancer Stem Cells are characterised by three key characteristics: differentiation; self-renewal; and homeostatic control[11], and are important for the proper functioning of the body serving as a basis for the regeneration of any tissue or organ cell during the life of an individual of specific importance to embryonic development. However, the same quality of tumour growth poses a significant concern to oncologists and poses a threat to patients.

Tumors consist of a heterogeneous group of cells, including cancer stem cells (CSCs), a minority subpopulation of undifferentiated cells capable of producing distinct cells that make up the bulk of the tumour. The CSC theory claims that cancers, as well as normal tissues, are produced from a group of cells called cancer stem cells or cancer-initiating cells by asymmetrical cell division, simultaneously preserving the stem population and creating multi-line differentiation [12].

Maintaining the stem population and establishing a multi-line distinction [12]. However, the origin of these CSCs is still unknown and two models have been suggested. One model notes that normal long-lived stem cells become malignant through the accumulation of genetic alterations, while others believe that mutations can give stem characteristics to a lineage-committed cell [13].

What seems increasingly apparent is that CSCs are implicated in tumors recurrence, metastatization, and drug resistance. The separation of leukaemia stem cells from acute myeloid leukaemia was the first data supporting the theory that tumors' are hierarchically arranged with tumorigenic properties. These tumorigenic cancer cells possess a specific profile of surface markers such as CD29, CD34+, CD38-, CD166, CD133+/-, Lin, stem cell antigen 1 (Sca-1), epithelial cell adhesion molecule or (EpCAM) associated with stemness

and that may be used to isolate them by means of fluorescence activated cell sorting (FACS) or other immunoselection procedures [20–22]. The use of all the described surface markers must be done with caution since some can also be found in non-cancer stem cells [23,24].

While we concentrate on the use of these markers as pharmacological targets, it should be remembered that they can be helpful in the prognosis, typically associated with more aggressiveness and invasiveness of the tumour. Following isolation, serum-free assays with a variety of additional factors or serially transplantable *in vivo* tumours may be used to test the stem properties of the chosen cells and propagate CSCs. Other three-dimensional systems, such as hydrogels, matrices consisting of biological substances [25,26], or scaffolds, structures mainly made of biopolymeric content [27]. New techniques for rising the proportion of cells with stem characteristics have appeared. These facts encouraged the analysis at the molecular level of this unusual population with the goal of recognising variations between typical stem cells as well as main stem cell dysfunctions.

To this end, three major CSC signalling pathways linked to self-renewal and differentiation, i.e. Notch, Wnt/ β -catenin and Hedgehog (Hh) pathways. Such essential signalling pathways in the CSCs are the TNF-5-007/NF- $\Delta\beta$, the transforming growth factor- β (TGF- β), the tyrosine kinase receptor (RTK) and the Janus kinase/signal transducer and transcription activator (JAK-STAT) pathways [28,29]. In addition, it has been shown that the induction of the epithelial-mesenchymal transformation (EMT) resulted in the development of cells with stemness characteristics.

3. Breast cancer stem cells (BCSC)

Globally, Breast cancer is the commonest type of cancer among females, approximately 500 million deaths linked with this type of cancer [30]. Surgery, radiotherapy and chemotherapy are the routine therapeutic approaches for breast cancer [31]. Unfortunately, cancer relapse and metastasis is usually occurs after treatment. Such phenomenon attributed to the presence of a subpopulation of cancer cells known as breast cancer stem cells (BCSCs). These type of cells characterized by their abilities of self-renewal and tumor initiation, allowing them to be drivers of metastases and tumor growth [32]. Their microenvironment is filled with residential inflammatory cells that provide the required signaling cues for BCSC-mediated selfrenewal and survival [33].

Additionally, metastasis of this type of cells is frequently mediated by the production of cytokines which makes them able to escape from the primary tumor and travel through the circulation to distant organs [34]. Due to their significant role in cancer initiation and recurrence, Molecular targeting of BCSCs considered a promising therapeutic strategy for management and eradication of breast cancer. Origins and Characteristics: Cancer stem cells in breast cancers were discovered in [35].

Several theories demonstrated the origins of these type of cells as follow; normal cells experience mutations that result in their conversion into BCSCs, [36]. on the other hand, other studies hypothesizes that these cells come from the misplacement of somatic stem cells *de novo*. Surface markers expression have been frequently used in identifying and isolating BCSCs. CD44, CD24 and aldehyde dehydrogenase (ALDH) are the most specific surface markers for BCSCs [37]. CD44, a cell surface glycoprotein, which essential for cell adhesion, migration and invasion of breast cancer cells. Additionally, this protein interacts with osteopontin accelerates tumor progression. ALDH, also facilitates the differentiation of stem cells. Recent studies exhibited that BCSCs are positive for both CD44 and ALDH, while negative for CD24 [38].

(ECM) and factors secreted by stromal cells. For example, interactions between hyaluronic acid (HA) and CD44 activates other signaling pathways which promote tumor malignancy such as Nanog, HER2 and NF- $\kappa\beta$ [39].

Signaling pathways activation induces proliferation, invasion and migration of BCSCs, this makes the primary breast tumors metastasizes towards distant organs. The epithelial-to-mesenchymal transition (EMT) and the mesenchymal-to-epithelial transition (MET) are key components of driving this metastasis process. As one may expect, BCSCs undergo both of these processes as they escape from the primary tumor site, enter the bloodstream and home to a new organ site to initiate tumor growth. These factors provides the signaling cues essential for the survival, growth and proliferation of BCSCs. Pathways that play key roles in embryonic development and adult tissue homeostasis have also been implicated in driving the phenotype of BCSCs. For instance, dysregulation of the Notch and Hedgehog pathways, which regulate normal stem cell differentiation and self-renewal [40].

These two pathways are frequently upregulated in breast cancer. Furthermore, some early work has shown that activation of these pathways can also be correlated to the resistance of BCSCs to therapy[41].

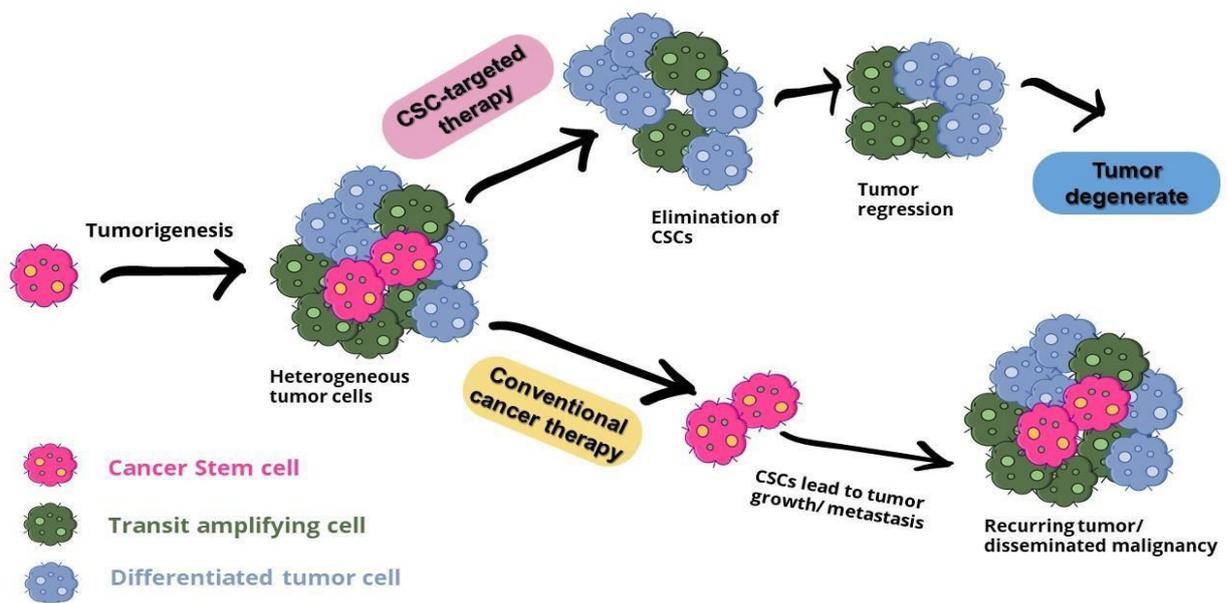


Figure (1): Therapeutic implications of cancer stem cells.

3.1. Breast Cancer Metabolism

Breast cancer stem cells are characterized from other tumor forming cells through expressing unique pattern of proteins and surface markers as fingerprints. Accordingly, breast cancer stem cells have distinctive metabolic properties to sustain their stemness and promote cancer progression [42]. ROS Level reduction promotes radio resistance and EMT phenotype of cancer stem cells, On the other hand, Notch signaling enhances breast cancer stem cell via interacting with cellular metabolism. Therapeutic Implications: Both pre-clinical and clinical studies have suggested a strong relationship between BCSCs and metastasis. Presence of CD44+/CD24- tumor cells in primary breast cancers powerfully induces metastases [43].

In addition, in vitro assays suggested that BCSCs increase motility and invasiveness [44]. Furthermore, there have been indications of the link between chemoresistance of CSCs and metastasis. Breast cancer metastases were enhanced by enriching them with CD44+/CD24- cells, indicative of a higher enrichment of BCSCs in these resistant tumors [45]. ATP-binding cassette transporters, ALDH activity and reactive oxygen species scavenging are regularly participate in inducing the therapeutic resistance of BCSCs [33]. As

a result, conventional therapeutic approaches routinely fail in eradicating breast tumors. Thus, discovering novel targeting approaches for BCSCs become a must.

3.2. Intrinsic Drug Resistance of CSCs

The routine conventional treatment failure attributed to drug resistance [46]. Drug resistance is characterized by resistance to a broad spectrum of drugs, which known as multidrug resistance (MDR) [47]. Two categories of resistance have been defined: (1) Acquired resistance, an induced response as a result of using specific treatment and (2) Intrinsic resistance, resistance is present even if the drugs in have never been used before [48]. Cancer stem cells characterized by a much higher endogenous against conventional therapies than non-cancer stem cells [49]. Therefore, chemotherapy and radiotherapy usually fail in eradicating CSCs, while they show great success in eliminating the bulk of the population of non-CSCs [50]. Glioblastoma, non-small cell lung cancer and colon cancer which involve cancer stem cells with CD133+ expression, presented worse 5-year overall survival and higher rates of chemotherapy and radiation resistance than the CD133-negative cells [51].

An increase with 2–4-fold in CSCs population usually occur after radiotherapy, that may attributed to a the activation of the DNA damage response. The main reason for drug resistance seems to reside in the similarities between CSCs and normal SCs [52]. Stem cells use various mechanisms to avoid death by apoptosis or cell senescence by the anti cancer drugs. The major mechanisms of MDR against commonly used therapeutic drugs are described below [53].

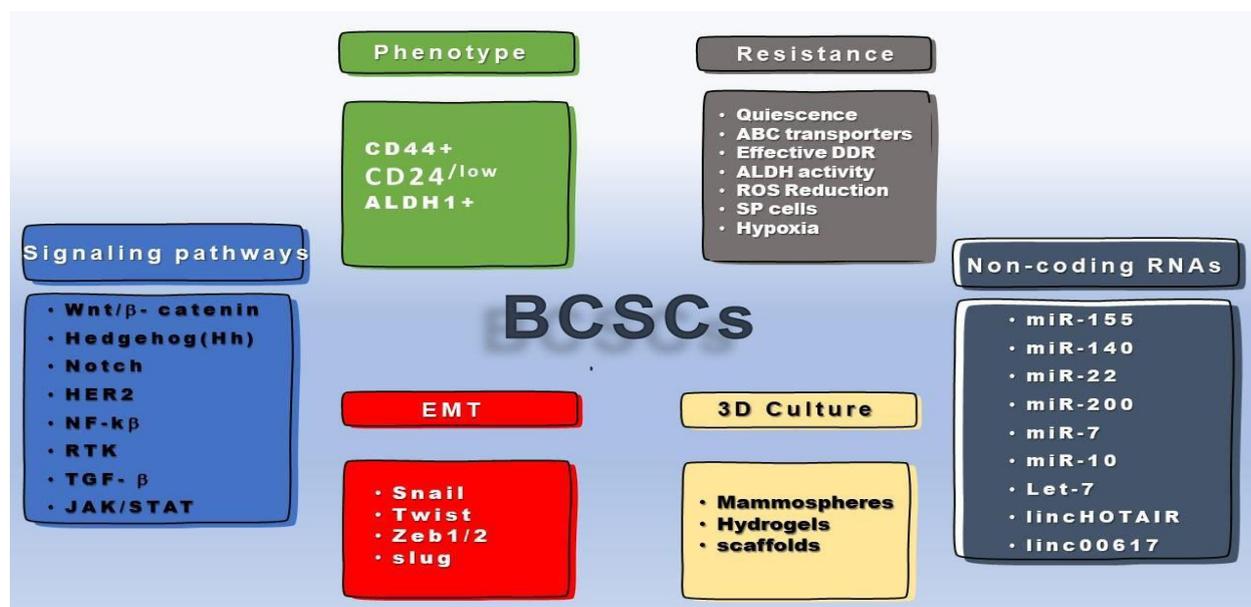


Figure (2): Summary of breast cancer stem cells (BCSCs) characteristics.

4. Novel strategies to target CSCs

Targeting strategies to inhibit self-renewal pathways in CSCs Conventional cancer treatment has the ability to target the bulk of sensitive tumor cells but they are not able to target cancer stem cells, which means that many tumor cells undergo apoptosis, while the CSCs survive by remaining in G0 phase and give rise to 'second-line tumors' with acquired resistance [54].

Therefore, current cancer research is interested in discovering novel targeting approaches for CSCs The embryonic signaling pathways enable the CSCs for maintaining their stemness properties even after being treated with anti cancer drugs. Normal Hh, Notch,

Wnt and BMI1 signaling pathways serve in maintaining the proper functionality in normal stem cells, while deregulated signaling pathways, is clearly associated with cancer stem cells, thus demonstrate their essential role in tumor initiating and maintainance[55].

As normal stem cells and CSCs share similarities in the signaling pathways, it would be extremely important while designing drugs to understand the complex biology of these pathways to destroy the CSCs and selectively sparing the normal stem cells.

4.1. CSC microenvironment inhibition

The major constituents of the tumor environment are CAFs, immune cells, multipotent stromal cells (MSCs), endothelial and perivascular cells, besides growth factors, cytokines, extracellular matrix (ECM) components, and extracellular vesicles, within a prevailing hypoxic region [56]. The tumor stroma serves in maintenance of CSCs and protecting the tumor from the immune system [57]. It also facilitates EMT induction, thus initiate tumors and promotes their progression, invasion, and metastasis. Furthermore, Multidrug resistance property of CSCs in usually developed in response to their interaction with the niche components in TME. Thus, targeting the TME is suggested to be a powerful and efficient way to prevent the phenomenon of multidrug resistance, a strategy which will successfully eradicate tumors.

4.2. Immunological approach to target CSCs

Immunotherapy is promising era of research that investigate several immunological mechanisms to target the tumor reinitiating ability of CSCs. For many years later, the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) was used by several immunotherapeutic approaches [58] or programmed death 1 (PD1)/programmed death receptor ligand-1 (PD-L1) [59]. In late stage cancer patients, blocking antibodies was of a great clinical response. Although the occurrence of such response, using single antibodies has a notable poor clinical outcomes. To achieve positive clinical outcomes and high recovery rate, several studies suggested using of combination therapeutic strategies [60]. PD-1 blockade promoted the antitumor efficacy of streptavidin-granulocyte-macrophage-CSF surface-modified bladder cancer stem cells vaccine. A recent study concluded that the CSC dendritic cell vaccine with CTLA-4 and PD-L1 blockades can successfully eradicate cancer in experimentally induced melanoma models [61].

In addition, CAR-T cells exhibited a great antitumor activity against different types of cancers [62]. In prostate cancer and NSCLC tumor models, CAR T-cells targeted against EpCAM and EGFR antigens could successfully eradicate CSCs and cancer cells [63].

4.3. The future of targeting metabolism to eradicate CSCs

Recent studies suggested to include metabolism as one of the cancer hallmarks and to consider the metabolic profile of CSCs a target for anticancer therapeutic approaches. One of the major reasons that facilitate designing pharmaceutical approaches to induce CSCs inhibition are their unique metabolic characteristics. Clearly, a unifying concept on which energy reactions are used by CSCs in response to stressful conditions like hypoxia, radiotherapy, and chemotherapy serve in identifying the metabolic Achille's heels which represent specific therapeutic targets. A great challenge for this is the inter-tumor heterogeneity. The CSCs isolation and cultivation techniques lead to diverse detected responses in analyzing their metabolic behavior[64].

The investigation of the metabolic phenotype of CSCs should be using highly recapitulates Experimental models using fresh patient or animal samples. Investigating and understanding the metabolic background of CSCs, its specific regulators and effectors may serve in facilitating designing of therapeutic approaches that specifically target CSCs, without

affecting the functions of normal stem cells, responsible for tissue homeostasis. Promising metabolism-based therapies are currently being investigated in characterizing and targeting CSCs[65].

4.4. Cancer stem cells and differentiation therapy

Exposure of ASCs and CSCs to specific signals of differentiation induce their differentiation into various cell types, including lineage-differentiated cells and nontumorigenic-differentiated cancer cells. Retinoic acid is Vit A lipophilic derivative which strongly induces normal stem cells to differentiate and proliferate. By interacting with RA, retinoic acid receptor (RAR) binds to the retinoic acid response element (RAREs) on DNA, as a result genes responsible for differentiation will be consequently undergo transcription [66].

Retinoic acid acts as an essential factor for intercellular signaling during vertebrate organogenesis (Marshall et al., 1996). Tretinoin is a pharmaceutical product of retinoic acid that was developed and conducted as an inducer in differentiation therapy for with acute promyelocytic leukemia (APL) patients [67]. The carboxylic acid form of vitamin A called (ATRA) Promoting differentiation by ATRA serves in enhancing sensitivity to therapies and reducing CSC motility and tumorigenicity by blocking angiogenic cytokines in glioblastomas [68].

ATRA based therapy to breast cancer stem-like cells, MCF7/C6, leads to cell differentiation, reduced invasion and metastasis, thus sensitivity to anticancer treatment increased. But in several glioma studies, ATRA induces imperfect CSCs differentiation, thereby glioma CSC frequently relapse [69]. BMP signaling is the most powerful inducer of differentiation. BMP is a secreted glycoprotein belonging to the transforming growth factor beta (TGF- β) superfamily. It is a ligand that binds to receptor BMPR2, which phosphorylates the neighbor receptor BMPR1. Then, phosphorylated BMPR1 induces phosphorylation of receptor-regulated Smads (R-Smads: including Smad1, Smad5, and Smad8). Furthermore, the phosphorylated R-Smads form heterodimers with co-Smad (Smad4) and translocate to the nucleus, where downstream genes are transcriptionally regulated [70].

A recent study also demonstrated that a variant type of BMP7 decreases tumor growth, angiogenesis, and invasion by inducing differentiation of glioma CSCs. Thus, induction of differentiation by activating BMP-associated signaling is a promising strategy for cancer treatment. A previous study showed that chromatin modifications of the BMPR2 promoter inhibit the expression of BMPR2 and that restoration of chromatin in the BMPR2 promoter or ectopic transduction of BMPR1B induces differentiation of CSCs and decreases tumorigenicity in glioblastomas[71].

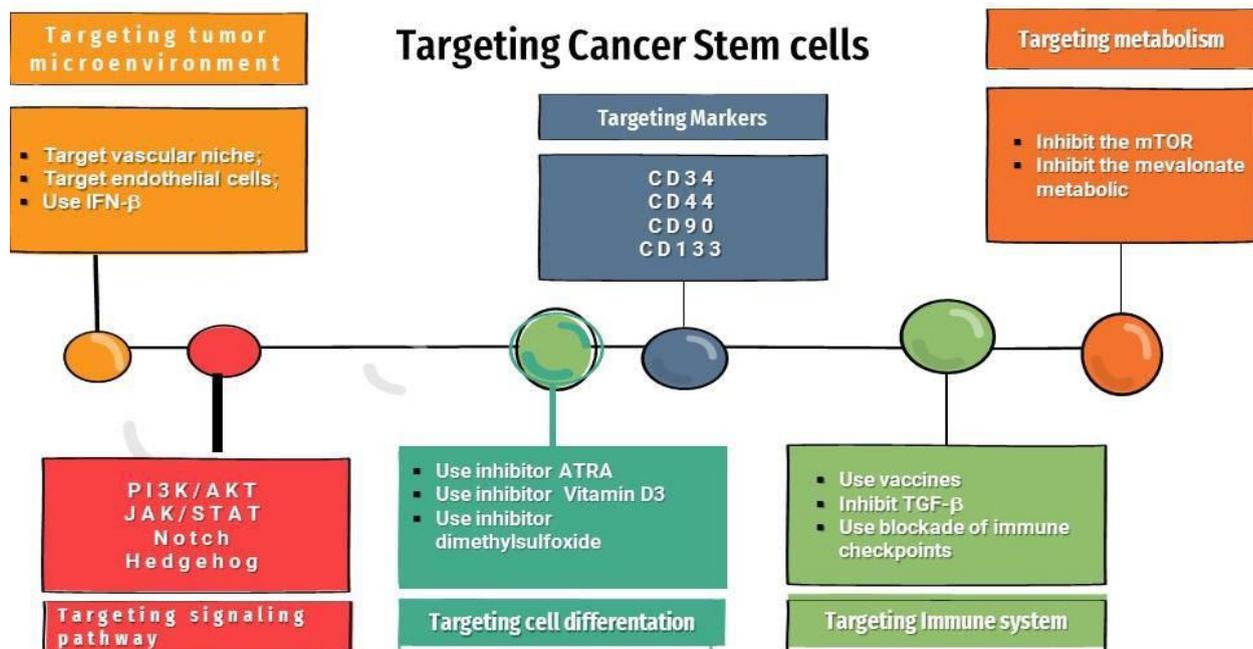


Figure (3): Cancer stem cells targeting strategies.

5. CONCLUSION

Cumulative evidence shows the presence of cancer initiating cells (CSCs) inside tumors accountable for drug resistance and conventional therapeutic failure and relapse. Confirmed developments in the identification, isolation, and characterization of BCSCs have been made. Consequently, many successful targeted therapeutic approaches for BCSCs have been effectively developed.

All of the existing targets here may be beneficial for developing effective targeted therapeutics for BCSCs, we also suggest that a combination of the pharmacological targets would be necessary as BCSC resistant populations may appear owing to the selection pressure resulting from monotherapy. In the same way, the plasticity of BCSCs to shift between stem-like and non-stem-like states suggests that the targeted therapy must not be limited to this small population, but rather to a combination treatment also addressing more distinguished progenitors and the cell population of bulk tumor.

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