NARRATIVE REVIEW

Comprehensive targeted treatment options available for Breast cancer stem cells; A literature review of the last 10 years' developments

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Abstract

Breast Cancer Stem Cells (BCSCs), unlike normal breast cells, exhibit the potential for self-regeneration and tumour formation and express unique markers. Studies have highlighted their role in tumour progression, recurrence, and treatment resistance. BCSCs can be one of the reasons that resistance is encountered despite recent advances in the treatment of breast cancer (BC). This review underlines the clinical implications at the molecular level of different cellular pathways, cellular level interactions in Tumour Micro Environment (TME), and types of markers and receptors involved in tumorigenesis. It accentuates the importance of comprehensive targeted treatment options available for BCSCs so that targeted modalities can be introduced to deal with treatment resistance. Stem cells (SCs) are a developing field, and limited data is available from our country to use stem cell-targeted treatment plans as a therapeutic option. Therefore, this literature review will provide insight for future research in this domain.

Keywords: Neoplastic, Stem Cells, Carcinogenesis, Transformation, Regeneration, Tumour, Breast Cancer

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Introduction

Stem cells in the human body are non-specialized cells that have the potential to differentiate into any cell type, self regenerates and is found in both adult and embryonic cells. Their potency to differentiate is reduced with each successive generation, with the highest potency in pluripotent stem cells (PSCs). Similarly, cancer stem cells(CSCs) harbour the same potential as SCs, with the difference only being the unregulated and unchecked differentiation and regeneration mechanism, which escapes many checkpoints and therefore results in the unregulated growth of abnormal progeny.[2] A team of researchers in 2006 discovered BSCs and proposed that a fully functional breast could form from a single breast stem cell(BSC)..[1] With more work in the field, CD44+CD24-/low lineage negative cancer cells were discovered in BC patients with the potential of tumour formation and labelled as BCSCs.[1] They are differentiated from other BC cells by expressing various cell surface markers, which include ALDH1(aldehyde dehydrogenase 1), (ABCG2)ATP-binding cassette subfamily G member 2, CD133, CD49f, (LGR5)Leucine-rich repeat-containing G protein-coupled receptor 5, $CD44^{+}$.[2]

The emergence of BCSCs, resistant to current treatment available, is an essential contributor to BC relapse and progression even after the treatment. Therefore, BCSCs are a new front in developing therapeutic options for BC patients.[3] Tumour microenvironment (TME) is crucial to study because different molecular signalling pathways inside TME control BCSCs, their regeneration, and tumorigenic and metastatic capacity.[3] Similarly, cellular interactions between BCSCs and surrounding cell populations in TME are widely studied, as it can directly through cell-cell interaction or indirectly through releasing factors causing the BCSCs to increase and grow.

Similarly, SCs become resistant by undergoing Mesenchymal to Epithelial Transition (MET). Breast cancer stem cells also escape many immune checkpoints by regulating their cell surface ligands/receptors and recruiting an immunosuppressive cell population to make their environment compatible with tumour growth and progression.[4]

This literature review is done to identify the clinical implications at the molecular level of different cellular pathways, cellular level interactions in TME, the role of EMT in tumorigenesis of BCSCs along with the significance of receptors and markers in BCSCs. Focus is given to new trends and developments relevant to BCSC-targeted treatment options available for BC patients who are non-responders and resistant to available therapeutic options and prospects in this field concerning comprehensive BCSCs-specific treatments.

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Targeted Treatment Options

Molecular level: Chemoresistance to conventional chemotherapeutics is a significant concern in BC treatment; however, ongoing research at the molecular level has attributed a part of this resistance to BCSCs. Therefore molecular-level signalling and the development of therapeutic options is a new strategy to overcome chemoresistance.

A study by Li et al highlighted one of the crucial molecular pathways. It demonstrated that in BCSCs, the levels of SOX21- AS1(long coding RNA) are deranged, which contributes to the stemness and proliferation of BCSCs, specifically CSC-MCF-7 lineage cells through inhibiting the HIPPO signalling pathway, which keeps the effector proteins YAP and TAZ unphosphorylated. It binds inside the nucleus to transcription factors resulting in genes which promote proliferation, stemness and invasion of the CSC-MCF-7 line of BCSCs.

Therefore, targeting SOX21-AS1 in BCSCs is a potential therapeutic option.[5]

MicroRNA like miR-223 acts as a tumour suppressor gene, and its concentration is downregulated in MDA-MB-231, MDA-MB-435 and TNBCSCs (CD44+ CD24-/low), while HAX-1 concentration is increased, which promotes tumorigenesis. However, by re-expressing mir-223, CSCs are re-sensitized to TRAIL-induced apoptosis by regulating the levels of HAX-1, so it has a potential role in inhibiting growth and progression in BCSCs. TRIAL selectively targets cancer cells without influencing normal cells; therefore, TRAIL is a potential anticancer agent, but its role is less effective when used alone for CSCs. Targeting miR-223 in combination with HAX-1 and TRAIL; has a potential therapeutic role in MDA-MB-231/435 and TNBC SCs.[6]

Another intracellular molecule, Src Kinase, is involved in the invasion and survival of many tumours; it is upregulated in many populations of BCSCs. SrcDN (Src Dominant Negative mutant gene) inhibits this molecular pathway and results in the inhibition of BCSCs.[7]

Similarly, the NOTCH pathway has a crucial role in maintaining BCSCs. Low levels of ALDH1A1 acetylation are associated with self-renewal capacity in BCSCs, especially in cells expressing high levels of ALDH1 activity (SCs). Acetylation of ALDH1A1 inhibits the CSCs population and its self-renewal capacity. NOTCH-induced SIRT2 activation causes ALDH1A1 deacetylation by enzymes PCAF and SIRT2, which promotes self-renewal and tumorigenesis in BCSCs. Therefore, inhibition of NOTCH reduces SIRT2 levels, resulting in the acetylation of ALDH1A1 and downstream inhibition of BCSCs activity.[8] In one of the clinical trials, compound PF-03084014, a selective gamma-secretase inhibitor which can target the NOTCH pathway in combination with docetaxel in TNBCSCs, showed antitumor activity against BCSCs.[9] Similarly, another trial demonstrated the efficacy of combination chemotherapy with BCSCs targeted therapy, i-e, gamma-secretase inhibitor GSI(MK-0752, an oral pharmaceutical) with docetaxel to target BCSCs. The results showed decreased expression of CD44+/CD24–, ALDH+ and mammosphere formation in the preclinical assessment of the BCSCs population by targeting the NOTCH pathway. Also, the role of notch ligand antibody Dll (delta-like ligand) as a combination treatment with paclitaxel reduced the BCSCs population in preclinical studies.[10]

Another pharmacological agent from the extract of ginger, 6-shogaol, is found to be effective in killing BCSCs lines. It inhibits the NOTCH pathway by using it in MCF-7 and MDA-MB-231 SCs. Also, concomitantly using it with γ-secretase inhibitor, DAPT further halts the progression of NOTCH pathway-mediated stemness; therefore, their combinatorial use is a potential therapeutic option to target BCSCs.[11]

A study showed TRAIL pathway-associated chemoresistance by downregulation of DR4 and DR5 receptors in the apoptosis pathway of the BT20 and MCF7 BCSCs line. The upregulation of COX-2 and PGE2 pathways also contributed to the chemoresistance of TRIAL-mediated apoptosis. Selective COX-2 inhibition using NS-398(COX-2 inhibitor) a crucial siRNA-mediated knockdown of COX-2, resulted in upregulation of DR5 and DR4 receptors, which reversed resistance to TRIAL-mediated cell apoptosis and decreased BCSCs population. Therefore, combination treatment inhibiting TRIAL and COX-2 had more promising results in treating the resistant BCSCs population.[12]

Fani et al. studied the role of Monobenzyltin Schiff base complex (compound C1), a non-platinum metal-based agent, on MCF-7 BCSCs by initiating apoptosis. It causes the release of mitochondrial cytochrome c, which activates caspase 9 and arrests MCF-7 BCSCs in the G0/G1 phase via expressing p21 and p27 proteins. Reduced levels of the ALDH1 population and MCF-7 BCSCs were seen while treatment with the C1 compound by causing apoptosis. Also, the compound downregulates self-renewal pathways of Wnt/β-catenin by reducing β-catenin and cyclin D1 levels and increasing β-catenin Ser33/Ser37/Thr4 levels in C1 treated cells.[13]

1-(3-chloro-4-(trifluoromethyl) thio) phenyl)-3-

(4(trifluoromethoxy) phenyl) urea (FND-4b) activates AMPK, which has been known to induce apoptosis and cease growth in many cancer cells. Johnson et al. used this novel drug to test its results on AMPK activation on BCs and found that higher dose treatment triggered death in MDA-MB-231 cells.[14]

Also, chemoresistance in BCSCs is correlated with Wnt/β-catenin pathway activation. The chemoresistance in TNBCSCs lines of MDA-MB-468 and MDA-MB-231 CSCs were associated with c-Myc induced over expressions of FZD8 levels (receptors for Wnt/β-catenin pathway), which regulates tumorigenesis in CSCs.[11] Another study depicted overexpression of Wnt pathway genes sFRP1 and b-CATENIN, especially in ER+ BCSCs, correlated it with stemness and metastasis in BC and predicted poor prognosis. Targeting the Wnt pathway using DKK1(specific Wnt inhibitor) reduced mammosphere formation in BCSCs population more in ER+ than in ER- BCSCs by inducing expression of sFRP1 and b-CATENIN. Therefore, inhibition of the FZD receptor of the Wnt pathway can prevent the activity of BCSCs in therapeutic doses.[15]

GTP-ase RhoC, from the Rho family, is an essential BCSCs metastasis regulator, especially in inflammatory BC. It promotes metastasis in SUM149 and MCF-10A BCSCs lines independent of their primary tumour status. It highly correlates with ALDH1 levels of the tumour, and its expression increases with BC progression and increases the metastatic potential of BCSCs.[16]

Similarly, upregulation of 3-hydroxy3-methylglutaryl-CoA synthase 1 (HMGCS1), a precursor enzyme in the mevalonate pathway, is found in the BCSCs population. It converts acetoacetyl-CoA to HMG-CoA in the mevalonate pathway. Its role in BCSCs enrichment, transformation and regulation in the basal subtype (MDA-MB-231 cell line) has been well studied. The pharmacological blockade of the mevalonate pathway by using statins and bisphosphonates resulted in a reduction of self-renewal and recurrences in BCSCs; however, the results by. Walsh et al. showed that selective targeting of HMGCS1 has more specific effects as compared to targeting the mevalonate pathway alone. Also, the levels of HMGCS1 are directly correlated with p53 mutation in tumour cell lines, higher tumour grades, aggressiveness and metastatic potential. Therefore, HMGCS1 is a promising therapeutic target in the basal type of CSCs.[17]

PARP 1 protein is involved in cellular functions of proliferation, apoptosis, transformation and DNA repair. In the study by Gilabert et al., BRCA 1 mutated ALDH+ cells showed over-expression of PARP1, which contributes to resistance in ALDH1 BCSCs. However, these cells showed resistance to olaparib, a PARP inhibitor. They explained that PARP1 contributes to some intrinsic DNA repair capacity of BCSCs in BRCA mutated cells, which is independent of PARP inhibition and contributes to resistance to OLAPARIB. They concluded that only a selected subunit of the CSCs population shows resistance to olaparib, which is also attributable to high PARP1 expression in these cells.[18]

Another trial studied the role of the combination treatment of taxane with chloroquine to target anthracycline-resistant metastatic BCSCs. Chloroquine halted BCSCs renewal by targeting Jak2 and STAT3 signalling pathways. Their results showed significant improvement when dual therapy was used against BCSCs targeting.[19]

NF-κB signalling is a pro-inflammatory pathway in TME. These are abundant in CSCs. Gomez-Cabrero et al. emphasised the role of IMD-0354, a drug that inhibits NF-κB and doxorubicin (using targeted nano-particles) and targets encapsulated tumour bulk cells. IMD-0354 derived inhibition of CSCs was evident by decreased sub-population of BCSCs in TME along with reduced expressions of Oct4, Nanog and Sox2 genes, and also appreciated its effect on survivin during the study. In addition, IMD-0354's effects on non-CSCs were also demonstrated by reduced viability and increased apoptosis in non-CSCs. Moreover, it reduces the cardio-toxicity of Doxorubicin, further encouraging its use in MDR breast cancer treatment.[20]

The above literature suggests that 6-Shagol, Chloroquine, PF 03084014, Gamma Secretase inhibitors (GSIs) have shown promising results in overcoming BCSCs stemness. Also, inhibition of cSrc, Sox21-ASI, Myc, WNT, ALDH1, PARP1, RhoC GTPase, HMG CSI and promoting miRNA 223/200c/205, ALDH1A1 acetylation will result in decreasing BCSCs population and resistance.

Epithelial-Mesenchymal Transition (EMT) level

Epithelial-mesenchymal transition (EMT) is a well-kept biological pathway that transforms epithelial(E) cells to become mobile mesenchymal(M) cells to acquire invasiveness. It does so by losing E-cadherins and overexpressing N-cadherins and Vimentins in EMT. With EMT, human neoplastic mammary epithelial cells develop stem cell-like characteristics.

Gonçalves et al, confirmed the role of these markers during EMT in CMT-U229 and MCF-7 BCSCs.[21] They also looked at how melatonin (N-acetyl-5-methoxytryptamine) therapy affected the regulation of

several stem cell molecular markers. According to their results, E-cadherin expression increased following melatonin therapy with a concomitant decrease in levels of N-cadherin and vimentin. Melatonin therapy was found effective in MCF-7 BCSCs as well. Melatonin's effectiveness as anti-metastatic and anti-invasive in many cellular models is well documentted.[21]

RNA-binding protein Lin28 is only produced in undifferentiated embryonic stem cells and is highly conserved. In many healthy adult tissues, Lin28 expression has been markedly downregulated. The ectopic expression of Lin28 is found to be associated with grave prognosis in BC. Liu et al. found Lin28 levels highly expressed in (M) type cells contrary to (E) types, where it downregulates Let-7a levels and causes EMT in BCSCs.[22]

Connective tissue growth factor WISP2, often called CCN5, is involved in various tumorigenic processes. However, the expression of WISP2 is correlated with less aggressive BCSCs, like MCF7. Loss of WISP2 is linked to EMT; therefore, it plays a critical role in maintaining less aggressive subtypes of BCs (hormone receptor-positive BC). The same is demonstrated by Ferrand et al., who showed that deletion of WISP2 expression causes a sharp rise in the proportion of CD44(high)/CD24(low) MCF7 cells, a key hallmark for stemness and metastasis in BC.[23]

Tumorigenesis is also attributable to deregulated miRNA expression. Patel et al. confirmed over expression of miR-15a, miR-200a, miR-200b, miR-203 and miR-429 with BMI1 down-regulation, a transcriptional repressor, in different BCSCs. BMI1 is associated with (EMT) transformation in many malignancies, promoting cell growth, invasion, and, ultimately, metastasis. The role of chemotherapy agent cisplatin was directly related to the upregulation of miRNAs, demonstrating strong potential as anticancer therapeutics against BCSCs.[24] Therapeutic strategies in inhibiting BC metastasis also look promising with the identification of Periostin (POSTN); this matricellular protein POSTN is over-expressed in various malignancies along with BC. By inhibiting stress-induced cancer cell apoptosis and enhancing angiogenesis by activating the Akt pathway, POSTN effectively promotes metastasis in colon cancer. Wang et al. demonstrated that POSTN therapy and its overexpression are associated with stemness and the EMT process in BCs. Therefore, inhibition of POSTN is a potential therapeutic option to target BCSCs.[25]

Another MicroRNA which has been studied is miR-200c; its low levels are correlated with the chemoresistance of the BT474 BCSCs line. Tyrosine kinase receptors TrkB and Bmi1 are target genes for mir-200c, which acquired chemoresistance by epithelial to the mesenchymal transformation of the target cells with each consecutive cycle of chemotherapy and produce low miR-200c levels, which maintain stemness in BCSCs via PI3K/Akt pathway. MDA-MB 436 CSCs, under modulation of miR-200c, acquire resistance through the PI3K/Akt pathway, which produces chemoresistance by producing TrkB and Bmi1 in CSCs and produce signals for survival; therefore, micro-RNA modulating anticancer agents can be a new front to overcome the resistance of stem cells.[26]

Similarly, Mayoral-Varo et al. worked on the role of miR205 in SUM159PT, a TNBCSCs line with mutated p53 and PIK3CA pathway. They correlated its reduced levels with tumour progression and stemness via increased expression for VEGFα, ErbB3, Zeb1, Fyn and Lyn A/B and Myc/cyclin-D1 levels (tumour promoters) and reducing expression of p27kip1 which inhibits tumour progression by Src Kinases/Stat3 axis activation. miR205 also inhibits epithelial to mesenchymal transition for stemness. Anti-miR205 co-expression during the experimental study reverted the cells to stemness and progression and ZEB1/2-induced EMT. Similar levels of miR205 were identified in MDA-MB-231 and Hs578t TNBCSCs. Modulating levels of miR205 is a potential therapeutic option in TNBCSCs, along with targeting Src Kinases/Stat3 axis.[27]

The role of TGF-β is well known in tumorigenesis and proliferation of BCSCs, specifically MCF7- sh-WISP2 BCSCs and SCs marker expression (Nanog and Oct 3 and 4). TGF-β induces genes like SNAI2, ZEB1 and SMAD3, which are involved in EMT and stemness of BCSCs. Hence, inhibiting the TGF-β pathway is a potential therapeutic option in BCSCs.[23]

Above stated studies suggest that targeting Lin28, WISP2/CCN5, POSTIN (an extracellular component of TME) and BMI1 has shown a reduction in BCSCs; also, melatonin can be a new therapeutic modality as it has been shown promising results in targeting BCSCs in experimental trials.

Tumour Micro-Environment (TME) level

A variety of cells are floating in an Extra-Cellular Matrix (ECM) to form the complex ecosystem known as the TME. Endothelium, cancer-associated fibroblasts (CAFs), and immune system cells are prominent examples of cell types. Multiple pathways control the behaviour of the BCSCs in the TME, including whether it engages in self-renewal, quiescence, apoptosis or maturation. The ECM provides a conduction medium to the various TME cells and acts as an architectural support. According to a study

by Cazet et al., CAFs are key cells in the medium and respond to Hedgehog (Hh) ligands stimulation. Hh signalling is active only in a subset of breast malignancies, particularly in TNBC subtypes. Neoplastic cells acquire a stem-like phenotype that is resistant to chemotherapy and is attributable to activated CAFs. In both animal/human models, the EDALINE trial demonstrated that Small Molecule Inhibitors like Vismodegib and Sonidegib acted on CAFs and increased the efficacy of docetaxel chemotherapy in TME.28

Sehl et al. made a stochastic model to investigate the static MET-like state and used CD44+/CD24- cells to convert it into an active MET-like state characterised by ALDH over-expression. They studied the frequency of interconversion between epithelial and mesenchymal BCSCs in TME and looked at the impact of treatments that target the BCSC niche and microenvironment. They concluded that the best treatment combinations to lower the number of BCSCs were the simultaneous suppression of HER2 and IL-6 inflammatory feedback loops.[29] In short, new treatment options might be available by targeting IL-6 inflammatory feedback loops, ALDH, and hedgehog signalling to CAFs inside TME.

Receptors and Tumour markers Level

Different receptor-related pathways and tumour markers play an essential role in carcinogenesis, metastasis, resistance to treatments and clinical recurrence if stem cells in the breast are involved. Accordingly, targeting these receptors and tumour markers can bring a notifiable improvement in the breast cancer treatment outcome. BCSCs most abundantly express the enzyme Aldehyde Dehydrogenase (ALDH) and are frequently characterised by CD24-/ CD44+ phenotype. ALDH+ BCSCs, which constitute only 0.1% to 10% of the bulk tumour, majorly exhibit chemokine receptor-1 (CXCR-1, a receptor for CXCL-8), unlike the bulk tumour. To reduce the incidence of breast tumour recurrence and to target BSCSs along with the bulk tumours, Schott et al., in their phase-1b pilot study, used Reparixin (an allosteric inhibitor of CXCR1/2) along with paclitaxel. This study highlighted the tolerability and safety of the paclitaxel + reparixin combination when given weekly in metastatic breast cancer.[30] In continuation, Goldstein et al., in their multi-centric clinical trials, observed that CD24−/CD44+ and ALDH+ expression was downregulated by ≥ 20% in HER-2-negative non-metastatic BC patients, who were treated with reparixin orally.[31]

Syndecan-1 (CD138), a co-receptor and a molecular marker for EMT, regulates WNT and IL-6/STAT3 signalling pathways in BCSCs. Ibrahim et al. tested BCSCs lines (MDA-MB-231 and MCF-7 cells) and concluded that the knockdown of Syndecan-1 is correlated with BCSCs reduction. Syndecan-1 is also involved in the down-regulation of STAT-3, NFkB, and WNT signalling co-receptor expression by >45%. These results highlight the importance of Syndecan-1 as a novel therapeutic target in breast cancer treatment.[32] Regarding inflammatory pathways, PPARa/HIF1a (Peroxisome proliferator-activated receptor-a and hypoxia-inducible factor-1a) receptors regulate IL-6, Carbonic anhydrase IX and apolipoprotein-E (pro-inflammatory cytokines) and promotes tumorigenesis in BCSCs. They also regulate miRNA130b/miRNA17-5p-dependent pathways and antagonise PPARc(gamma) receptors. Pioglitazone (PGZ) acts as an agonist to PPARc and up-regulates PPARc expression by affecting the PPARa/HIF1a interplay.[33]

BCSCs express Survivin protein, which is anti-apoptotic. Wanandi et al. discovered andrographolide, a compound with an antagonist activity against survivin. It activates apoptotic pathways inside BCSCs, proving it to be one of the potential therapeutic agents for breast cancer treatment.[34]

Nucleostemin (NS), a protein with an affinity for GTP, controls various RNA/DNA-related pathways and is a pivotal modulator to promote stemness in BCSCs. Manal M. Sami et al. identified that NS's expression correlates to HER-2 positive and TNBC BCSCs aggressiveness and signifies NS's importance as a therapeutic target in treatment-resistant and aggressive BC subtypes.[35]

Sodium Butyrate (NaBu), a potential anti-cancer agent, also induces arrest/apoptosis in BCSCs. Recently, a breast cancer subpopulation that is resistant to NaBu treatment has been identified. Sun et al., in their study, correlated c-MET expression in BCSCs with resistance to NaBu, especially in CD133+ cells. They also found that treating these cells with MET siRNA combined NaBu inhibited BC progression and incidence rate in these stem cells.[36]

In the adjuvant setting, trastuzumab addition to chemotherapy, has been shown to reduce recurrence rates and improve survival in Her2neu cells. This reduction of recurrence and long-term survival benefit has been linked to the supposition that BCSCs express HER2, an essential regulator of BCSC self-renewal.10 Riley et al. identified that even in cases where the tumour bulk does not over-express HER2, in due course HER2- targeted therapy (Trastuzumab), will eliminate the tumour by having a detrimental effect on the BCSCs' life span.[37]

Wang et al. identified the role of niclosamide in the down-regulation of the SCs pathway. It causes inhibition of the

Table-1: Summary of studies targeting BCSCs at molecular, Epithelial-Mesenchymal Transition, Tumor Micro-Environment and Receptor level in Breast Cancer.

Authors Names	Location of the study	Journal name	Key findings
Molecular level			
Ray et al.[11]	India	PLOS ONE	-This study investigated the inhibitory activity of the ginger-derived compound 6-shogaol against BCSCs and- The inhibitory action of 6-shogaol on spheroid growth, and sustainability was because of γ-secretase mediated down-regulation of Notch signalling.
Anand et al.[19]	Houston, USA	Clinical Breast Cancer	-In a phase II trial for breast cancer patients who were refractory to anthracycline-based chemotherapy, chloroquine was combined with Taxane or Taxane-like chemotherapy.-Overall response rate of the combination was 45% in contrast to 30% with chemotherapy alone.
Mayoral-Varo et al.[7]	Madrid, Spain	PLOS ONE	This study highlights c-Src functionality for breast cancer stem cell maintenance.
Li et al.[5]	Linyi, China	FEBS open bio	-SOX21-AS1 promotes the stemness of CSC-MCF-7 cells-SOX21-AS1 may promote the stemness viability, proliferation, migration and invasion of BCSCs by inhibiting the Hippo pathway.
Sun et al.[6]	Jinan, China	PLOS ONE	-miR-223 expression was down-regulated in CD44+ CD24-/low TNBCSCs compared withnon-CSCs.-MicroRNA-223 Increases the sensitivity ofTriple-Negative Breast Cancer Stem Cells toTRAIL-Induced apoptosis by targeting HAX-1
Fani et al.[13]	Malaysia	PLOS ONE	-Monobenzyltin Complex C1 Induces apoptosis in MCF-7 Breast Cancer Cells through the Intrinsic Signaling Pathway and the Targeting of MCF-7-Derived BCSCs via the Wnt/β-Catenin Signaling Pathway
Johnson et al.[14]	USA	PLOS ONE	AMP-activated protein kinase (AMPK) is a significant energy regulator suppressing tumour growth. This project aimed to test the effects of FND-4b treatment on AMPK activation, proliferation, and apoptosis in breast cancer, with a particular emphasis on Triple-negative breast cancer.
Zhao et al.[8]	California, USA	The Journal of Clinical Investigation	-Evaluation of breast carcinoma tissues from patients revealed that cells with high Aldehyde dehydrogenase (ALDH1) activity have low ALDH1A1 acetylation and are capable of self-renewal.-Acetylation of ALDH1A1 inhibited both the stem cell population and self-renewal properties in breast cancer.-NOTCH signalling activated ALDH1A1 by induction of SIRT2, leading to ALDH1A1 deacetylation and enzymatic activation to promote breast CSCs.
Locatelli et al.[9]	Italy, Belgium, USA	Onco-target	In this phase Ib, open-label, multicenter study (NCT01876251), they evaluated PF-03084014, a selective gamma-secretase inhibitor in patients with advanced triple-negative breast cancer and BCSCs.
Schott et.al.[10]	USA	Clin Cancer Res.	-This study evaluated the impact of gamma-secretase inhibitors (GSI) on the BCSC population and the efficacy of combining GSIwith docetaxel treatment.
Rosenthal et al.[16]	Michigan, USA	PLOS ONE	This study identifies RhoC GTPase as an important regulator of BCSC metastasis and highlights that RhoC modulates the frequency of BCSCs within a population.
Walsh et al.[17]	UK, Sweden, Canada	PLOS ONE	Mevalonate precursor enzyme HMGCS1 is a novel marker and key mediator of BCSCs enrichment in luminal and basal models of breast cancer.-Pharmacological inhibition of HMGCS1 could be a superior novel treatment approach for breast cancer patients via additional CSC blocking functions.
Chandrasekaran et al.[12]	USA	PLOS ONE	-This study accredits the TRAIL (TNF-alpha-related-apoptosis-inducing-ligand)- resistance and cancer stem cell phenotype observed in tumour spheroids to the upregulation of cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2) pathway.-Inhibition of the COX-2/PGE2 pathway by treating tumour spheroids with NS-398, a selective COX-2 inhibitor, reverses the TRAIL resistance and decreases the incidence of a CD44 and CD24population.
Lamb et al.[15]	UK	PLOS ONE	-This study highlights differential Wnt signalling in breast cancer subtypes and activity in patient-derived metastatic cancer stem-like cells, indicating a potential for Wnt-targeted treatment in breast cancers.
Gomez-Cabrero et al.[20]	USA	PLOS ONE	This study used IMD-0354, an NF-kB inhibitor, identified for targeting cancer stem cells (CSCs) in a combination therapy with doxorubicin encapsulated in targeted nanoparticles. IMD-0354 did target CSCs, evidenced by a decrease in the side population, demonstrated by the inhibition of the following: dye/drug efflux, reduction in ABC transporters, and colony formation in soft agar and low attachment plates.
Epithelial-Mesenchymal Transition Pathways:			
Gonçalves et al.[21]	Sweden	PLOS ONE	This study evaluates the formation of mammospheres from the canine and human breast cancer cell lines, CMT-U229 and MCF-7, and the effects of melatonin treatmenton the modulation of stem cell and EMT molecular markers: OCT4, E-cadherin, N-cadherinand vimentin, as well as on cell viability and invasiveness of the cells from mammospheres.

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Liu et al.[22]	Guangzhou, China	PLOS ONE	-This study demonstrates that the RNA binding protein Lin28 Induces Epithelial-to-Mesenchymal Transition and Stemness via Downregulation of Let-7a in Breast Cancer Cells.
Ferrand N et.al.[23]	Paris, France	PLOS ONE	-Loss of WISP2/CCN5 (associated with EMT) in Estrogen-Dependent MCF7 Human Breast Cancer Cells Promotes a Stem-Like Cell Phenotype
Wang et. al.[25]	Ohio, USA	PLOS ONE	-This study demonstrates that Periostin promotes a stem cell-like trait and a mesenchymal phenotype in human mammary epithelial cells and breast cancer cells.-Targeting POSTN and other extracellular matrix components of the tumour microenvironment may help to develop new therapeutical strategies to inhibit tumour metastasis.
Patel et.al.[24]	India	PLOS ONE	-This study demonstrates that regulating BMI1 expression via miRNAs promote Mesenchymal to Epithelial Transition (MET) and sensitizes breast cancer cell to a chemotherapeutic drug like cisplatin
Kopp et al.[26]	Munich, Germany	PLOS ONE	-Chemo-resistant BCSCs (to doxorubicin) display a mesenchymal-like phenotype with decreased levels of miR-200c.-miR-200c Sensitizes Breast Cancer Cells to Doxorubicin Treatment by Decreasing TrkB and Bmi1 Expression
Mayoral-Varo et al.[27]	United Kingdom	PLOS ONE	-that miR205 inhibited SUM159PT cancer-stem cell renewal, expression in mammospheres of CD44 and ALDH1 stem-cell markers, TAZ, and E2A.E12.
Niche and Microenvironment			
Sehl et. al.[29]	USA	PLOS ONE	-This study presents a mathematical model of the breast cancer stem cell (BCSC) niche to predict population dynamics during carcinogenesis and in response to treatment.
Cazet et al.[28]	Australia	Nature communication	-This study identifies Hedgehog signalling to CAFs as a novel mediator of CSC plasticity and an exciting new therapeutic target in TNBC.
Markers/Receptors:			
Ibrahim et. al.[32]	Germany	PLOS ONE	Syndecan-1 (CD138) is a molecular marker associated with the epithelial-mesenchymal transition during development and carcinogenesis. This study deciphered the functional impact of Syndecan-1 knockdown using RNA interference on the breast cancer stem cell phenotype of human triple-negative MDA-MB-231 and MCF-7 cells. Syndecan-1 depletion reduced the side population significantly in both cell types of stem cells.
Schott et. Al.[30]	USA	Clinical Cancer Research	Reparixin is an investigational allosteric inhibitor of chemokine receptors 1 and 2 (CXCR1/2), selectively expressed by breast cancer stem cells. This Phase Ib clinical trial showed that weekly paclitaxel plus reparixin in MBC appeared safe and targeted the BCSCs population with good outcomes.
Papi et. Al.[33]	Italy	PLOS ONE	Pro-inflammatory cytokine network envisages the potential role of anti-inflammatory molecules as new anti-cancer targets.This study reported the relationship between nuclear receptors (PPARa/PPARc) activity and the modulation of the pro-inflammatory phenotype in breast cancer stem cells.
Sami et. al.[35]	United Arab Emirates	Medicine®	Nucleostemin (NS) is thought to be a key molecule for cancer cell stemness. They found NS expression associated with the less differentiated and more advanced stage.
Wanandi et al.[34]	Indonesia	PLOS ONE	In this study, Andrographolide was selected as a lead compound through silico molecular docking with survivin, caspase-9, and caspase-3. The study revealed that andrographolide could be considered an anti-cancer compound that targets BCSCs due to its molecular interactions with survivin, caspase-9, and caspase-3, which induce apoptosis.
Wang et al.[38]	Taiwan	PLOS ONE	In this study, side population spheres (SPS) from breast cancer cell lines were produced, and the effect of niclosamide on SPS was studied. It shows that Niclosamide downregulated stem cell pathways, inhibited the formation of spheroids, and induced apoptosis in breast cancer SPS. The results of this proof-of-principle study may facilitate the development of new breast cancer therapies shortly.
Riley et al.[37]	USA	PLOS ONE	In this study, a mathematical model was developed to examine breast cancer stem cells(BCSC) population dynamics and predict the optimal therapy duration. It was found that lower susceptibility of BCSCs and increased rates of dedifferentiation entailed longer extinction times, indicating a need for prolonged administration of HER2-targeted therapy.
Zhou et al.[39]	China	PLOS ONE	This study investigated the potential of curcumin to reduce the breast cancer stem cell (BCSC) population for sensitising breast cancer cells to mitomycin C (MMC) both in vitro and in vivo. Curcumin improved the sensitivity of paclitaxel, cisplatin, and doxorubicin in breast cancer cell lines MCF-7 and MDA-MB-231 cell lines.
Sun et al.[36]	China	PLOS ONE	In this study, a specific breast cancer cell population resistant to Sodium Butyrate (NaBu) treatment was identified. They identified that NaBu-resistant cells express the cancer stem cell marker, the CD133, whereas only 10% of intact cells present the CD133 antigen. The CD133+ group also shows a higher level of c-MET. A combination treatment of MET siRNA and NaBu efficiently prohibited breast cancer progression, and the incident rate of the tumour decreased to 18%.
Goldstein et al.[31]	USA	Breast Cancer Research	CXCR1, one of the receptors for CXCL8, has been identified in breast cancer (BC) stem cells. Reparixin, an investigational allosteric inhibitor of CXCR1, reduced the cancer stem cell content of human breast cancer xenograft in mice. In this multicentre, single-arm trial, women with HER-2-negative operable BC received reparixin for 21 days before surgery. Reparixin appeared safe and well-tolerated. CSCs were reduced in several patients as measured by flow cytometry, suggesting targeting of CXCR1 on CSC.

spheroid formation and induction of apoptosis in BCSCs, highlighting the efficacy of this drug in Breast cancer treatment.[38]

Multi-drug-resistant transporters (MDR) that belong to the (ABC) transporter superfamily have a notifiable role in cancer cell drug resistance. Zhou et al., in their study, have underlined the imperative role of Curcumin, a compound to reduce the BCSCs population and sensitise CSCs by down-regulating ABC transporters G2 and C1 expression.

Moreover, Curcumin ameliorates the sensitivity of paclitaxel, doxorubicin and cisplatin in MDA-MB231 and MCF-7 BCSCs, by decreasing the Maximal Inhibitory Concentration of these drugs.[39]

It is summarised from the above-stated studies that inhibition of syndecan-1, nucleostemin, ABCC3, FAM83A, PPARa/PPARc, CXCR1/2, AMPK and receptors for survivin have an inhibitory effect on BCSCs. Also, the tumour response of BCSCs to treatment can be correlated using the TAZ receptors score with trastuzumab.

Molecular compounds like FND-46(targeting AMPK receptors), andrographolide (targeting survivin), reparixin(targeting CXCR1receptors) and combination treatments using curcumin with paclitaxel, niclosamide, MET siRNA with NaBu and modifying these treatments according to receptor subtypes and molecular level pathology will help change effective treatment plans to overcome BCSCs resistance and stemness.

Table I summarizes these studies in tabular form.

Conclusion

Understanding our knowledge regarding BCSCs at the molecular level will help us study the chemoresistance faced in current BC treatments, and along with it, new therapeutic options can be implemented in resistant BC cases. By focusing our research on targeting BCSCs, comprehensive targeted therapies can be formulated to treat breast cancer patients effectively.

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References

1. Visvader JE, Smith GH. Murine mammary epithelial stem cells: discovery, function, and current status. Cold Spring Harb Perspect Biol 2011;3:a004879. doi: 10.1101/cshperspect.a004879.
2. Wang L, Jin Z, Master RP, Maharjan CK, Carelock ME, Reccoppa TBA, et al. Breast Cancer Stem Cells: Signaling Pathways, Cellular Interactions, and Therapeutic Implications. Cancers (Basel) 2022;14:3287. doi: 10.3390/cancers14133287.
3. Izawa Y, Kashii-Magaribuchi K, Yoshida K, Nosaka M, Tsuji N, Yamamoto A, et al. Stem-like Human Breast Cancer Cells Initiate Vasculogenic Mimicry on Matrigel. Acta Histochem Cytochem 2018;51:173-83. doi: 10.1267/ahc.18041.
4. Xu Y, Dong X, Qi P, Ye Y, Shen W, Leng L, et al. Sox2 Communicates with Tregs Through CCL1 to Promote the Stemness Property of Breast Cancer Cells. Stem Cells 2017;35:2351-65. doi: 10.1002/stem.2720.
5. Li L, Meng D, Wang R. Long non-coding RNA SOX21-AS1 enhances the stemness of breast cancer cells via the Hippo pathway. FEBS Open Bio 2021;11:251-64. doi: 10.1002/2211-5463.13015.
6. Sun X, Li Y, Zheng M, Zuo W, Zheng W. MicroRNA-223 Increases the Sensitivity of Triple-Negative Breast Cancer Stem Cells to TRAIL-Induced Apoptosis by Targeting HAX-1. PLoS One 2016;11:e0162754. doi: 10.1371/journal.pone.0162754.
7. Mayoral-Varo V, Calcabrini A, Sánchez-Bailón MP, Martínez-Costa ÓH, González-Páramos C, Ciordia S, et al. c-Src functionality controls self-renewal and glucose metabolism in MCF7 breast cancer stem cells. PLoS One 2020;15:e0235850. doi: 10.1371/journal.pone.0235850.
8. Zhao D, Mo Y, Li MT, Zou SW, Cheng ZL, Sun YP, et al. NOTCH-induced aldehyde dehydrogenase 1A1 deacetylation promotes breast cancer stem cells. J Clin Invest 2014;124:5453-65. doi: 10.1172/JCI76611.
9. Locatelli MA, Aftimos P, Dees EC, LoRusso PM, Pegram MD, Awada A, et al. Phase I study of the gamma secretase inhibitor PF-03084014 in combination with docetaxel in patients with advanced triple-negative breast cancer. Oncotarget 2017;8:2320-8. doi: 10.18632/oncotarget.13727.
10. Schott AF, Landis MD, Dontu G, Griffith KA, Layman RM, Krop I, et al. Preclinical and clinical studies of gamma secretase inhibitors with docetaxel on human breast tumors. Clin Cancer Res 2013;19:1512-24. doi: 10.1158/1078-0432.CCR-11-3326.
11. Ray A, Vasudevan S, Sengupta S. 6-Shogaol Inhibits Breast Cancer Cells and Stem Cell-Like Spheroids by Modulation of Notch Signaling Pathway and Induction of Autophagic Cell Death. PLoS One 2015;10:e0137614. doi: 10.1371/journal.pone.0137614.
12. Chandrasekaran S, Marshall JR, Messing JA, Hsu JW, King MR. TRAIL-mediated apoptosis in breast cancer cells cultured as 3D spheroids. PLoS One 2014;9:e111487. doi: 10.1371/journal.pone.0111487.
13. Fani S, Dehghan F, Karimian H, Mun Lo K, Ebrahimi Nigjeh S, Swee Keong Y, et al. Monobenzyltin Complex C1 Induces Apoptosis in MCF-7 Breast Cancer Cells through the Intrinsic Signaling Pathway and through the Targeting of MCF-7-Derived Breast Cancer Stem Cells via the Wnt/β-Catenin Signaling Pathway. PLoS One 2016;11:e0160836. doi: 10.1371/journal.pone.0160836.
14. Johnson J, Rychahou P, Sviripa VM, Weiss HL, Liu C, Watt DS, et al. Induction of AMPK activation by N,N'-diarylurea FND-4b decreases growth and increases apoptosis in triple negative and estrogen-receptor positive breast cancers. PLoS One 2019;14:e0209392. doi: 10.1371/journal.pone.0209392.
15. Lamb R, Ablett MP, Spence K, Landberg G, Sims AH, Clarke RB. Wnt pathway activity in breast cancer sub-types and stem-like cells. PLoS One 2013;8:e67811. doi: 10.1371/journal.pone.0067811.
16. Rosenthal DT, Zhang J, Bao L, Zhu L, Wu Z, Toy K, et al. RhoC impacts the metastatic potential and abundance of breast cancer stem cells. PLoS One 2012;7:e40979. doi: 10.1371/journal.pone.0040979.
17. Walsh CA, Akrap N, Garre E, Magnusson Y, Harrison H, Andersson D, et al. The mevalonate precursor enzyme HMGCS1 is a novel marker and key mediator of cancer stem cell enrichment in luminal and basal models of breast cancer. PLoS One 2020;15:e0236187. doi: 10.1371/journal.pone.0236187.
18. Gilabert M, Launay S, Ginestier C, Bertucci F, Audebert S, Pophillat

M, et al. Poly(ADP-ribose) polymerase 1 (PARP1) overexpression in human breast cancer stem cells and resistance to olaparib. PLoS One 2014;9:e104302. doi: 10.1371/journal.pone.0104302.

19. Anand K, Niravath P, Patel T, Ensor J, Rodriguez A, Boone T, et al. A Phase II Study of the Efficacy and Safety of Chloroquine in Combination With Taxanes in the Treatment of Patients With Advanced or Metastatic Anthracycline-refractory Breast Cancer. Clin Breast Cancer 2021;21:199-204. doi: 10.1016/j.clbc.2020.09.015.
20. Gomez-Cabrero A, Wrasidlo W, Reisfeld RA. IMD-0354 targets breast cancer stem cells: a novel approach for an adjuvant to chemotherapy to prevent multidrug resistance in a murine model. PLoS One 2013;8:e73607. doi: 10.1371/journal.pone.0073607.
21. Gonçalves Ndo N, Colombo J, Lopes JR, Gelaleti GB, Moschetta MG, Sonehara NM, et al. Effect of Melatonin in Epithelial Mesenchymal Transition Markers and Invasive Properties of Breast Cancer Stem Cells of Canine and Human Cell Lines. PLoS One 2016;11:e0150407. doi: 10.1371/journal.pone.0150407.
22. Liu Y, Li H, Feng J, Cui X, Huang W, Li Y, et al. Lin28 induces epithelial-to-mesenchymal transition and stemness via downregulation of let-7a in breast cancer cells. PLoS One 2013;8:e83083. doi: 10.1371/journal.pone.0083083.
23. Ferrand N, Gnanapragasam A, Dorothee G, Redeuilh G, Larsen AK, Sabbah M. Loss of WISP2/CCN5 in estrogen-dependent MCF7 human breast cancer cells promotes a stem-like cell phenotype. PLoS One 2014;9:e87878. doi: 10.1371/journal.pone.0087878.
24. Patel N, Garikapati KR, Makani VKK, Nair AD, Vangara N, Bhadra U, et al. Regulating BMI1 expression via miRNAs promote Mesenchymal to Epithelial Transition (MET) and sensitizes breast cancer cell to chemotherapeutic drug. PLoS One 2018;13:e0190245. doi: 10.1371/journal.pone.0190245.
25. Wang X, Liu J, Wang Z, Huang Y, Liu W, Zhu X, et al. Periostin contributes to the acquisition of multipotent stem cell-like properties in human mammary epithelial cells and breast cancer cells. PLoS One 2013;8:e72962. doi: 10.1371/journal.pone.0072962.
26. Kopp F, Oak PS, Wagner E, Roidl A. miR-200c sensitizes breast cancer cells to doxorubicin treatment by decreasing TrkB and Bmi1 expression. PLoS One 2012;7:e50469. doi: 10.1371/journal.pone.0050469.
27. Mayoral-Varo V, Calcabrini A, Sánchez-Bailón MP, Martín-Pérez J. miR205 inhibits stem cell renewal in SUM159PT breast cancer cells. PLoS One 2017;12:e0188637. doi: 10.1371/journal.pone.0188637.
28. Cazet AS, Hui MN, Elsworth BL, Wu SZ, Roden D, Chan CL, et al. Targeting stromal remodeling and cancer stem cell plasticity overcomes chemoresistance in triple negative breast cancer. Nat Commun 2018;9:2897. doi: 10.1038/s41467-018-05220-6.
29. Sehl ME, Shimada M, Landeros A, Lange K, Wicha MS. Modeling of Cancer Stem Cell State Transitions Predicts Therapeutic Response. PLoS One 2015;10:e0135797. doi: 10.1371/journal.pone.0135797.
30. Schott AF, Goldstein LJ, Cristofanilli M, Ruffini PA, McCanna S, Reuben JM, et al. Phase Ib Pilot Study to Evaluate Reparixin in Combination with Weekly Paclitaxel in Patients with HER-2-Negative Metastatic Breast Cancer. Clin Cancer Res 2017;23:5358-65. doi: 10.1158/1078-0432.CCR-16-2748.
31. Goldstein LJ, Perez RP, Yardley D, Han LK, Reuben JM, Gao H, et al. A window-of-opportunity trial of the CXCR1/2 inhibitor reparixin in operable HER-2-negative breast cancer. Breast Cancer Res 2020;22:4. doi: 10.1186/s13058-019-1243-8.
32. Ibrahim SA, Hassan H, Vilardo L, Kumar SK, Kumar AV, Kelsch R, et al. Syndecan-1 (CD138) modulates triple-negative breast cancer stem cell properties via regulation of LRP-6 and IL-6-mediated STAT3 signaling. PLoS One 2013;8:e85737. doi: 10.1371/journal.pone.0085737.
33. Papi A, Storci G, Guarnieri T, De Carolis S, Bertoni S, Avenia N, et al. Peroxisome proliferator activated receptor-α/hypoxia inducible factor-1α interplay sustains carbonic anhydrase IX and apoliprotein E expression in breast cancer stem cells. PLoS One 2013;8:e54968. doi: 10.1371/journal.pone.0054968.
34. Wanandi SI, Limanto A, Yunita E, Syahrani RA, Louisa M, Wibowo AE, et al. In silico and in vitro studies on the anti-cancer activity of andrographolide targeting survivin in human breast cancer stem cells. PLoS One 2020;15:e0240020. doi: 10.1371/journal.pone.0240020.
35. Sami MM, Hachim MY, Hachim IY, Elbarkouky AH, López-Ozuna VM. Nucleostemin expression in breast cancer is a marker of more aggressive phenotype and unfavorable patients' outcome: A STROBE-compliant article. Medicine (Baltimore) 2019;98:e14744. doi: 10.1097/MD.0000000000014744.
36. Sun B, Liu R, Xiao ZD, Zhu X. c-MET protects breast cancer cells from apoptosis induced by sodium butyrate. PLoS One 2012;7:e30143. doi: 10.1371/journal.pone.0030143.
37. Riley L, Zhou H, Lange K, Sinsheimer JS, Sehl ME. Determining duration of HER2-targeted therapy using stem cell extinction models. PLoS One 2012;7:e46613. doi: 10.1371/journal.pone.0046613.
38. Wang YC, Chao TK, Chang CC, Yo YT, Yu MH, Lai HC. Drug screening identifies niclosamide as an inhibitor of breast cancer stem-like cells. PLoS One 2013;8:e74538. doi: 10.1371/journal.pone.0074538.
39. Zhou Q, Ye M, Lu Y, Zhang H, Chen Q, Huang S, et al. Curcumin Improves the Tumoricidal Effect of Mitomycin C by Suppressing ABCG2 Expression in Stem Cell-Like Breast Cancer Cells. PLoS One 2015;10:e0136694. doi: 10.1371/journal.pone.0136694.