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Self-renewal signaling pathways in breast cancer stem cells

Lakshmi Vineela Nalla, Kiran Kalia, Amit Khairnar*

Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gandhinagar, Gujarat, India

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ABSTRACT

Breast cancer, a death-dealing disease mainly affects the women populace in the world. Outmoded remedial treatments like chemo and radiotherapy against breast cancer have manifold limitations like systemic and local toxicity resulting in the failure of treatment and cancer relapse. Recurrence and treatment failure is due to the presence of the minor number of cells in the tumor called cancer stem cells (CSCs) stocked with the properties like epithelial-mesenchymal transition, drug resistibility, auto self-renewability, and stemness. But, the stemness in these cells is different from the normal stem cells with regards to their self-renewal signaling pathways which gets dysregulated due to genomic and epigenome changes. In the earlier period's headway in the cancer research led to the advancement of new targets by understanding the pathophysiological mechanism behind cancer progression but still, the mortality rate i. the breast cancer is at its peak due to their unclear understanding of the stemness signaling regulations. The present review highlights the current clinical limitations in treating cancer stem cells and discusses the recent writings of their stemness signaling regulations required in maintenance of self-renewal capability and metastasis. More importantly, it further describes the present clinical and preclinical updates targeting cancer stem cells pathways. A strong consideration of these signalings and developing the treatment strategies with the existed chemotherapy may possibly offer a promising approach to eradicate cancer stem cells for improving the cancer survival rate to persuade a long-term clinical response.

1. Introduction

According to the American Cancer Society in 2018, the projected number of new cases and deaths in breast cancer with both the sexes are 268,670 and 41,400 respectively making it a fatal cancer, affecting the wellbeing of most of the US citizens (Siegel et al., 2018). Lumpectomy, radiotherapy, hormone therapy, chemotherapy and other novel therapeutics are the available treatment options for treating the tumor bulk depending on the clinicopathological features of the individual patient. Tumor, sometimes considered as "wretch organ" encompasses cancer stem cells (CSCs), non-cancer stem cells and the surrounding tumor milieu which in turn contains extracellular matrix, immune cells (T cells, B cells, NK cells, MDSCs, DCs), TAMs, CAFs, and adipocytes. CSCs have the properties similar to the normal stem cells which have the capability to maintain its own stem pool and even gets differentiate into a multitude of cells aiding in fueling the tumor (Cahan and Daley, 2013; Chang, 2016; Shackleton, 2010). These innately resistant cells also possess a property called stemness which means aberrantly deregulated self-renewal signalings (STAT3, Notch, Wnt, Nanog, and Hedgehog) (Lathia and Liu, 2017) and these even supports in developing resistance to the innate immune system and chemotherapy (Kim et al., 2015). Further, the CSC milieu regulates the stemness and proliferation to

Abbreviations: 3′UTR, three prime untranslated region; AhR, arylhydrocarbon receptor; ALDH, aldehydedehydrogenase; Aph-1, ateriorpharynx-defective 1; BCCs, breastcancer cells; BCSCs, breastcancer stem cells; CDA-2, celldifferentiation agent-2; COX-2, cyclooxygenase2; CPT1B, carnitinepalmitoyltransferase 1B; CXCR1, chemokine receptor 1; CXCR7, CXCchemokine receptor 7; DNA, deoxyribose nucleic acid; EGFR, epidermal growth factor receptor; FAK, focaladhesion kinase; FAO, fatty acid β-oxidation; FGFR, fibroblastgrowth factor receptor; FOXC1, forkheadBox C1; GCSCs, gastriccancer stem cells; HIF-1α, hypoxiaInducible factor-1α; HMECs, humanmammary epithelial cells; IL-6, interleukin-6; IL-8, interleukin-8; JAK, januskinase; KLF4, kruppel like factor 4; LDHA, lactatedehydrogenase A; LEF-1, lymphoid enhancer binding factor 1; LncRNA, longnoncoding RNA; MCP-1, monocyte chemoattractant protein-1; Mel-18, melanomanuclear protein 18; mTOR, mammaliantarget of rapamycin; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclearfactor erythroid 2-related factor 2; OB-R, leptin receptor; OCSCs, ovarian cancer stem cells; PCSCs, prostate cancer stem cells; Pen-2, presenilin enhancer 2; PI3K, phosphatidylinositol3-kinase; PDK, pyruvatedehydrogenase kinase; PKM2, pyruvate Kinase M2; RALDH1, retinaldehydedehydrogenase 1; SCUBE2, signal peptide, CUB domain and EGF Like domain containing 2; shRNA, small hairpin RNA; SIRT2, Sirtuin 2; STAT, signal transducer and activator of transcription; TCF-4, transcriptionfactor 4; TNBC, triple negative breast cancer; TWIST, twist-related protein; WISP3, WNT1-inducible-signaling pathway protein 3; XIAP, X-linked inhibitor of apoptosis protein

* Corresponding author at: National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Palaj, Gandhinagar, 382355, Gujarat, India.
E-mail address: amitk@niperahm.ac.in (A. Khairnar).

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safeguard these stem cells from exhaustion.

A number of investigatory studies showed that CSCs are regulated by different factors and signalings in tumor milieu. So, targeting this milieu ruins self-restoration of CSCs and growth inhibition of tumor (Balkwill et al., 2012; Ye et al., 2014). Established cellular signalings involved in the self-restoration and differentiation of CSCs may not be sufficient and crosstalks within signaling and between various cells in the tumor milieu also exist which are to be considered for developing a targeted therapy against BCSCs. So, aiming these crosstalks and finding out new crosstalks provides a potential outcome for the survival of the patients. The current review seeks to discuss the present clinical limitations of CSCs and the role of its self-renewal signaling pathways in promoting the therapy failure in breast cancer.

2. CSCs and its influencing factors

Bonnet and Dick were the first to observe CSCs in the human acute myeloid leukemia (AML) in 1997 and stated these cells as a small set of the subpopulation with a potential role in tumor severity (Bonnet and Dick, 1997). Though CSCs were discovered several decades ago, their characterization and identification started from the last 15 years. These usually display a contrasting phenotype from the non-CSCs (tumor cells) in terms of following points- quiescence, slow cell cycle kinetics, capacity to repair DNA, upregulation of anti-apoptotic proteins, transporters for drug efflux, stemness signaling pathways, sphere-forming capacity to avoid anoikis (Cao et al., 2011), expression of embryonic stem cell markers, and detoxifying enzymes (Honoki et al., 2010; Verwey et al., 2016). All these features contribute to the resistance of conventional chemotherapeutics and radiotherapy with enhanced tumor recurrence and poor survival rate (Vinogradov and Wei, 2012). These cells have a high tumor-forming ability when implanted in the animals when compared to non-CSCs and also exhibit the high ALDH activity in comparison to cancer progenitor cells and non-CSCs. This feature makes the task easy in sorting these cells from the residing population of tumor to get a high purity of CSCs to study their characteristics (Shang et al., 2017). In addition to these features, cells even exhibits high activity of glycolysis and low activity of OXPHOS. These findings urged the researchers to work on the importance of cellular metabolism in cancer progression. Now a day's many researchers are interested in studying the cancer cell metabolism including the dysregulated enzymes (hexokinase, PKM2, LDHA, and PDK) and glutamate and lactate metabolism (Guo et al., 2017; Liu et al., 2014). In contradictory to the routine cancer cellular kinetics, a study described that cells with increased self-renewal (CSCs) exhibited higher mitochondrial function and are sensitive to oxidative phosphorylation inhibitor (Janiszewska et al., 2012; Lagadinou et al., 2013; Viale et al., 2014) speculating the need of OXPHOS for the CSCs survival, providing an alternate approach for targeting.

Existence of cellular signalings and growth factors in the tumor influence the CSCs functions. CSCs role in relapse of tumors critically depends on their two functional properties i.e. self-restoration and differentiation into a malignant subpopulation of tumor cells (Bao et al., 2013). Depending on the presence of different factors (hypoxia, TME) and crosstalks with the proteins involved in self-renewal signaling pathways, the features of CSCs gets altered. These were also speculated to be involved in metastasis whose presence is not well defined earlier. However, a study at the preclinical stage helped in elucidating their role in metastasis and the involvement of TME in its facilitation (Liu et al., 2010; Minn et al., 2005). In addition, hypoxic environment promoted EMT, stem-like properties (Cao et al., 2018), radio-resistance (Diehn et al., 2009), and drug resistance (Yan et al., 2018) in different cancers entangled with the therapy failure. HIF proteins affect the clinical consequences by promoting cellular adaptation and create resistant cells to therapy (Zhang et al., 2014). In addition to hypoxia, TME also plays a major role in endorsing CSCs growth. Castaño et al. postulated his two models by which tumor evolution is possible. First model states that CSCs niche and intra-tumoral heterogeneity were driven by TME factors and the second model states that CSCs create their own CSCs niche. Further, these two models act synergistically in driving the cancer progression (Castaño et al., 2012). Few studies have even proved that therapies targeting TME especially angiogenesis in combination with standard anticancer drugs turns out to be an important strategy for future therapy (Folkins et al., 2007). For the detailed information regarding the different approaches in targeting CSCs can be found in the reference article (Deshmukh et al., 2016).

3. Current CSCs clinical limitations

Research reports concerning CSCs enhanced our understanding towards their complex biology in the tumor bulk. However, due to the presence of heterogeneous cells in the tumor bulk, targeting these rare cells (CSCs) possess a bigger challenge to get an improved clinical outcome. Below discussed are the present major limitations in targeting CSCs

3.1. Plasticity and intra-tumor heterogeneity

It is so unacceptable that chemotherapeutics targeting CSCs shown only unassertive medical advantage even after 15 years of CSC research. These modest clinical limitations were in line to their renowned dynamic process like plasticity and tumor heterogeneity dictated by the TME through acquiring a continuous genetic and epigenetic alterations and such features signifies a major challenge in designing the therapies targeting CSCs in acquired therapy resistance (Ellsworth et al., 2017). Studies display that cells with better EMT plasticity are the metastatic initiating cells (MICs) that can initiate a primary tumor (Mani et al., 2008) and are even accountable for the intra-tumor heterogeneity along with the therapeutic failure (Clevers, 2011). Since intra-tumor heterogeneity is so engaged with the metastasis, targeting these mechanisms can control the progression of secondary tumors. Modular tumor model (MTM) has been proposed by Yakisich in which each module represents the interactions between the TME enriched with cells specific to different stemness phenotypes and determines the chemo-sensitivity towards anti-cancer drugs. It may help in guiding the development of anti-cancer treatment regimes in an added rational approach based on the understanding of complex tumor biology (Yakisich, 2012). Further, STAT3/G9a pathway (Chang et al., 2015), Notch1 (Zanetti et al., 2015), PAF-Wnt signaling axis (Wang et al., 2016), Hedgehog signaling to CAFs (Cazet et al., 2018; Das et al., 2013) were also involved with the maintenance of cancer plasticity.

3.2. Inadequate biomarkers

Another major limitation in breast cancer research includes inadequate biomarkers for the CSCs identification. In 2003, BCSCs were acknowledged for the first time and isolated with the help of $CD44^+/CD24^{-/low}$ Lin^- phenotype (Al-Hajj et al., 2003). Ever since this morphology of CSCs became a routine marker for separation of BCSCs and recently in a study this phenotype was correlated with the clinicopathological characteristics in human breast cancer specimens of 57 patients (Camerlingo et al., 2014). Also, recent studies showed CD338, EpCAM and Ganglioside GD2 as new surface markers in BCSCs sorting (Battula et al., 2012; Leccia et al., 2014; Tveito et al., 2011). Additionally, a recent report showed that a synthetic ligand 1 binds to $CD24^-/CD44^+/ALDH^+$ on CSCs of MCF-7 and MDA-MB-231 and can be used for affinity isolation of them (Chen, et al., 2018b). Although a broad gathering of surface markers has been studied (Bai et al., 2018; Bozorgi et al., 2015; Kim et al., 2016), none of them are specific for CSCs. So, development of novel surface markers in the identification of CSCs may hamper the enrichment of CSCs and can be targeted using the combination therapy with the existed anti-cancer regimens.

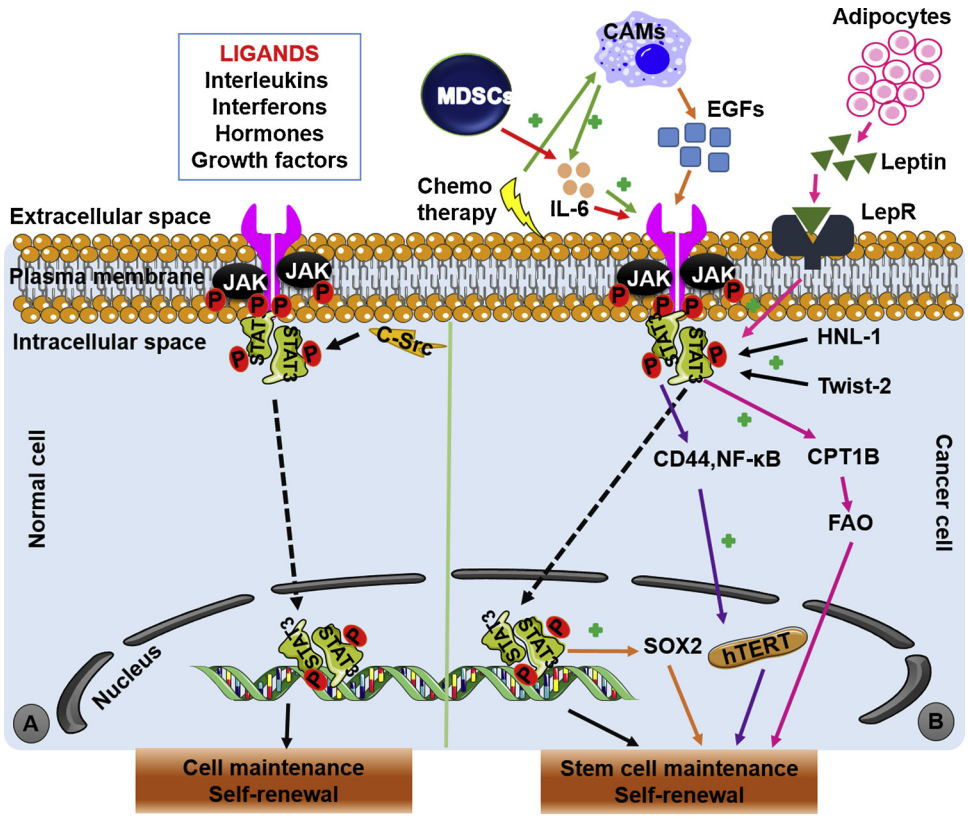

Fig. 1. Illustration representing the STAT3 regulation in normal cell and a cancer cell and its downstream signaling. A) Under normal physiological conditions, translocation of STAT3 to the nucleus occurs after its phosphorylation due to the activation and phosphorylation of receptor tyrosine kinase (JAK). In the nucleus, it acts as a transcription factor and starts its target gene expression and maintains the cells self-renewal. B) In cancer cells, different triggering factors like releases of cytokines, growth factors and hormones from different cells involved in the tumor microenvironment, chemotherapy-induced interleukins and several other proteins activate and phosphorylates JAK, in turn, activating STAT3 phosphorylation and promoting its nuclear functions which are involved in the maintenance and renewal of cancer stem cells.

3.3. Therapy resistance

Clinical margins of CSCs is only confined to the above discussed topics, but also with their starring role in promoting metastasis and resistance. CSCs are engaged with a collection of different resistance contrivances to attain therapy resistance. Recently, description of these resistance mechanisms from different malignancies suggested five different elements like quiescence, detoxification mechanisms in response to chemotherapy, DNA repair mechanisms induced by DNA damaging agents, survival promotion by anti-apoptotic mechanisms and adaptation to the clinical interventions (Kim et al., 2009). Quiescence cell is the first element in inducing the chemotherapy resistance which means the dormancy stage of the cells that reside in the G0 phase of the cell cycle and doesn't respond to the therapeutic drugs which usually act on highly proliferating cells (Saito et al., 2010). Even few studies found that these cells are even the observers of DNA damage. Tumor suppressors like p53, retinoblastoma protein and cyclin dependent kinases (p21) are involved in the regulation of this process (Cheung and Rando, 2013). Recently, the role of miRNAs (miR-126) in promoting dormancy and drug resistance has been recognized (Bliss et al., 2016). This dormancy state of CSCs guards them against the conventional chemotherapy targeting proliferation and assists in the CSC related resistance. Thus, it is an important strategy for developing the agents targeting only CSCs. The second element is the multidrug resistance and the best-characterized member is the family of ABC transporters which efflux the chemotherapeutic drugs and has been associated with different cancers (Januchowski et al., 2013). Even targeting the ABC transporters showed a therapy resistance in which the reductant ABC transporters got activated and continued the MDR mechanisms and hindered the clinical targeting and proved unsuccessful (Yu et al., 2013). The third element in this section includes the DNA repair mechanisms induced by DNA damaging agents. Induction of DNA damage response (DDR) by platinum-based chemotherapy is an example (Jung and Lippard, 2007) and even lead to p53 activation causing apoptosis via the induction of the apoptosis pathways. This property which is usually lost in most of the tumors leads to the fourth element i.e., survival response by helping the tumor cells to escape apoptosis induced by chemotherapy (Jung and Lippard, 2007). Post-intervention even directs to the fifth element i.e., adaptation of CSCs to the intervention and subsequently generating therapy resistant CSCs (Gasch et al., 2017). This explains the failure of pre-clinical success in the clinical trials. In addition, signaling pathways like a hedgehog (Das et al., 2013), STAT3 (He et al., 2018), Notch1/MVP pathway (Xiao et al., 2019) and Wnt/β-catenin (Shi et al., 2019) were also involved in promoting drug resistance.

As the above mentioned clinical limitations were related to their cellular signalings which are involved in developing the stemness properties, resistance, recurrence, and adaptation to the niche, a better understanding of these updated regulations of the signalings in detail is needed to develop novel therapeutics and to design new clinical trial strategies. So, this present review aims to discuss the recent writings of different studies involved with the BCSCs signalings.

4. Self-renewal pathways (SRPs) implicated with BCSCs

As mentioned before, the proliferating capacity of CSCs is maintained by an important property called self-renewal. In many cases, intrinsic and extrinsic mechanisms regulate the normal stem cell self-renewal and integration between these two mechanisms prevents the early exhaustion of stem cell pool by secreting growth factors and cytokines (Verga Falzacappa et al., 2012). As modifications in the genetics and epigenetics are engaged in unreserved growth, resistance and invasion in tumor cells, it is concerned that dysregulation of self-renewal pathways (SRPs) might be due to these modifications resulting in uncontrolled proliferation which is a hallmark of the early stage in the tumorigenesis process (Borah et al., 2015). SRPs in the normal stem cells are stringently regulated thereby controlling the development and expansion in the healthy tissue. Whereas, the BCSCs escapes such restraints and depends on the key dysregulated SRPs like JAK/STAT, Notch, Wnt, Hedgehog, and Nanog to mediate their self-restoration and

differentiation. So, these cellular signalings and the therapeutic molecules investigated at the level of preclinical and clinical were deliberated with boundless enthusiasm.

4.1. STAT3 signaling

JAK/STAT signaling plays a major function in cellular processes like apoptosis, metastasis, proliferation, angiogenesis, and invasion (Domanska and Brzezianska, 2012; Dutta and Li, 2013) and its dysregulation was reported in several other cancers. It is also involved in the maintenance of CSCs and germ-line stem cell population (Matsui, 2016). STATs were first described in 1994 and include a large family of transcription factors containing 7 members (STAT1-7) of which STAT3 plays a major role in breast cancer (Gkouveris et al., 2015). For the detailed signaling mechanism refer (Fig. 1). Notably, a study showed STAT3 as a functional marker for some subtypes of BCSCs and making it a good target in stem cell-targeted treatment (Wei et al., 2014).

The main cause for the greater mortality of breast cancer cases was metastasis which was found to be facilitated by the CSCs involving STAT signaling. For example, studies reported that factors like IL6/STAT3 and NO/NOTCH cross-talk between MDSCs and CSC (Peng et al., 2016), exogenous Twist2 (Fang et al., 2011) and IL-6 secreted from TAMs (Zhou et al., 2015) promoted the metastatic progression by inducing the pSTAT3 expression. Indeed, stem cell maintenance by leptin was also an important criterion for the survival of BCSCs (Thiagarajan et al., 2017). Further the influence of hypoxia (Abyaneh et al., 2018) and hematological and neurological expressed 1-like (HNL1) binding to the recognized sequences of microRNA-150, STAT3 and OB-Receptor persistently triggered OB-R/STAT3 signaling (Liu, et al., 2018b) and promoted CSCs. BCSCs regulate lipid metabolic genes for their self-renewal and differentiation through STAT3. In addition, the resistance of CSCs was found to be promoted by the STAT3-CPT1B-FAO pathway (Wang et al., 2018) and EGFR/Stat3/Sox-2 paracrine signaling by increasing the drug efflux capacity (Yang et al., 2013). Meanwhile, the physical interaction of STAT3 with hTERT even initiated the stem cell phenotype by the activation of the catalytic subunit of telomerase (hTERT) by acting as its transcription co-factor. This effect was reversed by pSTAT3 inhibition decreasing the $CD44^+$ cells (Fig. 1) (Chung et al., 2013) Table 1.

Recent studies involved in targeting the CSCs with natural compounds showed a greater therapeutic effect. Treatment with CHM-09 (Manupati et al., 2017) and cardamonin in parallel with or after the chemotherapy (Jia et al., 2016) perturbed EGFR downstream signalings and abolished chemotherapy-induced upregulation of STAT3, IL-6, IL-8, and MCP-1 respectively. In addition, Isoharringtonine (*Cephalotaxusharringtonia)* (Chen, et al., 2018c), Esculentoside A (*Phytolaccaesculenta*) (Liu, et al., 2018a) and catechol derived from lactic acid fermented Aronia juice (Choi et al., 2018) also inhibited BCSCs proportion by targeting STAT3 signaling. Even, an oral STAT3 inhibitor, TTI-101 (NCT03195699) entered the phase I clinical trial for the treatment of advanced cancer patients. So, by understanding the mechanisms of regulation in CSCs can bring the novel therapeutic drugs targeting towards the inhibition of STAT3 and which may achieve the BCSCs free breast cancer (Table 2). By the above studies, we can confirm that inhibition of STAT3 will achieve a greater therapeutic effect in the treatment of BCSCs.

4.2. Notch signaling

The translocation-associated Notch protein (NOTCH) signaling looks to be reactivated in epithelial cells contributing to the tumorigenesis in the initial phases of cancer by mainly controlling asymmetric divisions of CSCs and self-renewal (Yoo and Kwon, 2015). It has a grave role in preserving and differentiating stem cells and its unusual hyperactivation was reported to be involved in cancer development (Reedijk, 2012). For the detailed signaling mechanism refer (Fig. 2).

Hyper-proliferative response of Notch receptor was found and NOTCH1 levels were positively correlated with the metastasis and ALDH1 levels of CSCs indicating it as poor prognostic factor for the survival (Zhong et al., 2016) and its silencing resulted in the apoptosis and growth arrest in CSCs and non-CSCs (Suman et al., 2013). Relapse of the cancer is due to the stem cell pool expansion and maintenance of CSCs. Studies found that proteins like Enhancer of Zeste Homolog 2 (EZH2) (Gonzalez et al., 2014), fascin (Barnawi et al., 2016) and radiation (Lagadec et al., 2013) were found to activate NOTCH and promote stem cell expansion (Gonzalez et al., 2014). CCN6 or WISP3 found to get reduced in breast carcinoma (Huang, et al., 2016b) and was involved in regulating NOTCH through a crosstalk between the CCN6/Slug signaling axis and Notch1. In addition, the CCN6 levels were inversely correlated with the NICD1 in 69.5% invasive mammary carcinomas and its ectopic overexpression repressed tumor volume, CSC's and metastasis. Even hypoxia was found to positively regulate Notch and displayed reduced tumor growth and lung metastasis when HIF-1α was inhibited (Schwab et al., 2012). This explains how cancer cells obtain stemness in oxygen deficient conditions. However, EMT of CSCs was promoted by upregulated SCUBE2 expression by activating Notch signaling (Chen, et al., 2018a). Even, increased activity of SIRT2 promotes CSCs (Zhao, et al., 2014a). Identification of CSCs from the tumor population is a challenging task, for which $ALDH^+$ is one of the important characteristic features to isolate the CSCs. A study displayed sphingosine-1-phosphate (S1P), caused the activation of NOTCH via S1P receptor 3 (S1PR3) which enhanced the levels of $ALDH^+$ CSCs (Hirata et al., 2014). Inhibition of Notch cleavage in association with Akt or NF-kB with their respective inhibitors reduced the secondary mammospheres formation in CSCs (Hossain et al., 2017). Even the tumor microenvironment has a significant role in the progression of CSCs. Tsuyada et al. studied "cancer-stroma-cancer" circuit in which cancer cells induced the production of Chemokine (C-C motif) ligand 2 (CCL-2) in higher levels from cancer-associated stromal fibroblasts (CAFs). And the released CCL2 encourages Notch1 expression and regulates the CSC features in neighboring cancer cells (Tsuyada et al., 2012). Moreover, it has been found that Mel-18, a polycomb protein upregulates the Jagged-1 which is a Wnt/TCF target and activates Notch signaling to enrich the $CD44^+/CD24^-/ESA^+$ population (Won et al., 2012). Intrinsic resistance to mTOR inhibitor and the development of CSCs was due to the activation of Notch1 and FGF1 in association with the upsurge in mitochondrial metabolic rate and FGFR1 pathway. Abrogation of Notch1 activity by pharmacological inhibition revoked CSC development and tumor-propagating capacity brought up by mTORC1/2 inhibition (Bhola et al., 2016) (Fig. 2).

A well-explored Notch signaling inhibitor was the PF-03084014, which was a γ-secretase inhibitor and displayed inhibitory effect on metastasis and self-renewal in breast cancer xenograft model (Zhang et al., 2012). Other promising agents against γ-secretase inhibition in breast cancer which entered the ongoing Phase I/II clinical trials include RO4929097 (NCT01151449), MK-0752 (NCT00645333), LY3039478 (NCT02784795) and in future CB-103 which blocks the nuclear transcriptional activation of Notch signaling (NCT03422679) will be evaluated. In addition, monoclonal antibodies against delta-like ligand 4 such as MEDI0639 (NCT01577745) and REGN421 (NCT00871559) have entered the Phase I trials in treating the advanced solid tumors. All these inhibitors display an acceptable therapeutic response with tolerable toxicity. These awaiting results may offer a tough clinical proof with promising therapy for breast cancer.

4.3. Wnt signaling

The Wnt/Frizzled/-catenin signaling maintain the usual breast growth as well as implicated in cancer development and found to get over-activated in 50% of breast cancer patients accompanying to poor survival (Zhan et al., 2017). It controls the fate of cell during embryonic development and in adult age cells renewal (Takebe et al., 2011).

Table 1
Summary of published self-renewal signaling regulations studies regarding breast cancer stem cells.

	Inducer	Specimens used	Validation of CSCs		Outcomes of regulation	References
			Surface markers	Functional assays		
STAT3 REGULATION	MDSCs secreted IL-6	MDA-MB-231, MCF-7 cells, and Human breast cancer tissues	CD (3,4,5, 11b, 14, 15, 16, 19, 33, 45), HLA-DR and ALDH	Tumorsphere formation, Mammosphere Formation Assays,	Enhanced stem cell properties, T-cell activity suppression	(Peng et al., 2016)
	Twist2	MCF-7 cells and Human breast cancer tissues	$CD44^{high}/CD24^{low}$ cells	Mammosphere culture, Three-dimensional culture assay, Colony formation assay	Self- renewal enhancement	(Fang et al., 2011)
	Leptin	MDA-MB-231 and HCC70 cells	CD44 and CD49f, NANOG, SOX2, and OCT4	Limiting dilution assay	Induction and maintenance of TNBC CSCs	(Thiagarajan et al., 2017)
	HNL1	SUM159, MDA-MB-231, MDA-MB-468 cells and SCID-Beige mice	$CD44^{+}/CD24^{-/low}$ and ALDH	Cell Migration assay, Invasion Assay, Mammosphere formation assay	Sustain LEPR-STAT3 pathway activation	(Liu, et al., 2018b)
	TAMs	Apoptotic MCF-7 cells and peritoneal macrophages from mice	$CD44^{+}/CD24^{-}$	Cell proliferation assay	Increase in $CD44^{+}/CD24^{-}$ cancer stem-like cells, metastasis, and BC growth	(Zhou et al., 2015)
	TAMs	4T1, 4T07 cancer cells, and RAW 264.7 macrophage cells	Sca-1, c-Kit, ABCB1b or ABCG2	–	Upregulation of CSC phenotype	(Yang et al., 2013)
	STAT3	MCF-7, BBM2, BBM3, MB231-P, Hs578 T, MB436, HCC1500, BT20 and MB231-R cells	$CD44^{hi}$, $Sca\text{-}1^{hi}$	Tumorsphere formation and limiting dilution assay	Promotes cancer stemness and chemoresistance	(Wang et al., 2018)
	STAT3	BT474, SKBR3, MDA-MB-231 and MCF-7 cells	CD44	Tumorsphere formation assay	Promoted stem cell traits	(Chung et al., 2013)
NOTCH REGULATION	EZH2	Breast cancer tissues, (MMTV)-neu mouse model, SUM149 and MDA-MB-231 cells	$CD44^{+}/CD24^{-}$	Mammosphere assay, ALDEFLUOR assay	Accelerates breast cancer initiation	(Gonzalez et al., 2014)
	CCN6	MDA-MB-231, MDA-MB-436 cells and human carcinoma tissues	–	ALDEFLUOR assay, Tumorsphere formation assays	Suppress TICs	(Huang, et al., 2016b)
	Notch1	HEK293 T, HEPG2, MCF-7, MDA-MB-468 cells, primary breast cancer cell cultures, and NOD/SCID mice	–	Mammosphere assay, ALDEFLUOR assay	Promotes CSCs	(Zhao, et al., 2014a)
	CCL2	BT474, MDA-MB-361, MCF7 cells and human breast cancer tissues	–	PKH67 and ALDEFLUOR assays	Promotes CSCs phenotype	(Tsuyada et al., 2012)
	Radiation	MCF-7, SUM159PT, T47D cells and NOD SCID gamma (NSG) mice	$CD44^{+}$	Proteasome activity	Enrichment of BCSCs	(Lagadec et al., 2013)
	S1P	Balb/c nude mice, MCF-7 and MDA-MB-231 cells	$CD44^{+}/CD24^{-}$	ALDEFLUOR assays, Side population assay, Mammosphere assay,	Expansion of CSCs	(Hirata et al., 2014)
	Fascin	MDA-MB-231, T47D cells and nude mice	$CD44^{+}/CD24^{-}$, $ALDH^{+}$	Tumorsphere and colony forming assays, Limiting dilution xenograft assay	Maintenance of CSCs pool	(Barnawi et al., 2016)
	HIF-1α	Genetically modified mice	CD133, CD24, CD31	Limiting dilution assay and Tumorsphere formation assay	Primary tumor growth promotion	(Schwab et al., 2012)
	Mel-18 knockdown	MCF-7, MDAMB-231, and SK-BR-3 cells	$CD44^{+}/CD24^{-}/ESA^{+}$ cells	Tumorsphere formation and Colony formation assay	Self-renewal, cell proliferation, and tumorigenesis	(Won et al., 2012)
	Notch	MDA-MB-231 and MDA-MB-468 cells	$CD90^{hi}$, $CD44^{+}/CD24^{-}$	–	Regulation of BCSCs	(Hossain et al., 2017)
	TORC1/2 inhibition	SUM159, BT549, MDA 231, MDA468 and NCL/ADR-RES	$CD44^{+}$, $CD24^{-}$, $PORCR^{+}$, ESA^{+}, $CD133^{+}$	ALDEFLUOR assay, Mammosphere assay	Sustains CSCs pool and drug resistance to TORC1/2 inhibitors	(Bhola et al., 2016)
	SCUBE2	MDA-MB-231, Hs578 T cells, and NOD/SCID mice	ABCG2	–	Promoted EMT and metastasis in TNBC	(Chen, et al., 2018a)

(*continued on next page*)

Table 1 (*continued*)

	Inducer	Specimens used	Validation of CSCs		Outcomes of regulation	References
			Surface markers	Functional assays		
WNT REGULATION	FAK inhibition	Primary breast cancer specimens, MDA-MB-231, SUM159, and MCF-7 cells	–	Matrigel-on-Top (MoT) assay, Aldefluor assays, Side population (SP) analysis, and Tumorsphere assay	Reduce the proportion of CSCs	(Kolev et al., 2017)
	AhR	SKBR-3, MCF-7 and MDA-MB-231	CSCs specific markers	Aldefluor assays, Side population (SP) assay, mammosphere formation	CSCs self-renewal, chemoresistance	(Al-Dhfyan et al., 2017)
	NRP-1	MCF-7 and MDA-MB-231 cells	$CD44^+/CD24^-$	Mammosphere assay, and Soft agar colony formation assay	CSC related traits and chemoresistance	(Zhang et al., 2017)
	Let-7c miRNAs	MCF-7 and T47-D cells	–	Aldefluor assays, sphere formation assay	Self-renewal inhibition	(Sun, et al., 2016b)
	MiRNA600	SUM149, SUM159, S68 cells and human breast cancer samples	–	Aldefluor assay	Inhibition of self-renewal and expansion of CSCs	(El Helou et al., 2017)
	Nestin	Human carcinoma tissue specimens	$CD44^+/CD24^-$	Mammosphere formation assay, Transwell invasion assay	Regulation of survival and invasiveness of BCSCs	(Zhao, et al., 2014b)
	PKM2	Human carcinoma tissue specimens, MDA-MB-231, MDA-MB-435S, T47-D, and SK-BR-3 cells	$CD44^+/CD24^-$	Colony formation assay, Sphere formation assay, Holoclone assay, Aldefluor assay	Promotes stemness	(Zhao et al., 2016)
HEDGEHOG REGULATION	GTP2	MDA-MB-231, MCF7 cells, and Balb/c mice	$CD44^+/CD24^-$	Colony formation assay, aldefluor assay, and Soft agar colony formation assay	Tumorigenesis promotion	(Cao et al., 2017)
	FOXC1	MDA-MB-231, BT549 cells, and BALB/c nude mice	–	Aldeflour Assay	Regulation of breast cancer stemness	(Han et al., 2015)
	P63	Transgenic C57/BL6 J mice	–	Mammosphere assay and PKH-26 Assay	Regulation of Self-renewal and stemness of CSCs	(Memmi et al., 2015)
	Hh	MDA-MB-231, M6 murine mammary carcinoma cell,	EpCAM, CD45, CD31, BP1, TER119, CD24, CD29 and CD61	Limiting dilution assay and Tumorsphere assay	Drug-resistant and stem-like phenotype	(Cazet et al., 2017)
	LncRNA-Hh gene	MCF-7, Hs578 T, BT549, MDA-MB-231 cells and nude mice	–	Mammosphere formation assay	Increased CSCs and tumor formation	(Zhou et al., 2016)
NANOG REGULATION	PD-L1	MDA-MB-231, T-47D cells and nude mice	$CAM^+/ CD44^+/CD24^-$	Tumorsphere assay, limiting dilution assay	Maintenance of CSCs	(Almozyan et al., 2017)
	ALKBH5	MCF-7, MDA-MB-231, MDA-MB-435, T47D, BT474, SUM-149 and SUM-159 cells	–	Mammosphere assay and ALDH assay	Progression of BCSCs phenotype	(Zhang et al., 2016)
	CXCR7	MCF-7 cells and BALB/c-nul mice	$CD44^+/CD24^-$	Colony formation assay, proliferation assay	Stem cell-like cells induction	(Tang et al., 2016)
	IL-33	Carcinoma tissues, MCF-7 cells, and Female BALB/c nude mice	–	Mammosphere assay, tumorigenesis,	Induces CSCs property	(Hu et al., 2017)
	COX-2	Human BCCs (MCF-7, MDA-MB-468, MDA-MB-231, SKBR-3, Hs578 T, T47D), GUSB null/NOD/SCID/MPSVII mice, and human tissue samples	$ALDH^{high}/CD44^+CD24^-$	Proliferation and spheroid formation assays	Induces BCSCs	(Majumder et al., 2016)
	Bmi1	Breast cancer tissues, MCF7, and MDA-MB-231 cells	$CD44^+/CD24^-$	Self-renewal assay	Control self-renewal	(Paranjape et al., 2014)
	CX26	MDA-MB-231, HCC38, MDA-MB-157, MDA-MB-468, MCF10 A, MCF7, HCC70 BCCs and female mice	NANOG	ALDH assay, GJ dye diffusion assay, Limiting dilution assays	Self-renewal and maintenance of CSCs	(Thiagarajan et al., 2018)
	Leptin	M-Wnt cells from MMTV-Wnt-1 transgenic mice, C57BL/6 J mice, and MDA-MB-231 cells	$CD44^+/CD24^-$, CD49f	Tumorsphere assays	CSC like property in TNBC cells	(Zheng et al., 2013)

Table 2
Summary of published preclinical therapeutic studies against BCSCs.

Sr.no	Drug	Cell lines and animals	Inhibited mechanisms	References
1	Curcumin	SUM159 and MCF7 cells	CSCs markers, Wnt/β-catenin pathway	(Li et al., 2018)
2	Curcumin + Mitomycin C	MCF7 and MDA-MB-231 cells	Apoptotic activity	(Zhou et al., 2017)
3	Eugenol + Cisplatin	TNBC cells, BT-20, NBL-10, BM-4 cells and female nude mice	NF-κB binding at the IL-6 and IL-8 promoter regions	(Islam et al., 2018)
4	Chestnut leaf extract + paclitaxel	MCF7 cells	Nrf2 nuclear translocation	(Woo et al., 2017)
5	CHM-09	MDA-MB-231 cells	EGFR downstream signaling	(Manupati et al., 2017)
6	Melatonin	MCF-7 and MDA-MB- 231 cells	OCT4	(Lopes et al., 2017)
7	Citral	MDA-MB-231 cells	ALDH1A3 expression and its inducible genes	(Thomas et al., 2016)
8	SFN-loaded nanoparticles + docetaxel	Female Balb/c nude mice and MCF-7 cells	β-catenin pathway	(Huang, et al., 2016a)
9	Hyaluronic acid coated salinomycin and paclitaxel nanoparticles	MCF-7, MDA-MB-231 cells	_	(Muntimadugu et al., 2016)
10	SWCNT nanocarriers	MDA-MB-231 cells	_	(Al Faraj et al., 2016)
11	Pyrvinium pamoate	MCF-7, MDA-MB-231, MDA-MB-468 and SkBr-3 cells	Wnt pathway and CSCs markers	(Xu et al., 2016)
12	Reparixin + paclitaxel	MDA-MB-231 cells and Nude Balb/c mice	IL-8/CXCR1 pathway	(Brandolini et al., 2015)
13	Cardamonin	SUM190, MCF-7, MDA-MB-231, Cama-1 cell lines, and Athymic nude mice	IL-6, IL-8 and MCP-1 expression and activation of NF-κB/IKBα and STAT3	(Jia et al., 2016)
14	Mithramycin A	MCF-7, HBL-100 cells and primary tumor samples	Self-renewal and chemoresistance genes by inhibiting Sp-1 recruitment to its promoter region	(Saha et al., 2015)
15	Huaier aqueous extract	MCF-7 cells	Hh pathway, downregulation of NANOG, OCT4 and NESTIN	(Wang et al., 2014)
16	Nitidine chloride	MCF-7 and MDA-MB-231 cells	Hh pathway	(Sun, et al., 2016a)
17	Phosphosulindac	SK-3rd, HMLER cells and female BALB/c nude mice	Wnt pathway by β-catenin degradation	(Zhu et al., 2012)
18	Caffeic Acid Phenethyl Ester	MDA-MB-231 cells and Female Balb/c nude mice	Self-renewal inhibition	(Omene et al., 2012)
19	CDA-2	MCF-7 and MDA-MB-231 cells	Hedgehog signaling	(Lu et al., 2011)
20	Isoharringtonine	HCC1806, HCC1937 and MCF-7 cells	STAT3 signaling	(Chen, et al., 2018c)
21	Catechol from aronia juice	MCF-7 and MDA-MB-231cells	Stat3/IL-6 signaling	(Choi et al., 2018)
22	Esculentoside A	EMT6 and MCF-7 cells	Stat3/IL-6 signaling	(Liu, et al., 2018a)

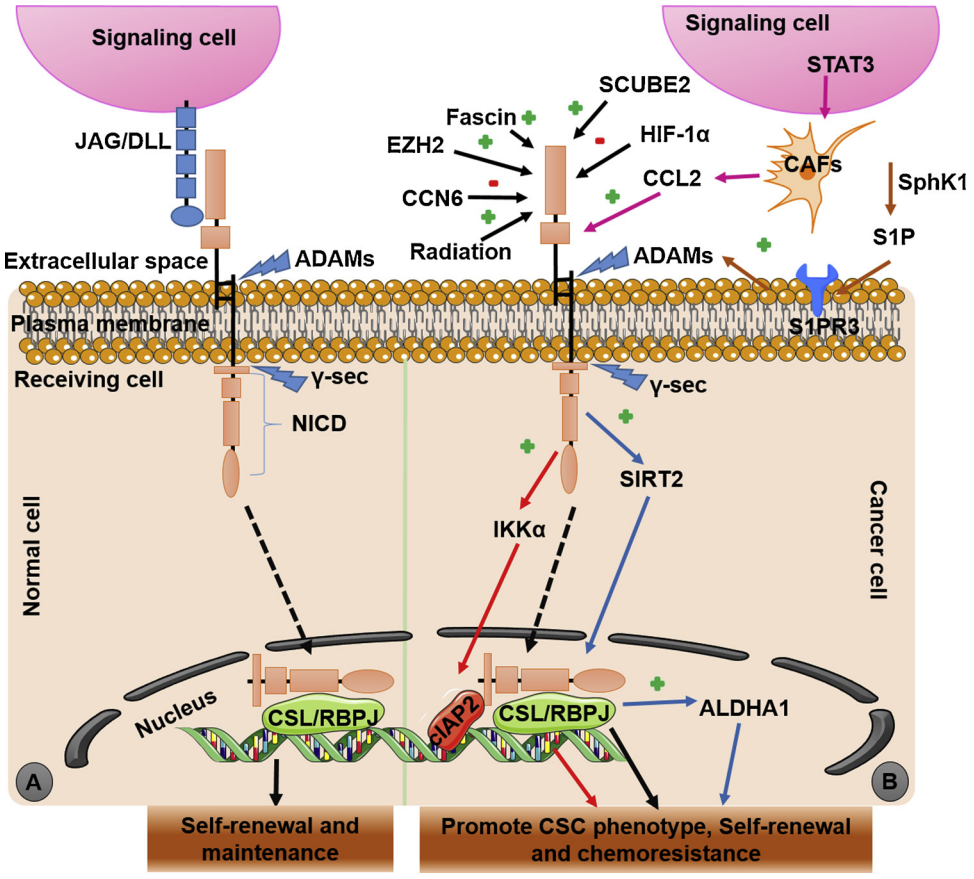

Fig. 2. Illustration representing the Notch signaling regulation in normal cell and a cancer cell. A) Under normal physiological conditions, translocation of NICD to the nucleus occurs after the cleavage of the notch by proteases (ADAMs and γ-sec). In the nucleus, NICD acts as a transcription factor and starts its target gene expression and maintains the cells self-renewal. B) In cancer cells, different triggering factors like releases of chemokines and other proteins in cytoplasm activate directly or indirectly NICD and its two-stage cleavage promoting its nuclear functions which are involved in the maintenance and renewal of stem cells.

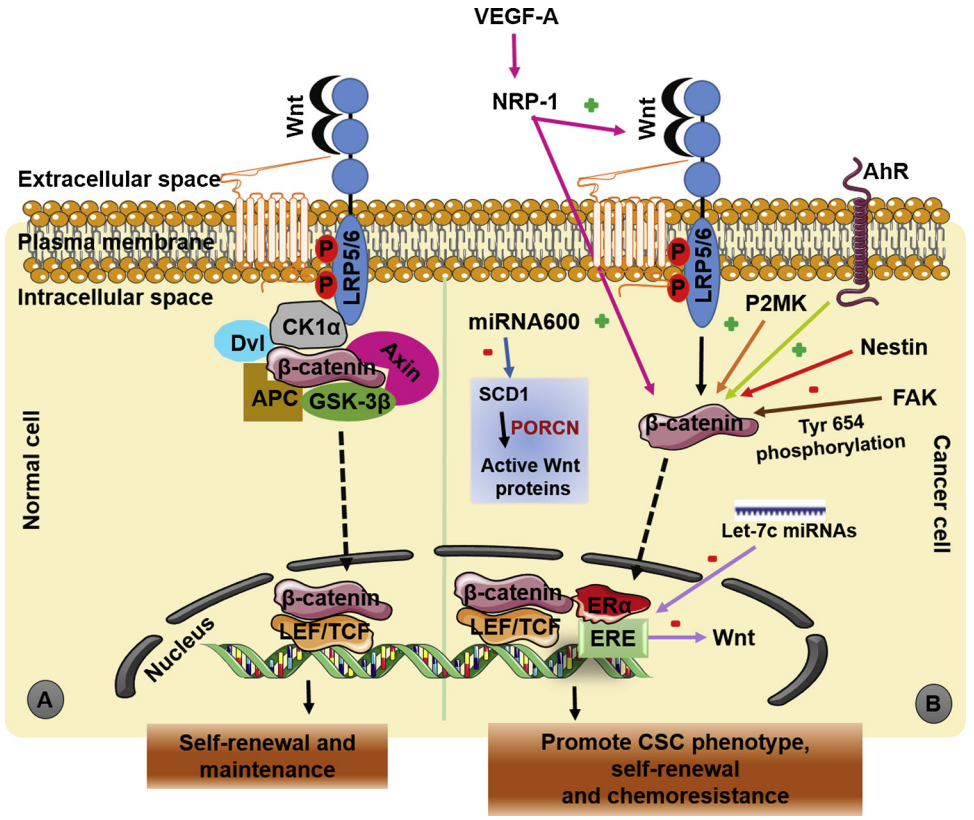

Fig. 3. Illustration representing the Wnt/β-catenin signaling regulation in normal cell and a cancer cell. A) Under normal physiological conditions, translocation of β-catenin to the nucleus occurs after the inhibition of β-catenin proteolytic degradation by disheveled family proteins. In nucleus, β-catenin acts as a transcription factor and starts its target gene expression and maintains the cells self- renewal. B) In cancer cells, different triggering factors like releases of growth factors, miRNAs and other proteins in cytoplasm activates β-catenin translocation and promotes its nuclear functions which are involved in the maintenance and renewal of stem cells.

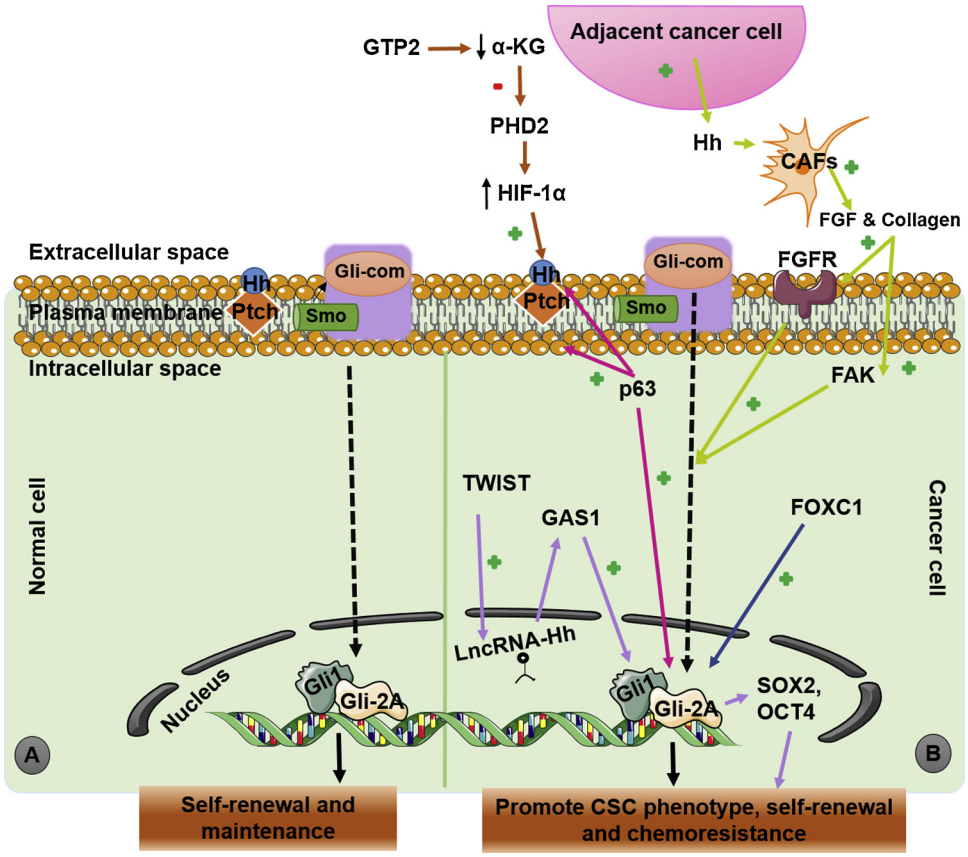

Fig. 4. Illustration representing the Hedgehog signaling regulation in normal cell and the cancer cell. A) Under normal physiological conditions, translocation of Gli-com to the nucleus occurs after the activation of the patched receptor by its hedgehog ligands. In the nucleus, Gli proteins act as a transcription factor and start their target gene expression and maintain the cells self- renewal. B) In cancer cells, different triggering factors like releases of growth factors, LncRNAs and other proteins in cytoplasm activate Gli-com translocation and promote their nuclear functions which are involved in the safeguarding and renewal of stem cells.

Proteins of the Wnt family trigger the transfer of β–catenin to the nucleus and starts the transcription of genes which are implicated in determining cellular differentiation, asymmetric division, migration and cell polarity (Fig. 3) (Matsui, 2016; Mohammed et al., 2016).

Inhibition of this signaling blocks mammary growth and alters proliferation induced by pregnancy. Even the upregulated Wnt signaling increased the expansion of preneoplastic progenitor cells in mammary glands of genetically modified mice (Lv et al., 2017). In comparison to normal stem cells, BCSCs show over activation of Wnt signaling, and its inhibition decreased the mammospheres in Estrogen Receptor dependent fashion (Lamb et al., 2013). Recently, authors found that β-catenin and PI3K/Akt signalings are controlled by AhR signaling which led to resistance to chemotherapy and renewal of CSCs (Al-Dhfyan et al., 2017). In fact, overexpression of neuropilin 1 (NRP-1), which is an activator of the Wnt/β-catenin pathway produced CSC like traits in breast cancer cells. (Zhang et al., 2017) and the silencing of Wnt1 in CSCs decreased stemness genes (CD44, Sca-1, and ALDH1) and tumor formation. Similarly, aldefluor assay revealed that $ALDH^+$ cells express high levels of LEF1, cyclin D, β-catenin and TCF-4 when compared to $ALDH^-$ cells signifying that the intensified Wnt signaling is essential for the stemness maintenance in cells of breast cancer. (Jang et al., 2015). In addition, nestin expression was found to associate with low survival of TNBC patients and its high expression resulted inefficient *in-vivo* tumor formation. Whereas, it's silencing in nestin- expressing CSCs downregulated β-catenin which diminished the mammospheres formation. This explains the role of Wnt pathway in cell proliferation, metastasis, and self-renewal (Zhao, et al., 2014b). Even the gain-and loss-of-functions of miRNAs like let-7 miRNAs facilitates the development of CSCs (Cai et al., 2013) and the inhibition of ERα by Let 7c miRNA induced Wnt signaling and repressed self-renewal of stemloids by binding directly to ERα 3′UTR region (Sun, et al., 2016b). Indeed higher expression of miRNA600 led to reduced self-renewal by inhibiting the active Wnt proteins. Similarly, silencing of miRNA600 led to the production of active Wnt proteins and CSCs expansion. Mechanistic understanding found that miRNA600 targets stearoyl desaturase1 (SCD1) enzyme which is an important factor in the production of lipid-modified active Wnt proteins (El Helou et al., 2017). In CSCs, there exists a crosstalk between β-catenin and FAK in which silencing of FAK resulted in blockade of β-catenin activation by decreasing the Tyr654 phosphorylation of the latter (Kolev et al., 2017). Even the tumor glycolytic enzyme PKM2 activates Wnt signaling and promotes stemness in BCCs (Zhao et al., 2016) (Fig. 3).

Therefore, drugs intervening the Wnt signaling pathway might give a better clinical outcome by targeting CSC's development. For example, sulforaphane loaded nanoparticles in combination with docetaxel (Huang, et al., 2016a), pyrvinium pamoate (Xu et al., 2016), and phosphosulindac (OXT-328) treatment intensely inhibited BCSCs self-renewal by downregulating β-catenin which suppressed CSCs, EMT and therapeutic resistance (Zhu et al., 2012) (Table 2). Even the clinical application of Wnt inhibitors against CSCs was promoted by a phase 1 study of OMP-54F28 which got completed in the year of 2017 (NCT01608867).

4.4. Hedgehog signaling

Signaling pathway of Hedgehog is important in proliferation regulation, cellular fate, stemness and maintenance of progenitor cell and their renewal capacity (Matsui, 2016). In fact, GLI1 upregulation gave an advantage to the progenitor population and tumor formation relating hedgehog signaling with stemloids (Fiaschi et al., 2009). Increasing the subpopulation of CSCs involves the overexpression of Glutamic pyruvate transaminase (GPT2) resulting in the constitutive activation of Shh signaling (Cao et al., 2017). In basal-like breast cancer (BLBC), FOXC1 overexpression enriched CSC properties through Gli2 (Han et al., 2015). A report even suggests that epithelial stem cells self-renewal is regulated by p63 in the mammary gland, the levels of which

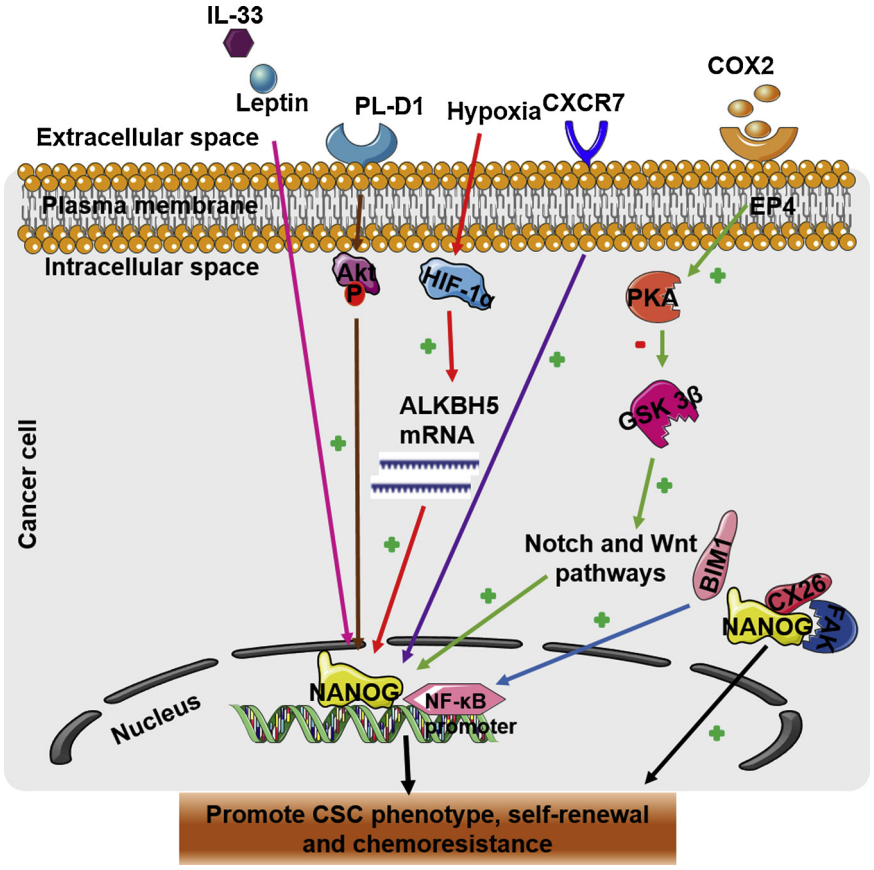

Fig. 5. Illustration representing the Nanog regulation in the cancer cell. In cancer cells, different triggering factors like releases of growth factors, hormones, interleukins and other proteins in cytoplasm activate Nanog nuclear functions which are involved in the maintenance and renewal of stem cells.

are increased compared to normal progenitor cells. This study also provided an additional link between p63 and Shh in which the former protein positively regulates the latter protein and promoted CSCs (Memmi et al., 2015). Consistently, CSCs can reprogram the CAFs and make the CAFs accompanying cancer cells to secrete fibroblast growth factor 5 (FGF5), and collagen. These accompanied cancer cells develop CSC phenotype and show resistance to chemotherapy (Cazet et al., 2017). Even lncRNA associated with Hh signaling (LncRNA-Hh) are involved in increasing the expression of OCT4 and SOX2 which plays the main role in CSC maintenance. While silencing of lncRNA resulted in the opposite effect indicating the important regulatory function in the stemness maintenance (Zhou et al., 2016) (Fig. 4).

Therapeutic targeting of Hh signaling shows the promising results in inhibiting CSCs. Currently in clinical practice, sonidegib and vismodegib are used for basal cell carcinoma as the antagonists of smoothened (SMO) (Basset-Seguin et al., 2015) but these drugs are less investigated in breast cancer due to their fine efficacy in tumors which harbors certain mutations in Hh signaling (O'Toole et al. (2011)). Even in the clinical trials sonidegib with paclitaxel (NCT01954355) and sonidegib with docetaxel (Martín et al., 2016) showed a tolerable effect in advanced breast cancer patients. Recently, some preclinical studies showed the therapeutic effect of huaier aqueous extract (Wang et al., 2014), nitidine chloride (Sun, et al., 2016a) and synergistic effect of CDA-2 with the thiotepa or paclitaxel in inhibiting CSCs proliferation (Lu et al., 2011) (Table 2). From the above preclinical and clinical studies the application of Hh inhibitors sheds the lights against breast cancer treatment targeting CSCs.

4.5. Nanog regulation

In dividing stem cells or progenitor cells, an equilibrium between the differentiation of stem cells and their self-renewal is a fundamental property to maintain homeostasis in a tissue. In a pluripotent state, the rapid proliferation of human and mouse embryonic stem cells occurs by a shortened G_1 phase (Coronado et al., 2013). In view of it, overexpression of NANOG1 induces proliferation and shortens G_1-S phase transition (Zhang et al., 2009). So, Nanog a homeobox protein displays an important role as a transcription factor in self-restoration and of embryonic stem cell pluripotency. It was also involved in motility and chemotherapeutic resistance (Pashaiasl et al., 2016) and exists in a mutual antagonism with Orthodenticle Homeobox 2 (OTX2) (Acampora et al., 2017). Nanog controls all these functions through complex interactions with innumerable factors such as OCT4, SOX2, and KLF4. Enhanced expression of proteins from Nanog family has been seen in various cancers, including breast cancer (Jeter et al., 2015; Palla et al., 2015). Though it is not able to induce mammary tumor alone, Nanog in conjunction with Wnt-1 developed mammary tumorigenesis and metastasis in a mouse model (Lu et al., 2014). Even, Programmed death-ligand 1 (PD-L1) expression showed chemoresistance and EMT by promoting NANOG and OCT4 in BCSCs (Almozyan et al., 2017). In addition, hypoxia-exposed CSCs promoted AlkB homolog 5 (ALKBH5), an m^6A demethylase downregulates the NANOG methylation and upregulates its mRNA and protein expression thereby encouraging the CSCs progression (Zhang et al., 2016). In fact, silencing of CXCR7 negatively affected the regulation of stem cell markers and enhanced apoptosis in the tumors (Tang et al., 2016).

IL-33 expression (Hu et al., 2017), and COX-2 co-localization with CSCs markers (Majumder et al., 2016) in mammary tumor cells induced breast cancer endocrine resistance and promotes breast cancer progression respectively. A recently identified special AT-rich binding protein-2 (SATB2) which is an epigenetic regulator was found to be greatly expressed in embryonic stem cells, breast cancer cells of human

origin, and CSCs but not in HMECs and normal breast tissues. ChIP assays identified that SATB2 bind directly to Bcl-2, c-Myc, Nanog, Klf4, and XIAP promoters and suggests its role in controlling the pluripotency and cell proliferation (Yu et al., 2017). Although, Bmi1 positively regulates NANOG activity (Paranjape et al., 2014) but CX26 complexes with NANOG and FAK (Thiagarajan et al., 2018) promoting the stabilization of NANOG and endorses the self-renewal of CSCs. However, leptin acts at the promoter site of NANOG promoting tumor cell proliferation and maintains the CSC properties (Zheng et al., 2013) (Fig. 5). So, disturbing the NANOG expression sensitizes the CSCs to current chemotherapeutics. For example, sensitization of CSCs to doxorubicin was achieved with Mithramycin A treatment perturbing the NANOG, OCT4 expressions and suppression of genes related to chemoresistance and self-renewal (Saha et al., 2015) (Table 2).

5. Novel therapeutics targeting CSCs

The existence of tumor cells with stemness features (CSCs) and their involvement with reversion and resistance has steered to the exploration for therapeutically active compounds to eradicate or modulate their stemness in the anticipation of treating cancer. Combination therapy is more significant than the single therapeutic intervention in the treatment of BCSCs. So far, studies related to BCSCs *in-vitro* and *in-vivo* were deliberated in the review. For example, the curcumin interventional effects on CSCs of breast cancer was explored (Li et al., 2018) and it even acted as a chemosensitizer in adjuvant with mitomycin C (Zhou et al., 2017). Indeed, eugenol in combination with cisplatin also achieved cytotoxicity and pro-apoptotic effects insignificant proportion when compared to drug alone (Islam et al., 2018). Similar study shows that chestnut extract in combination with paclitaxel increased the susceptibility of breast CSCs to chemotherapy (Woo et al., 2017). Besides a research study showed that melatonin downregulated a self-renewal factor and declined mammosphere formation in the BCCs (Lopes et al., 2017). A similar study considered the inhibitory effect of citral on ALDH1A3, a CSC marker and inhibited the ALDH1A3 mediated tumor growth (Thomas et al., 2016). Nanomedicine also brought revolutionary changes in the treatment of different malignancies. A good example of this is the paclitaxel nanoparticles, salinomycin nanoparticles coated with hyaluronic acid (Muntimadugu et al., 2016) and SWCNT nanocarriers (Al Faraj et al., 2016) targeting CSCs showed the highest cytotoxicity having a promising therapeutic use in eradicating CSCs. Cytokines (IL-8) released after chemotherapy acts on CXCR1 enriched CSCs and further promotes the survival and growth of CSCs. However, paclitaxel in combination with reparixin, a CXCR1 inhibitor counteracted the brain metastasis by targeting CSCs with anti-metastatic and pro-apoptotic activities respectively (Brandolini et al., 2015). A product from the honeybee, Caffeic acid phenethyl ester-CAPE also showed the inhibitory effect on mammary carcinoma by targeting self-restoration and progenitor formation of CSCs dose-dependently. It was also found to promote the CSCs cell cycle progress from G_0/G_1 to S phase (Omene et al., 2012) (Table 2).

6. Conclusion

Current data recommends CSCs as a key target in the treatment of breast cancer which remains a great challenge owing to their resistance, recurrence, and metastasis due to the complex natures and regulations. Even their changeability builds numerous challenges. So, we have abridged the cutting edges of several regulatory mechanisms in the self-renewal signaling pathways that may strengthen the preclinical research for developing newly targeted modalities targeting BCSCs. The forthcoming promising strategies should be to progress a molecular research to understand the unknown regulations and to develop a tumor niche mimicking systems which may advance the treatment options. Creating combined therapy regimens, in which novel CSCs targeting drugs with conventional drugs may offer cancer-free survival rates. So, an impending dose decrease of the chemotherapeutics might happen which helps in limiting adverse effects and toxicity by improving patient's quality of life. Furthermore, making the CSCs to differentiate signifies an effective method in eradicating BCSCs.

Competing interests

The authors declare no conflict of interest.

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