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SOX9: The Master Regulator of Cell Fate in Breast Cancer

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Abstract

SRY-related high-mobility group box 9 (SOX9) is an indispensable transcription factor that regulates multiple developmental pathways related to stemness, differentiation, and progenitor development. Previous studies have demonstrated that the SOX9 protein directs pathways involved in tumor initiation, proliferation, migration, chemoresistance, and stem cell maintenance, thereby regulating tumorigenesis as an oncogene. SOX9 overexpression is a frequent event in breast cancer (BC) subtypes. Of note, the molecular mechanisms and functional regulation underlying SOX9 upregulation during BC progression are still being uncovered. The focus of this review is to appraise recent advances regarding the involvement of SOX9 in BC pathogenesis. First, we provide a general overview of SOX9 structure and function, as well as its involvement in various kinds of cancer. Next, we discuss pathways of SOX9 regulation, particularly its miRNA-mediated regulation, in BC. Finally, we describe the involvement of SOX9 in BC pathogenesis via its regulation of pathways involved in regulating cancer hallmarks, as well as its clinical and therapeutic importance. In general, this review article aims to serve as an ample source of knowledge on the involvement of SOX9 in BC progression. Targeting SOX9 activity may improve therapeutic strategies to treat BC, but precisely inhibiting SOX9 using drugs and/or small peptides remains a huge challenge for forthcoming cancer research.

Graphical Abstract

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Authors' contributions

SJ, BMK, JS: prepared original draft of the manuscript; DH, RS, SA, SSS: reviewed and edited the manuscript. All authors read and approved the final manuscript.

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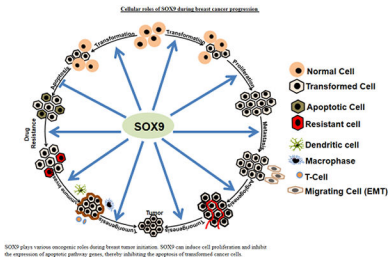

Keywords

Breast cancer; SOX9; microRNA; Chemoresistance; Therapy

1. Introduction

Breast cancer (BC) is the leading cause of cancer incidence and the second leading cause of cancer mortality in women [1]. Despite advances in early detection and treatment, about 50% of patients with BC will either fail to respond to initial chemotherapy or will rapidly acquire resistance to chemotherapeutic agents; these data correspond to global or entire world patients. Drug resistance remains a major obstacle to the successful treatment of BC. Chemoresistance and metastasis are responsible for ~90% of deaths in patients with solid tumors, including those originating in the breast [2]. To more effectively tailor treatments to individual patients; it is important to identify potent targets at the molecular level.

In the early 1990s, a novel transcription factor was discovered and found to be involved in testis determination. The gene encoding this transcription factor, termed the sex-determining region Y (SRY) gene due to its location on the Y chromosome, has a distinctive DNA-binding domain [3, 4]. With its conserved high-mobility group (HMG) domain, the SRY protein always binds to specific DNA sequences. Since then, 20 different so-called SOX genes, which contain the HMG domain, have been identified in the mouse and human genomes based on their sequences and functions [5]. These genes have been divided into eight subgroups: SOX A-H, with 1–3 members each [6]. SOX9 belongs to subgroup E, along with SOX8 and SOX10. The subgroup E proteins share high sequence similarity and mediate cell fate by regulating diverse functions that maintain pluripotency, terminal differentiation, cell lineage restriction, and tissue homeostasis, depending on the cell and tissue types in which they are expressed [5]. In recent years, mounting evidence has found that SOX9 can regulate diverse cellular processes, including cell proliferation [7], apoptosis [8], migration [9], invasion [10], chemoresistance [11], stem cell [12], autophagy [13], angiogenesis [14], immune escape [15] and metastasis [16], by regulating the expression of several targeted genes. Extensive studies also demonstrated the involvement of SOX9 in the development of various cancers, such as bladder cancer [17], brain cancer [18], BC [19], cervical cancer [20], colon cancer [21], chondrosarcoma [22], esophageal cancer [23], endometrial cancer [24], gastric cancer [25], head and neck cancer [26], liver cancer [27], lung cancer [28], melanoma [16], ovarian cancer [29], prostate cancer [30], pancreatic cancer [31], renal cell carcinoma [32], and thyroid cancer [33], suggesting that it has a general role in tumor development and progression. SOX9 has also been discovered to play

a role in regulating multiple signaling pathways during cancer progression [34–56] (Figs 1 and 2A). In this review, we discuss recent advancements regarding the role of SOX9 in BC pathogenesis through its mediation of important mechanisms, including tumor initiation and proliferation, apoptosis, migration, invasion, angiogenesis, chemoresistance, immune escape, stem cell maintenance, and regulation of the tumor microenvironment (Fig 2B). We also discuss the prognostic value of SOX9 expression and the potential of targeting SOX9 for BC therapy.

2. SOX9 structure and functions

To understand the molecular mechanisms underlying the role of SOX9 in development and the progression of various diseases, it is essential to establish a comprehensive picture of the structure and function of the protein. The expression of SOX9, a SRY family gene located a 3-Mb region on chromosome 17, is directed in a complex manner by individual enhancers in different tissues [57]. Generally, SOX9 contains a SRY-related HMG domain with three α helices (Fig 2A) with ~50% amino acid similarity [58]. SOX9 exhibits a high rate of translocation between the nucleus and cytoplasm, as its DNA-binding HMG domain contains two nuclear localization signal (NLS) sequences [59] and one nuclear export signal (NES) sequence enriched with leucines [60] (Fig 2A). During development, this structural arrangement ensures the translocation of SOX [61]. The HMG domain contains a conserved, 79 amino acid-long DNA-binding motif that binds with the minor groove of DNA containing the consensus sequence (A/TA/TCAAA/TG), forming an L-shaped complex [62]. SOX9 is in subgroup E of the SRY family, along with SOX8 and SOX10. These proteins share the highest level of similarity within the HMG domain and contain two other functional domains: 1) a dimerization domain (DIM), which enables homodimerization through dimerization with the HMG box of another SOX [63]; and 2) another C-terminal domain called the transactivation domain (TAC), which interacts with transcription machinery or co-activators associated with transcription. Like other SOX proteins, SOX9 undergoes post-translational modifications like phosphorylation, acetylation, and ubiquitination at different amino acid sequences (Fig 2A).

SOX9 protein, like other proteins in its subgroup, functions by interacting with various partners and shows stimulation and suppression activities in different cell types [58]. SOX9 was first reported in relation to campomelic dysplasia (CMPD), a severe skeleton malformation syndrome in which it is mutated [64]. Differential expression of SOX9 has also been associated with testis development disorder and sex reversal [65], and it is also known to play a critical role in chondrogenesis [66] and Sertoli cell differentiation [67]. SOX9 is also known to control the development of hair follicles [68], the central nervous system [69], the retina [70], the lungs [71], the pancreas [72], the heart [73], and the kidney [74]. In summary, SOX9 is important for cell lineage determination during various developmental processes. Recent studies have also found that SOX9 plays an essential role in the development of several tumor types, including BC. The present review focuses on the role of SOX9 in BC growth and progression.

3. Molecular mechanisms of SOX9 regulation

3.1 Transcriptional regulation

3.1.1 Epigenetic—Anomalous DNA methylation, which may aberrantly regulate transcriptional activity, is a hallmark of cancer [75]. DNA in cancers may be hypermethylated or hypomethylated, dictating the under- and overexpression of genes, respectively, depending on the coverage of the CpG islands in their promoter regions. Previous work has demonstrated that the methylation status of the SOX9 promoter region is dysregulated in several progressing tumor types. The very first study was done in bladder cancer and revealed that SOX9 was hypermethylated in 56.4% of cases and correlated significantly with advanced grade and poor overall survival (OS) [17]. Since then, several similar studies have been conducted in different cancers [76–80]. However, few studies have been performed to assess methylation in BC. One study revealed that several genes associated with BC, but not SOX9 or NFE2L3, were hypermethylated in BC [81]. Another interesting study revealed that the stem cell-associated genes ALDH1A, WNT5A, and SOX9 were significantly hypomethylated after neoadjuvant chemotherapy in all samples tested, including BC cells and tumor tissues [82]. Therefore, methylation may prove to be a valuable regulator that can modulate the expression of SOX9 in BC, but this remains to be evaluated.

3.1.2 Transcription factor—It is well documented that SOX9 expression is elevated in most types of cancer, including BC. Transcription factors that directly activate SOX9 expression have been identified in several cancers, including BC. For example, using a global transcriptomic approach, it was demonstrated that HDAC9 (histone deacetylase 9) regulates the expression of SOX9 [83]. Previous work demonstrated that PML (promyelocytic leukemia) protein was highly expressed in BC-initiating cells, and particularly in highly aggressive BC cells. This work also determined that SOX9 is the downstream modulator of PML via close proximity binding to its promoter region as because it has no canonical DNA-binding domain [84]. In addition, whole-genome EVI1 ChIP data documented the correlation of EVI1 within SOX9 binding sites, and EVI1 exhaustion reduced the expression of SOX9 in BC cell lines [85]. Additionally, Jeselsohn R *et al.* found that the expression of SOX9 in BC cells is modulated by the RUNX2-ER complex, resulting in induction of the stemness-mediated endocrine resistance of BC cells [86].

3.2 Post-transcriptional regulation

3.2.1 microRNA (miRNA)-mediated—miRNAs are short 22bp noncoding RNAs that regulate the expression of genes by directly binding to their 3’UTR sequences, modulating various cellular pathways, including those involved in proliferation, migration, and development [87]. Emerging studies show that several miRNAs contribute to BC pathogenesis by dysregulating the expression of various genes [88, 89]. Several studies have demonstrated that SOX9 expression is regulated by multiple miRNAs in various cancers, including BC [26, 90]. miR-101 was the first miRNA reported to regulate the expression of SOX9 by directly binding to its 3’UTR sequence in human hepatocellular carcinoma [27]. In BC, miR-140 was the first miRNA found to regulate the expression of

SOX9 by direct binding, resulting in reduced self-renewal of cancer stem cells (CSCs) and tumorforming capacity of BC cells [47]. In contrast, another study found that miR-3134, in combination with the RNA binding protein HuR, stabilized the AU-rich element (ARE)-bearing transcript SOX9 in a BC cell line [91]. Furthermore, based on a study by Gernapudi R *et al.*, it was shown that SOX9 is dysregulated by the preadipocyte-derived exosomal miRNA, miR-140 [92]. In addition, an interesting study revealed that miR-206 expression was dysregulated particularly in TNBC (triple negative BC) and regulated the expression of SOX9, along with VEGF and MAPK3. Thus, the downregulation of miR-206 promoted TNBC invasion and angiogenesis [14]. In contrast, CSCs and chemoresistant cells were found to secrete exosomal miR-155, inducing the expression of stem cell factor SOX9, but the molecular mechanisms behind this effect is still unknown [93]. In another study using *in vitro* and *in vivo* models, it was revealed that SOX9 expression is regulated by tumor-suppressive miR-133b, which reduces the cell proliferation, migration, and invasion abilities of BC cell. This study also revealed that the simultaneous expression with miR-133b reduced the oncogenic defects of SOX9 [94]. Consistent with these findings, it is known that miR-511 plays an important role in breast tumor growth and metastasis reduction by directly regulating the expression of SOX9 and deactivated the oncogenic PI3K/Akt signaling pathway [95]. Yu Y *et al.* further explored the expression of SOX9 in BC. They revealed that the expression of miR-190 was downregulated in BC samples, and the enforced expression of miR-190 inhibited the Wnt/β-catenin signaling cascade by directly modulating the expression of SOX9, increasing the sensitivity of BC cells to tamoxifen (TAM) *in vitro* and *in vivo* [96]. Additionally, the role of chemotherapy drug-induced extracellular vesicles (EV)-contained miRNA secretion as a positive regulator of SOX9 was revealed by Shen M *et al.* They observed that drug-treated BC cells secreted EV-contained miR-9-5p, miR-195-5p, and miR-203a-3p, which dysregulated the expression of ONECUT2 by direct binding, resulting in upregulated expression of CSC phenotype and stem cells factors, including NOTCH1, SOX9, NANOG, OCT4, and SOX2 in BC cells [97]. Finally, Gao JB *et al.* found that enhancing the expression of the tumor suppressor miR-215-5p could decrease the aggressiveness of BC cells by regulating the expression of SOX9 [98]. These findings suggest that miRNAs can be considered potent regulators of SOX9 expression (Table 1), providing a promising strategy to reduce SOX9 expression in BC.

3.2.2 Long noncoding RNA (lncRNA)-mediated—lncRNAs are noncoding RNAs longer than 200 nucleotides. [99]. Numerous reports have revealed that lncRNAs play crucial roles in all cellular processes, including proliferation, differentiation, apoptosis, migration, invasion, and metabolism, by regulating the expression of various genes and miRNAs [99, 100]. In the very first report of lncRNA-mediated SOX9 regulation, Meng Q *et al.* showed that an oncogenic lncRNA, LncRNA-RMRP, induced the expression of KRAS, FMNL2, and SOX9 by inhibiting the expression of miR-206 [50]. Since then, many lncRNAs have been found to deregulate the expression of SOX9 in several cancers, including some that induce the expression of SOX9 [52, 56, 101] and others that reduce the expression of SOX9 [102, 103]. However, a recent study by Tariq A *et al.* revealed the regulatory function of lncRNAs on SOX9 in BC, stated that lncRNA linc02095 and SOX9 are expressed in basal type BC cell lines in a co-regulated manner. They also discovered that linc02095 induced the SOX9 transcription by regulating the tenancy of RNA pol II at

the SOX9 gene and the induced linc02095 help in the export process of SOX9 mRNA to cytoplasm [56]. More studies will be required to uncover the mechanisms through which SOX9 is regulated by lncRNAs during BC progression.

4. Role of SOX9 in BC tumorigenesis

Emerging studies have revealed that SOX9 can control several important phenomena in BC during tumorigenesis, including proliferation, metastasis, drug resistance, stem cell maintenance, immune evasion, and modulation of the tumor microenvironment (Figs 2B and C). In this section, we describe each parameter that is regulated by SOX9 during BC progression.

4.1 BC initiation and proliferation

SOX9 is one of the most important genes upregulated during early tumor formation [45, 104]. The contribution of SOX9 to tumor initiation is associated primarily with cell cycle progression and cell proliferation. Contrasting results have been reported regarding the role of SOX9 in cell cycle regulation during BC progression. The very first report on this topic revealed that SOX9 is involved in the G0/G1 arrest of T47D BC cell line [34]. The tumor-suppressive role of SOX9 was further supported by the finding that the SOX9 mediates the HES-1 expression in BC cells during treatment with anti-proliferative retinoic acid (RA) [105]. In contrast, the involvement of SOX9 in BC cell proliferation was first observed by Chakravarty G *et al.,* who demonstrated that cytoplasmic SOX9 expression was highly associated with the expression of the proliferative maker Ki67, specifically in invasive ductal breast tumors [106]. Additionally in basal-like BC cells, the inhibition of SOX9 expression reduced proliferation by controlling the expression of LRP6 and TCF4 [46]. Moreover, SOX9 expression reduced by fucoxanthin and fucoxanthinol in MDA-MB231 cells, but not in MCF7 cells, and may be involved in cell viability [107].

Interestingly, it was also revealed that SOX9 is an HDAC9 target gene, and the expression of both genes was highly correlated in the basal subtype of BC. The study also revealed the significance of SOX9 in maintaining the mitogenic activity of HDAC9, resulting in the dysregulation of cell proliferation [83]. Also, it was claimed that EVI1 and SOX9 cooperatively regulate transcriptional reprogramming, promoting the upregulation of pathways involved in BC tumor initiation [85]. Meanwhile, it was discovered that SOX9 was upregulated and promoted the proliferation of endocrine-resistant BC cells through the RUNX2-ER complex [86]. Interestingly, a recent study determined that the expression of SOX9 was higher in tumor spheres than in adherent BC cells and may be associated with their proliferation status [108]. Notably, one study showed that reducing the expression of SOX9 inhibited the proliferation of BC cells [94]. Furthermore, SOX9 was found to induce the activity of PI3K/Akt signaling pathway thus promoting the proliferation of BC *in vitro* and *in vivo* [95]. Recently, Domenici G *et al.* also found that reducing the expression of SOX9 could decrease the soft agar colony formation of TAM-resistance BC cells *in vitro* and tumorigenicity of TAM-resistance BC cells *in vivo*. They also observed that the soft agar colony formation, invasion, and invasion from spheroid were decreased upon lower the expression of SOX9 in TNBC cell lines [109]. Additionally, Yu Y *et al.*

confirmed the involvement of SOX9 in BC cell proliferation; they observed the reversal of the proliferative effects of SOX9 on BC cells by co-treatment with tumor-suppressive miR-190 [96]. Consistent with these findings, SOX9 was shown to be involved in cell proliferation and colony formation in BC cells [98]. In conclusion, SOX9 has a potential role in controlling cell proliferation and may be a crucial molecular target to hamper the proliferative nature of BC cells.

4.2 BC apoptosis

Tumor cell apoptosis is a vital step in cellular development. Apoptosis is stimulated intrinsically and extrinsically through two different pathways that trigger cell death by activating various proteins, resulting in the elimination of damaged, aged, or autoimmune cells [110]. The initiation and progression of tumor growth is supported by the reduction of apoptosis in tumor cells. An increasing number of studies have shown that reduced SOX9 expression is correlated with apoptosis and that SOX9 may function as an essential regulator of cancer cell death. The apoptosis of BC cells was promoted by fucoxanthin and fucoxanthinol, and fucoxanthinolmediated apoptosis was more significant than others in MDA-MB231 cells. In the same report, researchers described the dysregulated expression of nuclear SOX9 after treatment with this compound [111]. Another study by the same group revealed reduced expression of members of the NFκB pathway and SOX9 after treatment with fucoxanthinol, which they associated with the apoptosis-inducing effect of this compound [107]. In addition, SOX9 may inhibit apoptosis by inducing the activity of its target gene HDAC9 (83). SOX9 has been consistently shown to be involved in reducing apoptosis in BC, resulting high rates of growth and metastasis rate [95]. From these studies, it is becoming clear that SOX9 may play an anti-apoptotic role in BC by targeting the apoptotic pathway.

4.3 BC migration and invasion

Metastasis was the most essential sequel in the advancement of BC and represents the main cause of mortality in BC patients, accounting for ~90% of deaths in patients within solid tumors [2]. The phenomenon of metastasis involves a series of sequential and organized events; however, migration and invasion play central roles in metastasis. Important evidence has highlighted the role of SOX9 in these complex processes. Interestingly, SOX9 has been identified as a pro-metastatic gene by Endo Y *et al.* They demonstrated that SOX9 is involved in the transcriptional regulation of vascular endothelial (VE)-cadherin by directly binding to its promoter region after RA treatment, thereby regulating the endothelial-like differentiation of BC cells and suggesting that SOX9 probably supports the incorporation of cells into a growing organ or tumor [112]. It was found that SOX9 expression was increased in estrogen receptor (ER)-negative and higher-grade human breast tumors, and its cytoplasmic accumulation was associated with the lymph node metastasis of invasive ductal carcinomas [106]. Meanwhile, SOX9 was discovered to induce micro- and macro-metastases within the lungs during BC progression, and abating SOX9 reduced the rate of macro-metastases to the lungs [20]. In addition, it was documented that SOX9 was positively regulated the metastatic outgrowth of latency-competent cancer (LCC) cells from BC cell lines during tolerant conditions [15]. Furthermore, the role of SOX9 as a positive regulator of metastasis was confirmed by Mateo F *et al.* They found that the dysregulation of SOX9

directly mediated the metastatic signature of BC cells to the lungs [85]. Similarly, the ability of restored miR-133b expression to inhibit BC invasion was halted by the subsequent overexpression of SOX9, suggesting that SOX9 is involved in maintaining micro-metastases and the weight of the lungs during BC progression [94]. Furthermore, the inhibition of SOX9 was shown to suppress the migration and invasion capacity of BC cells [95]. In addition, the induction of invasion caused by SOX9 was elucidated by Gao JB *et al.*, who found that the dysregulation of SOX9 was directly involved in the regulation of BC migration and invasion [98]. Thus, SOX9 dysregulation might be associated with the process of metastasis, though further investigation is needed to understand the mechanisms underlying this association during BC progression.

4.4 BC angiogenesis

Tumor angiogenesis, the formation of new blood vessels, is an important hallmark [113] that is significant for the growth and metastasis of cancers, including BC [114]. The association between angiogenesis and tumor growth has become a major interest in the field of cancer research. Substantial work has demonstrated that SOX9 might support the development and progression of cancers through its role in angiogenesis. VE-cadherin, one of the most important among many endothelial genes, has been associated with angiogenesis [115]. Endo Y *et al.* found that SKBR3 cells undergo network formation in matrigel, forming mixed structures when co-cultured with human umbilical vein endothelial cells during RA treatment. They also found increased expression of VE-cadherin and SOX9 after the same treatment. In addition, they observed that the induction of VE-cadherin expression by the SOX9-ER81 transcriptional complex resulted in morphological changes that may lead to angiogenesis throughout RA treatment [112]. TNBC has higher rates of primary and secondary metastases compare to other BC subtypes due to greater angiogenic potential [116]. More recently, SOX9 has been proposed to be involved in TNBC invasion and metastasis [14]. Collectively, whether SOX9 enhances angiogenesis and may be appealing as a therapeutic target in BC remains to be explored further.

4.5 BC drug resistance

About 80% of BCs are ER-positive, constituting the predominant subtype of BC. The main treatment strategies to treat ER-positive BC generally involve endocrine therapy. Unfortunately, not all patients with ER-positive BC are responsive to anti-estrogen therapy, and many acquire resistance [117]. Several studies have established the significant role of SOX9 in drug resistance. The very first report of SOX9-mediated drug resistance was on endocrine resistance, demonstrated by Jeselsohn R *et al.* They observed upregulation of RUNX2 in TAM-resistant BC cells, and the resistance against TAM was mediated by a set of genes transcribed by RUNX2 – ER complex. They also observed the most abundant gene transcribed by this complex was SOX9, which contributed to the development of endocrine resistance [86]. For instance, SOX9 was found to be significantly upregulated in doxorubicin (DOX)- and paclitaxel (PTX)-resistant MCF7 BC cells. The same study also revealed that the expression of SOX9 was upregulated in MCF7 cells that received exosomes from CSCs and DOX- and PTX-resistant MCF7 cells [93]. Moreover, upregulated SOX9 has also been observed in tumor initiating cells (TICs) and $ALDH^+$ cells, and correlated with the expression of FXYD3. It has been also found that SOX9 directly regulating the

expression of FXYD3 in a positive manner and that FXYD3 play an important role in chemoresistance of ER^+ BC cells [118]. Furthermore, SOX9 was elevated in patient's samples, and its expression level was significantly high in ER negative tissue samples than ER positive tissue, and its downregulation reduced the growth of TAM-resistant BC cells *in vivo* [109]. Interestingly, a recent study identified that SOX9 is the primary modulator of the Wnt/β-catenin signaling pathway, increasing resistance of BC cells against anti-estrogen therapy [96]. Consistent with these results, a recent study reported that EVs secreted by BC cells treated with chemotherapeutic agents (docetaxel and DOX) induced the expression of SOX9 in recipient BC cells and led to drug resistance [97]. The studies mentioned above focused primarily on TAM resistance (Table 2a). Nevertheless, increasing evidence indicates that BC patients are distressed from multi-drug resistance, the molecular mechanisms of which remain to be explored.

4.6 BC stem cells

CSCs are important players assumed to have significant roles in tumor formation, chemoresistance, metastasis, and disease recurrence [119]. It has been well documented that breast tumors contain a population of self-renewing CSCs [120] that are involved in predetermining BC cells fate [121]. First, Guo W *et al.* identified that SOX9, collaboratively with Slug, regulates the mammary stem cell (MaSC) state, and inhibition of either protein inhibit MaSC activity. Conversely, the ectopic expression of both proteins induces the conversion of luminal cells into MaSCs by regulating luminal progenitor genes like cKit, CXCR4, ELF5, and LBP [19]. For instance, SOX9 was found to be significantly upregulated in DCIS (ductal carcinoma *in situ*) cancer stem-like cells and involved in maintaining their mammosphere-forming capacity [47]. Furthermore, the expression of SOX9 was induced in WISP2/CCN5-knockdown BC cells and may be involved in maintaining stem cell properties and tumor growth [122]. In addition, it was acknowledged that SOX9 is positively regulated by NKG2D (natural killer group 2 member D) and stimulates cancer cell plasticity by inducing stem cell phenotypes [123]. Additionally, it was found that SOX9 was increased in lymphangioleiomyomatosis (LAM), a neoplasm characterized by proliferative ERα-negative and PR-positive smooth muscle-like cells with lung-metastatic capabilities. The study revealed that the biomarkers associated with LAM to BC stemness and lung metastasis [124]. As the very first study on this topic determined, Slug and SOX9 are two transcription factors that are important for regulating MaSCs. Consistent with these results, Fazilaty H *et al.* showed that SOX9 and Slug cooperatively regulate the expression of tenascin-C and periostin, two essential factors associated with tumor initiation and metastatic niche formation [125]. At the same time, Malladi S *et al.* found that SOX9 and SOX2 are involved in maintaining the stem cell-like characteristics of latency competent cancer (LCC) cells derived from human BC cell lines, resulting in the sustained tumor-instigation properties of LCC cells during the latent metastatic stage [15]. Interestingly, it was also announced that PML protein positively regulates the expression of the stem cell factor SOX9 by directly binding to its promoter region in BC initiating cells, thus regulating cancer initiation and metastatic seeding abilities [84]. Furthermore, the induction of EVI1 was shown to regulate the expression of SOX9 and maintain stem cell-like phenotypes, and cooperatively with EVI1, SOX9 also increased the expression of the mTOR cascade components REHB and RAPTOR, as well as the lung metastasis mediators and FSCN1

and SPARC, in BC cells [85]. Notably, the function of SOX9 in lineage maintenance was observed by Wang C *et al.*, who found that a distinct population of SOX9-positive stem cells always developed into and maintained an ER-negative lineage of cells [126]. Meanwhile, SOX9 was discovered to associate with cadherins, particularly CDH4 and CDH17, and to maintain stem cell phenotypes in TNBC [127]. Meanwhile, it was also discovered that RUNX2-ER complexes regulate the expression of the stem cell factor SOX9 in endocrine-resistant BC cells, and the induction of SOX9 controlled resistance against TAM [86]. Also, it was claimed that human BC cell-derived M13HS hybrid clone cells and their parental cells express SOX9 and Slug and possess CSC-like properties [128]. Moreover, it has been observed that SOX9 is more highly expressed in tumor spheres than in adherent BC cell lines [108]. Interestingly, a recent study determined that FXYD3 (an estrogen-inducible gene) was notably overexpressed in luminal CSCs, particularly the ER-positive subtype. This study also revealed that the stem cell factor SOX9 directly regulates the expression of FXYD3, which regulates the nuclear translocation of SOX9 in BC (118). Additionally, Domenici G *et al.* confirmed the involvement of SOX9 in human breast CSCs and breast luminal progenitor cell maintenance. They observed that SOX2 is an upstream modulator of SOX9 and that SOX9 was involved in modulating luminal progenitor cell content and expression of ALDH1A3 in breast CSCs. They also observed that CRISPR-mediated knockout of SOX9 diminished the growth of TAM-resistant breast tumors *in vivo* [109]. Meanwhile, in another study, it was proclaimed that SOX9 rescues TAM resistance and the stemness properties of BC cells treated with tumor-suppressive miR-190. The study also acknowledged that the tumor-suppressive properties of miR-190 were due to its regulation of SOX9 activity by direct targeting [96]. Strekalova E *et al.* disclosed that the restriction of methionine in TNBC cells repressed mammosphere formation and the CSC population by regulating MAT2A/SOX9 axis [129]. Recently, Shen M *et al.* found enhanced expression of SOX9 and other stem cell-related transcription factors in BC cells treated with EVs secreted by chemotherapy-treated BC cells. They also found that this effect was due to EV miRNA-mediated regulation of ONECUT2 [97]. Taken together, these results endorsed the functional importance of SOX9 in BC progression and stem cell maintenance (Fig 2C).

4.7 BC immunomodulation

Recent studies have demonstrated that immune cells play an important role in the initiation and progression of cancer by suppressing immune rejection and thus supporting enhanced tumor growth and metastasis [130]. The pivotal role of SOX9 in immune evasion was described for the first time by Malladi *et al.* in 2016. They observed that SOX2 and SOX9 maintained the long-term survival and tumor-initiating properties of LCC by maintaining stem cell-like characteristics. They also observed that these two transcription factors are essential in LCC cells for maintaining a quiescence state at secondary metastasis sites and evading immune surveillance under immune-tolerant conditions [15].

4.8 BC microenvironment

Recently, it has been shown that the tumor microenvironment plays a crucial role in cancer development/progression and treatment efficacy, which are associated with advanced stages and drug resistance [131]. A previous study implicated preadipocytes in breast tumor initiation and metastasis through their exosomes-mediated communication with BC

cells. The study also revealed the role of preadipocyte-secreted exosomal miR-140 in the regulation of stem cell renewal, cell migration, and tumor formation during the early stages of BC. Furthermore, based on the same study, it was proclaimed that the SOX9 is the main regulator of these processes in the tumor microenvironment and thus promoted tumorigenesis *in vivo* [92].

5. Clinical significance

In the clinic, a simple biomarker to improve early disease detection and prognosis is highly desirable. There have been numerous reports on the prognostic roles of various genes in BC; however, no presently used biomarkers can perfectly predict outcomes in BC. Critical evidence has demonstrated that the aberrant expression of SOX9 in BC, supporting the role of SOX9 in various cancer-associated pathways. BC tumor tissues overexpress SOX9, whereas normal tissues have very low expression of SOX9. This study also revealed that the expression of SOX9 was directly associated with hormone receptor expression and advanced grade, with higher expression in ER-negative BC. The higher expression was also associated with shorter overall survival (OS). In addition, it was observed that the cytoplasmic accumulation of SOX9, which was linked to the elevated proliferation of IDC (invasive ductal carcinoma) and metastatic BC, also contributes to poor clinical outcomes for patients with invasive BC [106]. Guo W *et al.* confirmed that the overexpression of SOX9 and Slug in BC patient samples was associated with poor OS. Their study also revealed that the co-expression of SOX9 and Slug promotes the tumorigenicity and metastatic seeding abilities of BC cells [19]. In another study using immunohistochemistry, it was revealed that SOX9 is an aggressive basal-like BC signature gene. Its expression was associated with histological grade and may function as an independent factor indicating the poor prognosis of patients with TNBC [46]. Meanwhile, SOX9 was discovered within the stroma of BC patients, and strong expression of SOX9 within the stroma after chemotherapy was associated with shorter OS [132]. Moreover Sox9 expression was closely related with expression of ER, PR, Ki67, p53, and lymph node metastasis. High expression of SOX9 was also associated with BC stem cells and both were correlated with poor overall and disease free survival and related with worst prognosis [133]. Additionally, one group found that SOX9 was highly expressed in patient with TNB, but it was not significantly correlated with disease outcome [134]. Subsequently, Richtig G *et al.* found that the expression of SOX9 may be a strong indicator of poor 5-year, relapse-free survival [108]. Furthermore, Kündig P *et al.* reported that the cytoplasmic expression of SOX9 was significantly associated with histological grade and may function as a prognostic marker of BC patients [135]. All of the above studies indicate the potential of SOX9 as a prognostic marker (Table 2b). These studies opened a new way of thinking about SOX9 as a diagnostic marker, and more studies will be needed to confirm this activity.

6. Conclusions

SOX9 plays a crucial role in the regulation of several different processes during cancer pathogenesis, such as cell growth, apoptosis, migration, invasion, stemness, drug resistance, and immune escape. The expression of transcriptional factor SOX9 is significantly induced in BC patient samples. However, one investigation using a BC cell line determined that

SOX9 was involved in growth retardation. In most cases, the activity of SOX9 during BC progression is maintained by tumor-suppressive miRNA, but a few proteins can also regulate its expression. Various studies have demonstrated that SOX9-related breast carcinomas exhibit treatment failure due to drug resistance and induction of stemness; therefore, SOX9 contributes to a poorer prognosis for patients with BC. Considering the functional activities of SOX9 during BC pathogenesis, it is apparent that SOX9 may represent a critical target for drug development. Additionally, measurable evidence for the expression of SOX9 protein within the exosome might be imperative from a prognostic point of view. However, several vital questions must be addressed to elucidate the influence of SOX9 during BC development and to inform the design of SOX9-targeting drugs, which may open a new era for combinatorial treatment with existing endocrine therapies.

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Abbreviations:

3'UTR	3'untranslated region
ALDH1A	aldehyde dehydrogenase 1 family member A1
ALDH1A3	aldehyde dehydrogenase 1 family member A3
ARE	AU-rich element
BC	breast cancer
CDH17	cadherin 17
CDH7	cadherin 7
cKit	KIT proto-oncogene, receptor tyrosine kinase
CMPD	campomelic dysplasia
CSCs	cancer stem cells
CXCR4	C-X-C motif chemokine receptor 4
DCIS	ductal carcinoma *in situ*
DIM	dimerization domain
DOX	doxorubicin
ELF5	E74-like ETS transcription factor 5
ER	estrogen receptor
ER81/ETV1	ETS variant transcription factor 1

ERα/ESR1	estrogen receptor 1
EV	extracellular vesicle
EVI1	ecotropic viral integration site-1
FMNL2	formin-like 2
FSCN1	fascin actin-bundling protein 1
FXYD3	FXYD domain-containing ion transport regulator 3
HDAC9	histone deacetylase 9
HMG	high-mobility group
HuR/ELAVL1	ELAV-like RNA binding protein 1
IDC	invasive ductal carcinoma
KRAS	KRAS proto-oncogene
LAM	lymphangioleiomyomatosis
LBP	lipopolysaccharide binding protein
LCC	latency-competent cancer
lncRNAs	long noncoding RNAs
LRP6	LDL receptor related protein 6
MAPK3	mitogen-activated protein kinase 3
MaSC	mammary stem cell
MAT2A	methionine adenosyl-transferase 2A
miRNAs	microRNAs
NANOG	Nanog homeobox
NES	nuclear export signal
NFE2L3	nuclear factor, erythroid 2-like 3
NKG2D	natural killer group 2 member D
NLS	nuclear localization signal
NOTCH1	notch receptor 1
OCT4/ POU5F1	POU class 5 homeobox 1
ONECUT2	one cut homeobox 2
OS	overall survival

PI3K	phosphoinositide 3-kinase
PML	promyelocytic leukemia
PR	progesterone receptor
PTX	paclitaxel
RA	retinoic acid
RAPTOR	regulatory associated protein of mTOR complex 1
RUNX2	RUNX family transcription factor 2
SNAI2 (Slug)	snail family transcriptional repressor 2
SOX2	SRY-box transcription factor 2
SOX9	SRY-related high-mobility group box 9
SPARC	secreted protein acidic and cysteine rich
SRY	sex-determining region Y
TAC	transactivation domain C-terminus
TAM	tamoxifen
TCF4	transcription factor 4
TF	transcription factor
TNBC	triple-negative breast cancer
VEGF	vascular endothelial growth factor A
WISP2/CCN5	cellular communication network factor 5
WNT5A	Wnt family member 5A

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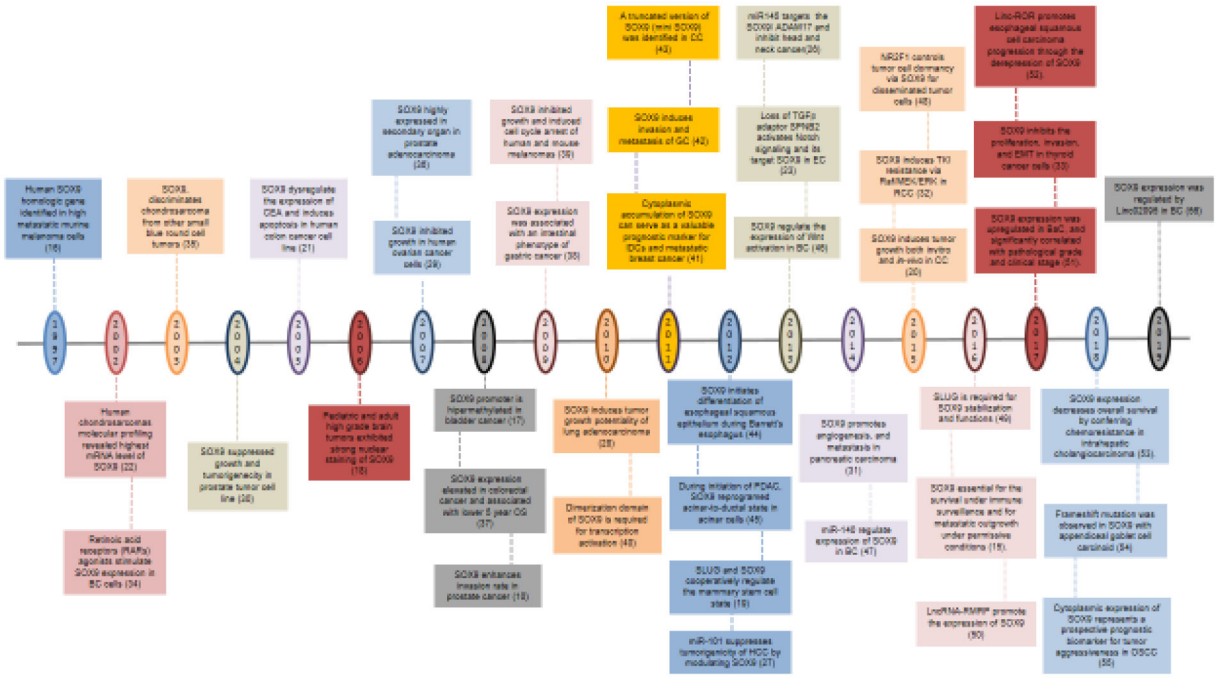

Figure 1.

Timeline of the most important findings on the role of SOX9 in tumor progression since its discovery.

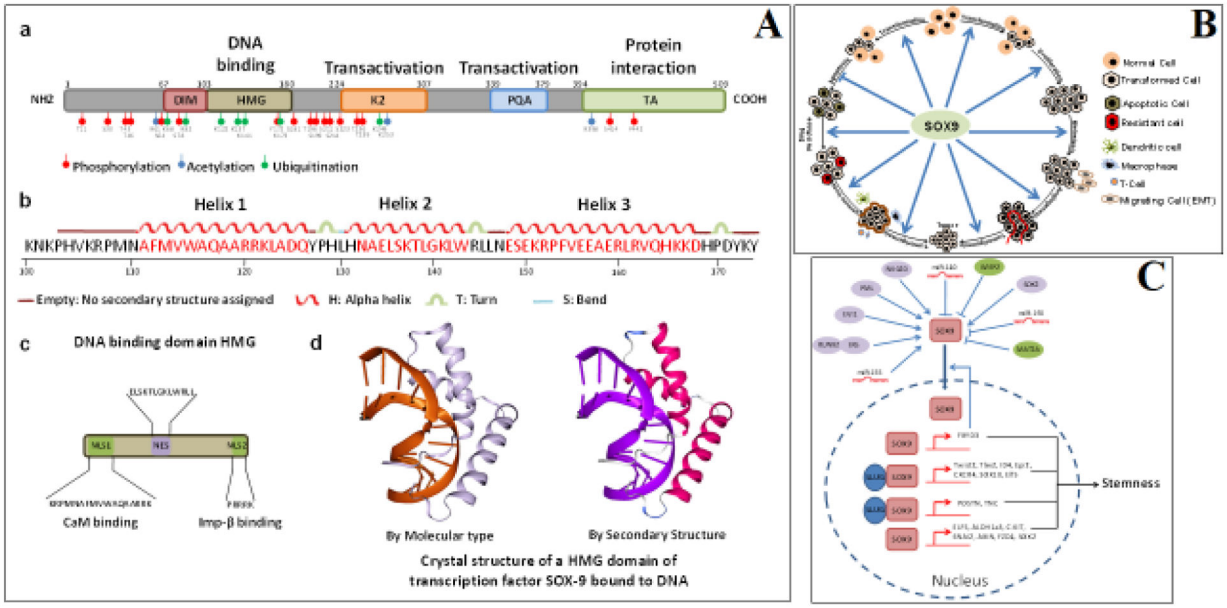

Figure 2.

(Panel A) Schematic drawing and crystal structure of SOX9 Schematic structures of SOX9 protein. SOX9 protein has five different domains: the dimerization domain (DIM), followed by the DNA-binding high-mobility group (HMG) domain, two transactivation domains (K2 and PQA) located in a central position, and one at the C-terminal domain (TA). Post-translational modifications identified by phosphorylation sites (red), acetylation sites (blue), and ubiquitination/sumoylation sites (green) are highlighted (**a**). Schematic diagrams of the SOX9 DNA-binding HMG domain, showing the amino acid sequence involved in the production of its secondary helix structure (**b**), two independent nuclear localization signal (NLS) sequences that interact with calmodulin (CaM) and importin-β, and nuclear export signal (NES) sequences (**c**). Crystal structural illustrations of the SOX9 HMG domain (PDB ID: 4EUW) bound to DNA (**d**). **(Panel B) Cellular roles of SOX9 during BC progression** SOX9 plays various oncogenic roles during breast tumor initiation. SOX9 can induce cell proliferation and inhibit the expression of apoptotic pathway genes, thereby inhibiting the apoptosis of transformed cancer cells. Moreover, SOX9 induces metastatic signaling involved in progression of tumorigenesis. SOX9 contributes to tumorigenesis (both metastasis and chemoresistance) by regulating BC stem cells. Further, SOX9 contributes to tumorigenesis by promoting angiogenesis and the immune evasion of tumor cells. **(Panel C) SOX9 proteins are involved in the induction of stemness of BC cells** Upstream regulators and targets of SOX9 in the regulation of the stem cell properties of BC. RUNX2, RUNX family transcription factor 2; ERα, estrogen receptor alpha; EVI1, ecotropic virus integration 1 site protein; PML, promyelocytic leukemia; NKG2D, natural killer group 2D; CCN5, cellular communication network factor 5; SOX2, SRY-box transcription factor 2; MAT2A, methionine adenosyl-transferase 2A; miR-155, miR-140, miR-190. FXYD3, FXYD domain-containing ion transport regulator 3; TWIST2, twist family bHLH transcription factor 2; TBX2, T-box transcription factor 2; ID4, inhibitor of DNA binding 4, HLH protein; EGR2, early growth response 2; CXCR4, C-X-C motif chemokine receptor 4; SOX10, SRY-box transcription factor 10; ELF5, E74-like ETS transcription

factor 5; POSTN, periostin; TNC, tenascin-C; ALDH1a3, aldehyde dehydrogenase 1 family member A3; cKit, KIT proto-oncogene, receptor tyrosine kinase; SNAI2 (Slug), snail family transcriptional repressor 2; AXIN, axin 1; FZD4, frizzled class receptor 4.

Table 1.

SOX9 Targeting miRNAs in Human Breast Cancer

miRNA	Patient samples	*In-vitro* model	*In-vivo* model	Functions	Refs
miR-140	43 tumor samples and 5 normal samples	MCF10A, MCF10DCIS, SUM225CWN and SUM102PT cells	Immuno-deficient nude female mice	miR-140 is significantly downregulated in cancer stem-like cells and enforced expression reduced CSC self-renewal and tumor formation by directly targeting ALDH1 and SOX9	[47]
miR-3134		MCF7 cells		miR-3134 (ARE binding miRNA) induced the expression of SOX9 in collaboration with ARE binding protein HuR	[91]
miR-140		MCF10DCIS and mouse preadipocytes (3T3L1, MBA1)	Immuno-deficient Nu/Nu female mice	miRNA-140 was upregulated after shikonin treatment and reduced the expression of SOX9	[92]
miR-206	40 tumor samples	MCF7, MDA-MB361, MDA-MB231, MDA-MB435, and HCC1395		miR-206 expression was downregulated in TNBC. Ectopic expression of miR-206 inhibited cell invasion and angiogenesis of TNBC by downregulating the expression of VEGF, MAPK3, and SOX9	[14]
miR-155		MCF7 and MDA-MB231		miR-155 was induced in exosomes isolated from CSCs and resistant cells and that exosomes induced the expression of SOX9	[93]
miR-133b	38 paired tissues	BT549, MDA-MB231, BT474, SKBR3, HCC1937	BALB/c female nude mice, SCID/beige mice	miR-133b was downregulated in tumor and associated with advanced grade. Forced expression of miR-133b inhibited cell growth, invasion both *in-vitro* and *in-vivo* by directly modulating SOX9	[94]
miR-511	51 pair	MDA-MB231, MCF7	BALB/C nude mice	miR-511 was downregulated in BC tissues and cell lines and associated with lymph node metastasis and tumor stage. Ectopic expression inhibited cell growth and metastasis invite and also attenuated tumor growth *in-vivo* by directly targeting SOX9 mediated PI3K/Akt pathway	[95]
miR-190	30 paired tissues	T47D, MCF7, MDA-MB231, MDA-MB435, MDA-MB468	Female BALB/c nude mice	miR-190 induced TAM sensitivity of BC cells both *in-vitro* and *in-vivo* by targeting SOX9 resulted introverted Wnt/β-catenin signaling. The expression of miR-190 inversely correlated with SOX9 in BC samples	[96]
miR-9-5p, miR-195-5p, and miR-203a-3p	12 pre and post NT	MDA-MB231, MCF7, BT474	NOD/SCID/IL2R γ-null (NSG) mice	Chemotherapy induced EV miRNA which targeted ONECUT2 resulting induction of CSC phenotype through induction of NOTCH1, SOX9, NANOG, OCT4, and SOX2	[97]
miRNA-215-5p	39 pair of breast carcinoma tissues	MCF10A, MDA-MB468, MDA-MB231 and MCF7	BALB/c nude mice	miR-215-5p was downregulated in BC tissue samples. Upregulation of miR-215-5p inhibited BC cells growth and metastasis both *in-vitro* and *in-vivo* by directly targeting SOX9	[98]

BC, breast cancer; TNBC, triple negative BC; NT, neoadjuvant therapy

Table 2a.

Summary of SOX9-Mediated Resistance to Therapy

Cancer cell subtype	SOX9-mediated resistance to therapy	Major upstream/downstream target involved	Refs
Luminal and basal type	Inhibition of Rapamycin (mTOR)	EVI1, RHEB, mTOR, RAPTOR, FSCN1, and SPARC	[85]
Luminal type	Endocrine resistance	RUNX2, ERα, and NCOA3	[86]
Luminal and basal type	Doxorubicin and Paclitaxel	miR-155	[93]
Luminal type	Endocrine resistance (TAM)	FXYD3, pERK, Src, and ERα	[118]
Luminal type	Endocrine resistance (TAM)	SOX2, Estrogen (negative regulation) ALDH1A3, and Wnt signalling	[109]
Luminal and basal type	Endocrine resistance	ZEB1, ERα, miR-190, β-Catenin, c-Myc, CD44, TCF4	[96]
Luminal and basal type	Doxorubicin and docetaxel	ONECUT2, Notch1, Nanog, Oct4, SOX2	[97]

TAM, tamoxifen; EVI1, ecotropic viral integration site-1; mTOR, mammalian target of rapamycin; RAPTOR, regulatory associated protein of mTOR complex 1; FSCN1, fascin actin-bundling protein 1; SPARC, secreted protein acidic and cysteine rich; RUNX2, RUNX family transcription factor 2; ERα/ESR1, estrogen receptor 1; NCOA3. nuclear receptor co-activator 3; FXYD3, FXYD domain containing ion transport regulator 3; pERK, phosphor extracellular-signal-regulated kinase; SRC, proto-oncogene tyrosine-protein kinase Src; SOX2, SRY-box transcription factor 2; ALDH1A3, aldehyde dehydrogenase 1 family member A3; ZEB1, zinc finger E-box binding homeobox 1; cMyc, MYC proto-oncogene; TCF4, transcription factor 4; ONECUT2, one cut homeobox 2; NOTCH1, notch receptor 1; NANOG, Nanog homeobox; OCT4/ POU5F1, POU class 5 homeobox 1

Table 2b.

Molecular Prognostic Effect Associated with SOX9

Prognostic role	SOX9 expression	Sample size	Patients sample type	Effect	Refs
Poor	Up	>200	Tissue	Associated with higher grade and ER negative tumor. Significantly shorter OS. Cytoplasmic accumulation associated with high proliferation for IDC and metastatic BC	[106]
Poor	Up	306	Tissue	Co-expression of Slug and Sox9 associated with poor overall survival	[19]
Poor	Up	114	Tissue	Associated with tumor subtype (high expression in TNBC) and histological grade	[46]
Poor	Up	84	Tissue (stroma)	Strong stromal expression after chemotherapy associated with shorter overall survival	[132]
Poor	Up	420	Tissue	High expression with high stem cell population associated with poor OS and DFS	[133]
	Up in TNBC	617	Tissue	No significant association	[134]
Poor	Up	3951	Tissue	Strong indicator of poor 5-years relapse free survival	[108]

OS, overall survival; DFS, disease free survival; BC, breast cancer; IDC, invasive ductal carcinoma; TNBC, triple negative BC