Role of RUNX2 in breast cancer development and drug resistance (Review)

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Abstract. Breast cancer is the most common malignancy and ranks second among the causes of tumor-associated death in females. The recurrence and drug resistance of breast cancer are intractable due to the presence of breast cancer stem cells (BCSCs), which are adequate to initiate tumor formation and refractory to conventional remedies. Runt-related transcription factor 2 (RUNX2), a pivotal transcription factor in mammary gland and bone development, has also been related to metastatic cancer and BCSCs. State-of-the-art research has indicated the retention of RUNX2 expression in a more invasive subtype of breast cancer, and in particular, triple-negative breast cancer development and drug resistance are associated with estrogen receptor signaling pathways. The present review mainly focused on the latest updates on RUNX2 in BCSCs and their roles in breast cancer progression and drug resistance, providing insight that may aid the development of RUNX2-based diagnostics and treatments for breast cancer in clinical practice.

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Contents

1. Introduction

Breast cancer, a phenomenon of uncontrolled proliferation of breast epithelial cells caused by carcinogenic factors, is the most common malignancy and ranks second among the causes of tumor-associated death in females; it has become a public health issue and endangers women's health worldwide (1). According to the data released by the International Agency for Research on Cancer of the World Health Organization, breast cancer surpassed lung cancer in 2020 and became the world's most significant cancer type with the most newly-diagnosed cases and deaths among females (2). Women with early breast cancer or locoregional relapse are usually able to be cured by multidisciplinary remedies, such as surgery, radiotherapy, chemotherapy and pharmacotherapy. However, for those with metastatic breast cancer, the clinical treatment outcomes are still far from satisfactory and only palliative care (e.g., focusing on the prolongation of survival and alleviation of symptoms) may be possible, largely due to the metastatic heterogeneity and the elusive pathogenesis of breast cancer. For instance, as reviewed by Li *et al* (3), triple-negative breast cancer (TNBC) may have worse cause-specific survival and overall survival than the non-TNBC counterpart in all stages and substages, regardless of influencing factors from univariate and multivariate analyses (e.g., tumor grade, age, ethnicity, surgery and radiation treatments).

Runt-related transcription factor 2 (RUNX2) belongs to the RUNX family (including RUNX1-3) and has been recognized as a key modulator and master transcription factor

for osteogenesis, as well as prostate and skeletal development (4-7). To date, RUNX2 has been involved in diverse physiological processes, including osteogenic differentiation of mesenchymal stem/stromal cells, chondrocyte hypertrophy, immunomodulation, vascular invasion and endothelial cell migration via modulating a variety of signaling cascades (e.g., MAPK and NK-κB pathway macrophage reprogramming) (8-11). Meanwhile, the dysregulation and alteration of RUNX2 expression or activity may result in arteriosclerosis, skeletal dysplasia (e.g., cleidocranial dysplasia) and tumorigenesis (4,12-14). For instance, RUNX2 is involved in the progression of various tumor types, such as osteosarcoma, renal cell carcinoma, gastric cancer and breast cancer (15-20). For instance, a recent study by our group reported the facilitating effect of RUNX2 during aggressiveness and chemoresistance of TNBC cells via activating MMP1, which was significantly associated with poor prognosis (21). Of note, other studies have also indicated the involvement of RUNX2 in breast cancer stem cells (BCSCs) and breast cancer progression (22,23). For instance, Zhang *et al* (23,24) found that RUNX2 was required for the activity of $CD44^+/CD24^{-/low}$ BCSCs during breast cancer development, while miR-205/RUNX2 axis was further identified with s negative regulatory effect upon the activity of $CD44^+/CD24^-$ BCSCs. Taken together, these studies indicated the involvement of RUNX2 in BCSCs and its roles in breast cancer diagnosis and drug resistance, revealing its promising prospective clinical application and utility as an antitumor drug target in the future.

The present review article mainly focused on the roles of RUNX2 and BCSCs in the progression and management of breast cancer. Furthermore, current advances in therapeutic approaches for breast cancer, ranging from RUNX2-based remedies to drug resistance, were summarized. Collectively, the systematic and detailed research on RUNX2 has constituted a prospective area of diagnosis and treatment strategy innovation for breast cancer.

2. Breast cancer and classification

Breast cancers are divided into different molecular subtypes, including luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-positive and basal-like breast cancer. Among them, luminal A is a class of breast cancer with an immunohistochemical index showing estrogen receptor (ER) positivity, progesterone receptor (PR) positivity and HER2 negativity. Of the aforementioned subtypes, luminal A breast cancer is the most common type of breast cancer, which may be targeted by hormonal therapy with a favorable prognosis (25,26).

Distinct from luminal A breast cancer, the $HER2^+$ luminal B subtype features poor differentiation and a worse prognosis (27). HER2-positive breast cancer accounts for 12-20% of all invasive breast cancers, which exhibits multifaceted deteriorative characteristics, including a higher degree of malignancy, faster disease progression, greater likelihood of relapse and metastasis, and poor prognosis (28). HER2-targeted therapies have been widely used for the treatment of HER2-positive breast cancer, such as monoclonal antibodies, kinase inhibitors and antibody-drug conjugates (ADCs). Basal-like breast cancer is positive for basal cytokeratin and epidermal growth factor receptors and/or c-Kit expression, and it accounts for ~15% of breast cancers. It usually features rapid development of local and distant metastases, and thus, it has relatively high mortality rates (29). Gene expression profiling typically classifies triple-negative breast cancer (TNBC) as a major subtype of basal-like breast cancer without the expression of ER, PR or HER2 (30), which is clinically characterized by a strong aggressive nature, high metastatic potential, proneness to relapse and poor prognosis (31). Although poly(ADP ribose) polymerase inhibitors (e.g., programmed cell death-1 monoclonal antibody, trophoblast cell surface antigen 2 ADC) have been used for a subset of TNBCs, the prognosis of patients with TNBC is still far from satisfactory (Table I).

3. Status of clinical treatment of breast cancer

Breast cancer is a heterogeneous disease with highly aggressive and complex biological features, and the clinical treatment and prognosis of different patients vary greatly (32). As mentioned above, patients with early breast cancer may be effectively cured and the focus of treatment is to avoid overtreatment and undertreatment (33). However, metastatic breast cancer cannot be radically cured by current clinical remedies and its management mainly focuses on prolonging survival time and maintaining quality of life instead (34).

Currently, the major clinical remedies of breast cancer are endocrine therapy, molecular targeted therapy and chemotherapy (26). For instance, patients with stage I and II breast cancers are recommended to undergo breast-conserving surgery and radiation treatment, whereas those with node-positive breast cancer are systemically treated with chemotherapy, endocrine therapy and trastuzumab. As to patients with stage III breast cancer, chemotherapy is applied to facilitate breast-conserving surgery (35).

However, as the molecular phenotypes vary across breast cancers, breast cancer is not sensitive to either conventional endocrine therapy or molecular targeted therapies (36). Therefore, chemotherapy remains the primary strategy for breast cancer management. However, the efficacy of conventional postoperative adjuvant chemotherapy is unreliable, and residual metastatic lesions eventually lead to tumor recurrence, which largely attributes to secondary tumor recurrence and metastasis caused by acquired chemotherapy resistance (37,38). Thus, there is an urgent need to explore the drug resistance mechanisms and to identify novel therapeutic targets to finally overcome the resistance of breast cancer to chemotherapy, which is of great clinical significance for clinical practice in the future. Of note, state-of-the-art updates have indicated the role of the transcription factor RUNX2 in BCSCs and drug resistance in breast cancer, which will provide overwhelming new references for the development of the next generation of therapeutic targets and innovative drugs for breast cancer management.

4. The RUNX family

RUNX is a highly conserved transcription factor family that has an important role in the regulation of gene expression involved in embryonic development and cell differentiation. Recent studies have demonstrated that members of the RUNX

Table I. Molecular typing of breast cancer.

Subtype	ER	PR	HER2
Luminal A	+	+	-
Luminal B (HER2-negative)	+	-	-
HER2-positive (ER-positive)	+	Any	+
HER2-positive (ER-negative)	-	-	+
Basal-like (Triple negative)	-	-	-

The information presented is derived from Ref. (132). ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

transcription factor family are involved in the differentiation of a variety of hematopoietic cells. RUNX2 is required for osteogenesis, whereas RUNX1 and RUNX3 control blood cell development at different stages of cell lineage specification.

RUNX1 is also known as acute myeloid leukemia gene 1. Mutations in this gene are common in patients with lymphoblastic and myeloid leukemia (39). It has been suggested that RUNX1 deficiency is associated with familial platelet disorders. Familial platelet disorders predispose to myeloid leukemia and are associated with thrombocytopenia, as well as marked reductions in B-lymphoid, T-lymphoid and myeloid lineages (40).

RUNX2 has a key role in the development of bone and cartilage tissue, while abnormal expression of RUNX2 is associated with a variety of cancer types, and in particular, the process of tumor bone metastasis (41). To date, RUNX2 has been proven to be involved in melanoma, thyroid cancer and hepatocellular carcinoma (42-44). For instance, Guan *et al* (44) found that circular (circ) RNA_102272 promoted cisplatin resistance of hepatocellular carcinoma via inhibiting the targeting effect of microRNA (miR)-326 upon RUNX2. Of note, Matthijssens *et al* (45) reported that RUNX2 was able to significantly induce glycolysis and oxidative phosphorylation, and resulted in increased mitochondrial activity, which collectively suggested the promoting effect of RUNX2 in accelerating tumor cell metabolism, and in particular, in benefiting the invasion and migration of leukemic cells via mediating the interaction between glycolysis and mitochondrial respiration. RUNX2 also functions in the development of gastric cancer, including tumor invasion and metastasis by simultaneously facilitating the proliferation of gastric cancer cells and increasing the self-renewal potential (19). Ji *et al* (46) found that metastasis associated lung adenocarcinoma transcript 1 was able to modulate the transcription and translation of RUNX2 to further increase the metastasis of recurrent colorectal cancer via binding to miR-15 family members, inhibiting LDL receptor related protein 6 expression and enhancing β-catenin signaling, or binding to splicing factor proline and glutamine rich (SFPQ) and dissociating the SFPQ/polypyrimidine tract binding protein 2 dimer. Collectively, RUNX2 holds the potential to function as an important indicator for the detection of multiple cancers in the early stage, which also serves as a promising therapeutic target for clinical practice.

RUNX3 has a significant role in the occurrence and development of a variety of human cancers. Zhang *et al* (47) indicated that RUNX3 inhibits colorectal cancer proliferation and metastasis. Liu *et al* (48) found that RUNX3 inhibits glutamine metabolism in gastric cancer. A study identified a NO•/RUNX3/kynurenine metabolic axis, which enhances disease aggressiveness in pancreatic cancer (49).

To summarize, the RUNX family not only plays a role in normal physiological functions, but abnormal expression of any member of the family may cause a variety of different diseases.

5. Physiological and pathological roles of RUNX2

RUNX2, together with RUNX1 and RUNX3, is an essential transcription factor of the RUNX family (50). To date, studies have indicated the role of RUNX2 in numerous physiological and pathological processes, such as osteoblast differentiation, chondrocyte maturation (51), skeletal and mammary gland development (22), osteoarthritis, osteosarcoma, prostatic carcinoma, gastric cancer and even breast cancer (19,52-55).

Roles of RUNX2 in physiological processes. RUNX2 has important roles in various types of cells, such as chondrocytes, osteoblasts and mesenchymal stem/stromal cells (MSCs) (56). Meanwhile, RUNX2 has been reported essential for breast development (57), which also exhibits good interaction with twist family BHLH transcription factor (TWIST)1 in regulating cranial neural crest-derived cell fate and thus guides craniofacial muscle development (58).

For decades, RUNX2 has been recognized as a key transcription factor for bone formation and osteoblast differentiation, as identified by cell sorting, lineage tracing and single-cell transcriptome analysis. For instance, Shu *et al* (59) developed a dual-recombinase fate-mapping system for the capture of the spatio-temporal skeletal progenitor transition during postnatal bone formation. During intramembranous ossification, RUNX2 promotes the differentiation of MSCs into anterior osteoblasts and immature osteoblasts (12). Furthermore, Liu and Lee (60) reviewed the regulatory network of key transcription factors governing bone formation, such as Msh homeobox 2, TWIST and promyelocytic leukemia zinc-finger protein. During endochondral ossification, RUNX2 maintains the survival of terminal hypertrophic cartilage and promotes its trans-differentiation into osteoblasts (61). Despite the fast-growing understanding of the transcriptional mechanisms of osteogenesis, the detailed mechanisms of RUNX2-related skeletal development and precise orchestration of osteogenesis remain largely elusive.

RUNX2 has also been reported to have a crucial function during breast development via directly regulating the expression pattern of a number of genes related to mammary gland development (62). Studies have revealed the expression of RUNX2 in mammary tissues, including basal cells and luminal cells, which thereby participates in breast development. For instance, Inman and Shore (63) and Sato *et al* (64) verified that osteopontin may act as a target of RUNX2 and exert its function in breast differentiation in breast epithelial cells during pregnancy and lactation.

RUNX2 is associated with breast cancer. Besides its critical role in physiological development, RUNX2 also functions as an oncogene in numerous types of cancer. RUNX2 has been associated with a wide variety of processes, including tumor progression and heterogeneity, via mediating the responses of cells to signaling pathways hyperactive in tumors (65), such as the connective tissue growth factor-RUNX2-RANKL axis (66), miR-130a-5p-RUNX2-serine/threonine kinase 32A network (67), the bone morphogenetic protein (BMP)/TGF-β-RUNX2 loop (65) and the Zic family member 2-RUNX2-nucleolar and coiled-body phosphoprotein 1 signaling axis (68). To date, RUNX2 has emerged as a key mediator in the metastasis of cancers with preinvasive and promigratory behaviors in osteosarcoma, breast cancer, thyroid cancer, prostatic carcinoma and melanoma cells (69). Furthermore, numerous investigations of the molecular mechanisms of RUNX2 have revealed the mode of action in tumor metastasis and growth via activating the expression of bone matrix and adhesion proteins, matrix metalloproteinase (MMP) and angiogenic factors in cancer cells (70,71).

As mentioned above, breast cancer is composed of distinct subtypes with multiple stages during tumor progression. Of note, RUNX2 has been reported to have an important role in bone metastasis, which is the final stage of breast cancer development (72). For instance, phosphorylation of RUNX2 mediated by tyrosine kinase ABL is adequate to promote breast cancer invasion (73), while the interaction with core binding factor subunit β protein is sufficient to guide breast cancer cell invasion (53).

During tumor metastasis, cancer cells function via autophagy to recover nutrients to maintain their own survival and RUNX2 promotes autophagy by increasing acetylation of α-tubulin sub-units of microtubules, and thus promotes the metastasis of breast cancer cells (74). It has been indicated that the integrin subunit α5 (ITGA5)β3 expressed on the surface of breast cancer cells was able to anchor cells to osteoblasts by binding the tripeptide Arg-Gly-Asp motif of bone matrix proteins (75). During the aforementioned process, RUNX2 facilitated breast cancer cell recruitment and colonization in bone in an ITGA5-dependent manner (76). SET domain containing 7, histone lysine methyltransferase (SET7)/9 in breast cancer is able to activate target gene expression through histone methylation or directly act as a target via non-histone methylation. It has also been indicated that SET7/9 is able to promote multiple malignant biological behaviors in the development of breast cancer by activating RUNX2 (77). TGF-β is indispensable in normal physiological function (78) and had a complex 'double-edged sword' role in the occurrence and development of tumors (79). Furthermore, RUNX2 may promote bone metastasis in breast cancer cells through the activation of the TGF-β signaling pathway (80). In addition, accumulating evidence has indicated that multiple miRNAs (e.g., miR-30, miR-135, miR-203, miR-205, miR-505-3P and miR-590-3P) are adequate to inhibit the development and metastasis of breast cancer via targeting RUNX2 (23,71,81-84). Taken together, abnormal expression of RUNX2 may be associated with the occurrence and progression of breast cancer, and the underlying molecular mechanisms, including target genes and the concomitant signaling pathways, remain to be fully elucidated.

6. BCSCs

CSCs and epithelial-mesenchymal transition (EMT) are the major elements contributing to the metastasis and recurrence of cancers (85,86). In general, CSCs are unique subpopulations of solid tumors (e.g., breast cancer, lung cancer and stomach cancer) with stem cell-related characteristics, such as self-renewal, differentiation and tumorigenic potential, which have re-emerged as a hot topic of increased interest in the field (87,88). In breast cancer, BCSCs promote angiogenesis in mammary tissues by dedifferentiating to endothelial cells and secreting proangiogenic or angiogenic factors, which collectively facilitates metastasis and therapy resistance of breast cancer (89). Of note, studies have demonstrated that a series of signaling pathways are involved in orchestrating the phenotypes of CSCs, including the Hippo, Wnt, Notch and Hedgehog pathways (90). Meanwhile, drug efflux transporters and multi-drug resistance genes expressed in BCSCs also confer resistance against conventional chemotherapeutic drugs (90).

Breast cancer and BCSCs. Despite the inspiring advancements in radiation and chemotherapies, breast cancer is still an intractable disease owing to tumor relapse and drug resistance caused by BCSCs (90). BCSCs participate in the occurrence and development of breast cancer and usually result in cancer relapse with enhanced aggressiveness (91). Although only a small proportion of breast cancer cells may lead to tumors xenografts, these tumor-initiating cells are able to reconstruct tumors with a similar heterogeneity to that of the primary tumor, which indicates the distinct stem cell-like plasticity of BCSCs (92). To date, a large number of studies have indicated the pivotal role of the different pathways involved in the regulation of BCSCs, such as Wnt, Hedgehog and Notch signaling. For instance, Katoh (93) summarized Wnt signaling cascades cross-talk with the fibroblast growth factor, Notch, Hedgehog and TGF-β/BMP signaling cascades and regulate the expression of functional CSC markers. Furthermore, Ibrahim *et al* (94) verified syndecan-1 as a novel biomarker for inflammatory TNBC and modulating the BCSC phenotype via orchestrating the IL-6/STAT3-Notch and EGFR signal cascades.

According to the currently recognized stem cell markers for breast cancer, BCSCs may be divided into the $CD44^+/CD24^-$ and aldehyde dehydrogenase $(ALDH)^+$ subtypes (95). The most rapidly proliferating $ALDH^+$ BCSCs have an epithelial cell morphology, whereas the $CD44^+/CD24^-$ mesenchymal subsets display declined proliferation but high invasion and metastasis (96). In general, based on the expression levels of CD44, breast cancers of different molecular subtypes show variations in the proportion of $CD44^+/CD24^-$ BCSCs (97). For instance, the proportion of $CD44^+/CD24^-$ BCSCs in basal-like breast cancer is significantly higher than that in luminal A and luminal B breast cancers (98), which may be accountable for the worse prognosis of basal-like breast cancer as compared with that of the luminal A and luminal B subtypes (Table II). Collectively, the existence of BCSCs has tremendously increased the metastasis, angiogenesis and therapy resistance of breast cancer, as well as the secondary tumor formation in patients. Therefore, devising therapeutic interventions with

Table II. BCSC markers.

First author, year	Marker	BCSCs	Location	Function	(Refs.)
Dzobo, 2021	CD44	+	Cell membrane	Cell-cell interactions, cell adhesion and migration	(133)
Fillmore, 2007	CD24	-	Cell membrane	Cell differentiation	(134)
Tomita, 2016	ALDH1	+	Cytoplasm	Oxidize xenobiotic and intracellular aldehydes	(135)

BCSC, breast cancer stem cell; ALDH1, aldehyde dehydrogenase 1.

multidisciplinary strategies to target BCSCs would be of great help in boosting patients' survival rates and in increasing the sensitivity to anti-tumor drugs for breast cancer.

RUNX2 and BCSCs. State-of-the-art renewal has indicated the involvement of RUNX2 in various cancer types. The pro-cancer role of RUNX2 in breast cancer is known to be related to BCSCs (7). Furthermore, several studies have indicated a potential link between RUNX2 and CD44. For instance, in colorectal cancer, RUNX2 interacts with brahma-related gene 1 to target CD44 for promoting invasion and migration of colorectal cancer cells (99). In prostate cancer cells, RUNX2 forms a complex with intracellular domain of CD44 as a co-transcriptional factor, activates the expression of metastasis-related genes, and contributes to migration and tumor formation (54).

As a surface marker of BCSCs, CD44 rather than CD24 is positively correlated with RUNX2 expression, which suggests the association of RUNX2 with $CD44^+/CD24^-$ BCSCs. For instance, Zhang *et al* (24) found that RUNX2 promoted the malignant biological behavior of breast cancer cells by regulating the proportion of BCSCs. As a common event in breast cancer progression, PR Ser294 phosphorylation is required to maintain BCSCs fate via cooperation with the growth factor-initiated signaling pathway and key phosphor-PR targets (e.g., solute carrier family 37 member 2 and RUNX2). With the aid of the microsphere formation test, RUNX2 has been proven to act as an important driver of BCSC formation *in vitro* (100). In detail, RUNX2 has a critical influence in both EMT and BCSCs, and the ectopic expression of RUNX2 may subsequently induce the occurrence of EMT via the regulation of key pathways (e.g., TGF-β, Wnt) (101). Meanwhile, RUNX2 also has a critical role in $CD44^+/CD24^-$ MCF10AT1 cells with BCSC characteristics (102).

Taken together, RUNX2 has a critical role in promoting the development and metastasis of breast cancer via regulating the proportion of BCSCs, which provides new insight for the further dissection of the pathogenesis and therapeutic remedies for breast cancer.

7. Drug resistance in breast cancer

Attributed to the rapid development of technologies, the diagnosis and treatment of breast cancer have markedly improved; however, drug resistance in breast cancer remains an issue, as the mechanisms comprise disorders in the orchestration of hormones, apoptosis and efflux pump activation, signaling pathways and oncogenes (103).

Relationship between CSCs and drug resistance. CSCs are self-renewal cells with high tumorigenic potential. CSCs are able to adapt to changes in the surrounding environment and are more resistant to radiotherapy and chemotherapy than other cells in the tumor. Several studies have indicated that the dual effects of intrinsic and extrinsic factors contributes to CSC-mediated therapy resistance.

CSCs have a very slow cell cycle and possess an anti-apoptotic machinery, DNA repair systems and persistent stemness, which lead to drug resistance during cancer treatment (104). Besides, CSC drug resistance may also be caused by external factors, such as the tumor microenvironment (TME). When the TME is always in a state of nutrition, metabolism and oxygen deficiency, it promotes the adaptation of CSCs to the TME (105). CSC drug resistance is complex. CSCs promote tumor progression, treatment resistance and disease recurrence, which may achieved by their sustained proliferation, invasion into normal tissue, promotion of angiogenesis, evasion of the immune system and resistance to conventional anticancer therapies (106,107).

BCSCs and drug resistance in breast cancer. Cancer metastasis and drug resistance currently remain major challenges in cancer therapy (106). Increasing evidence suggests that CSCs favor cancer metastasis and drug resistance, leading to relapse of cancer and death of patients (108). On the one hand, BCSCs have been considered the result of overactivation of mutant normal breast stem cells during self-renewal. On the other hand, BCSCs are resultant of the dedifferentiation of cancer cells caused by somatic mutations or microenvironmental components during treatment (109).

The proportion of BCSCs was found to be significantly increased in chemoresistant and radioresistant breast cancer cell lines and human tissues (110). Furthermore, overactivation of the anti-apoptotic PI3K signaling pathway and the anti-oxidant nuclear factor E2-related factor 2 signaling pathway collectively contribute to cellular resistance and resistance of BCSCs to radiation-induced ROS attack and apoptosis compared with non-BCSCs (111,112). Further mechanistic studies revealed that BCSCs with a high level of free radical scavenger expression were more tolerant to the hypoxic environment and thus conferred a radiation-resistant phenotype by reducing the accumulation of intracellular ROS (113). In addition, certain cell surface pumps in BCSCs (e.g., ATP binding cassette subfamily G member 2) are able to impair the intracellular accumulation of anticancer drugs (114). Collectively, the variations in BCSCs are responsible for increased drug resistance of breast cancer.

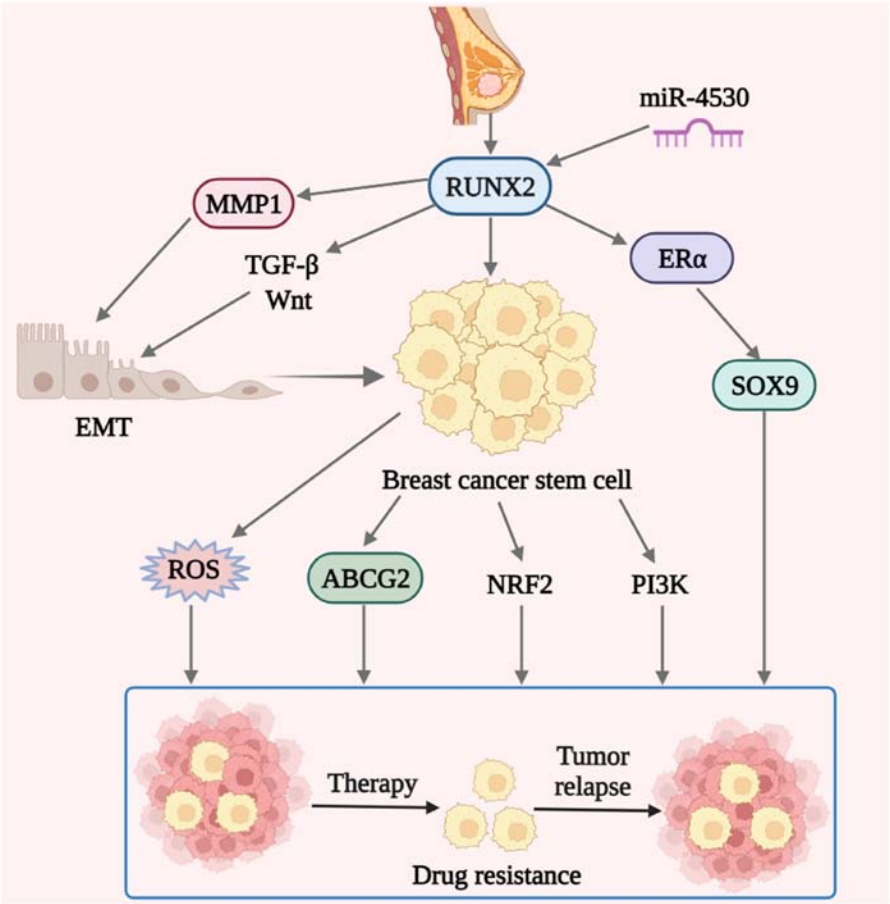

Figure 1. Schematic diagram of possible drug resistance mechanisms of RUNX2 in breast cancer. In breast cancer, aberrant RUNX2 expression contributes to drug resistance. Elevated serum miR-4530 levels may sensitize breast cancer to drugs by suppressing RUNX2. The drug resistance of breast cancer may be initiated by the direct transcription of SOX9 induced by the interaction of RUNX2 with ERα. In addition, RUNX2 may regulate BCSCs, which cause drug resistance through protein ABCG2 or the PI3K and NRF2 signaling pathways. ROS in BCSCs also have a certain role. Finally, RUNX2 may affect EMT through secreted protein MMP1 or signaling pathways, such as TGF-β and Wnt, and EMT regulates BCSCs and eventually leads to breast cancer resistance. RUNX2, Runt-related transcription factor 2; miR, microRNA; ER, estrogen receptor; NRF2, nuclear factor erythroid 2-related factor 2; BCSC, breast cancer stem cell; ROS, reactive oxygen species; ABCG2, ATP binding cassette subfamily G member 2; EMT, epithelial to mesenchymal transition.

RUNX2 and drug resistance of breast cancers. Overexpression of RUNX2 commonly leads to reduced sensitivity of cancer cells (e.g., osteosarcoma) to chemotherapy, which may thus be used as a reliable marker for the evaluation of chemotherapy resistance (115). Conversely, loss of RUNX2 expression is adequate to increase the sensitivity of osteosarcoma cells to the chemotherapeutic agent doxorubicin (116). First, Sugimoto *et al* (117) found that silencing RUNX2 was able to enhance the sensitivity of AsPC-1 cells in P53-deficient human pancreatic cancer to gemcitabine (GEM) by stimulating Tap63 (isoform of p63 gene)-mediated cell death. Furthermore, Ozaki *et al* (118) verified that increased Tap73-dependent cell death mediated by RUNX2 depletion enhanced the sensitivity of P53-mutant pancreatic cancer MiaPaCa-2 cells to GEM. In addition, RUNX2 was found to weaken the proapoptotic activity and enhance the sensitivity to GEM drugs of P53-mutant Panc-1 pancreatic cancer cells via suppressing Tap63 expression (118). These findings demonstrate that RUNX2 is associated with drug sensitivity to GEM in pancreatic cancer cells lacking functional P53.

In breast cancer, Tamoxifen (TAM) resistance is responsible for a large proportion of breast cancer-associated mortalities. Jeselsohn *et al* (119) found that RUNX2 was able to interact with ERα to directly induce SOX9 transcription and reduce the sensitivity of breast cancer cells to TAM. Of note, Geter *et al* (120) identified RUNX2 as the only RUNX family member that was transcriptionally or translationally upregulated in both TAM-resistant LCC9 cells and patient-derived xenograft derived from tamoxifen-resistant cell lines, suggesting that RUNX2 has a core role in the drug resistance of breast cancer. The study's results revealed that RUNX2 promoted the metastasis of breast cancer, and knockdown of RUNX2 was able to significantly restore the sensitivity of breast cancer to TAM (120). Furthermore, Othman *et al* (121) found that silencing of RUNX2 increased the sensitivity of breast cancer cells to microtubule-targeting agents. Wang *et al* (122) demonstrated that serum miR-4530 may sensitize breast cancer to taxane- and anthracycline-based neoadjuvant chemotherapy by suppressing RUNX2. A recent study revealed that RUNX2 directly targeted MMP1 and facilitates aggressiveness and chemoresistance of TNBC cells (21).

However, the specific mechanisms of RUNX2-associated drug resistance in breast cancer still require confirmation and further elucidation. Systematic and detailed dissection of the underlying mechanisms will contribute to conquering drug resistance and provide new references for the treatment of breast cancer (Fig. 1).

8. Discussion

RUNX2 has been recognized as a master transcription factor for osteogenesis and bone metastases of invasive breast cancer (72). In highly metastatic breast cancer, RUNX2 phosphorylation has associations with the invasive capacity of MDA-MB-231 cells by modulating the activity of tyrosine kinase ABL, which is involved in the activation of the classical BMP-SMAD signaling cascades (17). It was further indicated that RUNX2 facilitates the aggressiveness and chemoresistance of TNBC and the concomitant tumorigenesis and cancer progression via activating MMP1, which thus provides a basis for developing RUNX2-MMP1 axis-based novel candidates for breast cancer diagnostics (21).

Longitudinal studies have highlighted the potential utility of RUNX2 as a pivotal contributor to bone-specific metastasis during breast cancer. For instance, Li *et al* (76) verified the facilitating effect of RUNX2 and the targeted ITGA5 during the adhesion and attraction of breast cancer cells, as well as osteotropism and bone colonization. In contrast to the aforementioned studies indicating the critical role of RUNX2 in bone-specific metastasis or progression of breast cancer, the present review mainly focused on the latest updates on the interaction between RUNX2 and BCSCs and their biofunctions, which contribute to the aggressiveness and drug resistance of breast cancer. Furthermore, the systematic and detailed investigation of the RUNX2-BCSCs axis in breast cancer and knowledge regarding its utility in diagnosis and treatment are still far from satisfactory and further preclinical and clinical investigations are required in the future.

Of note, current progress has also highlighted the potential application of developing computational models based on the data fusion paradigm for identification of the disease-related non-coding RNAs (ncRNAs) as a future direction in disease assessment (123,124). For instance, Huang *et al* (123) summarized the advances in miRNAs and algorithm design for complex diseases by computational models, which may help overcome the obstacles to disease prediction. Simultaneously, Chen *et al* (125) and Wang *et al* (126) put forward the limitations and future directions of the disease prediction utility of long ncRNAs (lncRNAs) or circular RNAs (circRNAs) for complex diseases by combing experimental technology with computational prediction algorithms. Similarly, a series of ncRNAs have been identified to be linked to the disease progression of breast cancer or TNBC, such as the miR-20a-5p/high mobility group AT-hook 2 axis, lncRNA-CDC6/miRNA-215 and circRNA_002502/miR-182-5p/forkhead box O3a axis (127-130). In addition, Yin *et al* (131) performed a weighted gene co-expression network analysis and competing endogenous RNA network analysis based on the Cancer Genome Atlas database and identified the prospective association of 3,301 key modules and 453 genes with breast cancer prognosis. Therewith, it would be of great interest and importance to perform further studies to predict the association of RUNX2 and BCSCs with breast cancer development and drug resistance based on powerful computational model construction, which may also benefit the development of the framework of oncology diagnostics and therapeutics from a different perspective in the future. The present review mainly summarized the research progress on RUNX2 and BCSC in terms of breast cancer progression and diagnosis, may benefit the identification of key mechanisms of breast cancer and supply references for the concomitant anticancer treatment and drug development in the future.

As a malignant tumor type with high morbidity and mortality rates, breast cancer persistently endangers the health and lives of females. While chemotherapy remains the major remedy for breast cancer, drug resistance severely limits the efficacy and usually results in tumor metastasis and recurrence.

In recent years, RUNX2 in breast cancer has attracted considerable attention, as increasing evidence indicated its pivotal role in tumor development and metastasis, as well as its association with BCSCs-related drug resistance. Based on the current literature, it may be presumed that RUNX2 enhances drug resistance in breast cancer by regulating the proportion and biofunction of BCSCs, which will vastly enhance the current understanding of the pathogenesis and provide reliable targets for clinical treatment and drug development in the future.

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Availability of data and materials

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Authors' contributions

WS and FL designed the study, performed data analysis and interpretation, wrote the manuscript and gave final approval of the manuscript. WS and CK performed the literature search and wrote the manuscript. FL and LZ reviewed the manuscript. All authors contributed to the review article, and have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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