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Review Article

Dual Therapeutic Strategy Targeting Tumor Cells and Tumor Microenvironment in Triple-negative Breast Cancer

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Abstract

Objective: Triple-negative breast cancer (TNBC) is characterized by a lack of estrogen receptors (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu). Only 30% of TNBC patients show a pathologic complete response, and the other 70% of patients exhibit a less pronounced response followed by relapse and metastasis to distant organs after neoadjuvant chemotherapy. Achievements of immunotherapy targeting programmed cell death 1 ligand 1 (PD-L1) in clinical trials for treating melanoma, nonsmall-cell lung cancer, renal cell carcinoma, and TNBC suggest that targeting the interaction of tumor cells with tumor microenvironment is highly beneficial for cancer treatment. Finding a novel dual-targeting therapy against tumor cells and the tumor microenvironment (TME) may provide options for improved responses in TNBC patients. **Data Sources:** We searched the potential targeted therapy candidates that regulate tumor cells as well as the TME of cancer diseases, including TNBC, based on our previous and recent other publications. **Study Selection:** We selected the potential targeted therapies supported by relevance clinical data, *in vitro* and *in vivo* studies. **Results:** In this review, we found the KDM5B, Cadherin 11, β-catenin, CDK2, signal peptide CUB-EGF domain-containing protein 2, and PDL1 regulate the tumor cells and TME of TNBC cells. In addition, we also highlighted the *Antrocin, Ovatodiolide,* and *Pterostilbene* as natural small compound possess anti-cancer through the disruption of tumor cell–TME interactions. **Conclusion:** The new therapy approach targeting tumor cells-TME interaction may improve the response and survival rate of TNBC patients. Later, natural small compounds could provide alternative therapy options for TNBC patients.

Keywords: Targeted therapy, triple-negative breast cancer, tumor cell, tumor microenvironment

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Introduction

Triple-negative breast cancer (TNBC) is characterized by a lack of ER, PR, and HER2. It has more aggressive clinical features and a worse prognosis than HR+ and HER2+ breast cancer subtypes. TNBC constitutes approximately 15% of all breast cancer subtypes. Cytotoxic chemotherapy is the current standard therapy for TNBC. Among the breast cancer subtypes, TNBC is the most responsive to chemotherapy; however, disease relapse occurs in very early followed by distant metastasis, leading to death.[1]

The standard treatment for TNBC is neoadjuvant chemotherapy (NAC), which involves a combination of anthracycline-and taxane-based drugs.[2] Only approximately 22% of TNBC patients achieve a pathologic complete response (pCR) after NAC completion.[3] Another study showed that approximately 30% of TNBC patients achieve pCR.[4] Although the pRC rate is higher in TNBC patients compared with other breast cancer subtypes in response to NAC, 3-year progression-free survival and 3-year overall survival decline sharply in TNBC patients after NAC.[3] After NAC, 30%–50% of TNBC patients develop a resistant phenotype, leading to early recurrence, distant metastasis, and death.[2] The mechanism behind the chemoresistance of TNBC is still unclear.

Because of the heterogeneity of the cells in both genetically and phenotypically, TNBCs are clinically more aggressive than non-TNBC cells. The tumor microenvironment (TME) is a niche consisting of cellular and noncellular parts surrounding tumor cells, which determines tumor initiation and disease progression. The complex interaction of tumor cells and the TME defines whether the primary tumor has been eradicated, relapsed, or developed into distant metastasis after getting treatment completion. A single therapy approach targeting either tumor cells or TMEs is insufficient to achieve a high rate of pCR against TNBC. In this review, we discuss the heterogeneity of TNBC cells as well as the TME. Furthermore, we detail several potential targeted therapies that regulate tumor cells and the TME of TNBC [Table 1]. In addition, we discuss the anticancer potential of natural small compounds through tumor cell inhibition and TME regulation against TNBC.

Tumor Cell Heterogeneity

Carcinogenesis is the process by which somatic cells randomly mutate, leading to a change in their phenotype. This process repeatedly occurs to generate different clones. The unique fitness of cell clones is called the bulk of tumor cells.[5] The heterogeneity of each cell clone in bulk tumor cells due to nonuniform genetics during carcinogenesis provides the cells with different sensitivity levels to treatments, including chemotherapy. Although chemotherapy acts as to eradicate tumor cells, it also promotes molecular changes in tumor cells through a shift in the mutational spectrum. Following these mutational events, the new clones arising exhibit chemoresistant phenotypes.[6] TNBC cells have more genetic variations than any other breast cancer subtype. This phenomenon may explain the high early recurrence of TNBC after NAC. Clonal heterogeneity takes an important role in tumor progression. The drug-resistant clonal cells could become a dominant lead to relapse and distant organ metastasis.[7] Identifying a new approach that targets the most aggressive clonal cells in the bulk tumor is crucial for increasing the pCR of TNBC patients.

Table 1: Potential targeted therapy for triple-negative breast cancer

Targeted therapy	Role in TNBC	References
CDH11	Epithelial to mesenchymal transition, cancer stem cell, cell proliferation, and immune suppression	[21,25,26]
β-Catenin	Cancer stem cell and immune cell suppression	[31,32,35]
KDM5B	Epithelial to mesenchymal transition, cancer stem cell, and immune cell suppression	[39,41,42]
SCUBE2	Cancer stem cell, cell proliferation, and immune cell suppression	[47-49,51]
CDK2	Cell proliferation, evasion of growth suppression, and immune cell suppression	[53,54]
VEGF	Angiogenesis	[67-69]
PD-L1	Immune T-cell activation	[61,71-75]

CDH11: Cadherin 11, SCUBE2: Signal peptide CUB-EGF domain-containing protein 2, TNBC: Triple-negative breast cancer, VEGF: Vascular endothelial growth factor, PD-L1: Programmed cell death 1 ligand 1

Cancer Stem Cells

Initially, cancer stem cells (CSCs) have been identified in patients with hematology-related cancers. This finding was followed by the first isolation of CSCs from solid tumors, breast cancer. This subset population of cells exhibits self-renewal ability and multidirectional differentiation. The breast CSCs express cluster of differentiation 44 positive (CD44+) and the cluster of differentiation 44 negative (CD24−). These cells could generate tumor bulk tissue when injected into immunodeficient mice. However, these CD44−/CD24+ lineage cells failed to grow in immunodeficient mice. This finding suggests the CD44+/CD24− exhibit self-renewal ability and differentiation ability generate the new tumor bulk tissue.[8] Both markers CD44 and CD24 have been widely used to isolate breast CSCs for revealing the role of breast CSCs in tumor progression, relapse, and distant metastasis. Growing evidence indicates that CSCs also exist in other solid tumors.[9]

In TNBC patients, the CD44+/CD24 − populations of cells at the tumor site have significant prognostic value. TNBC patients with a high expression of breast cancer stem cell (BCSC) markers have a significantly poorer overall survival rate than those with a low expression of BCSC markers. An *in vitro* study showed that BCSCs exhibit high resistance to chemotherapy drugs.[10] Another study has revealed that BCSCs express more

multidrug-resistant proteins than non-BCSCs.[11] Moreover, circulating cancer cells and new distant organ metastasis tumor tissues express more CSC markers than a primary tumor tissue from the same patient.[12]

CSCs also exhibit a unique metabolic phenotype. Many studies revealed that CSCs are more glycolic than non-CSCs in the tumor. The presence of glucose in the tumor microenvironment significantly increases the specific glucose metabolism pathway associated-genes (c-Myc, Glut-1, HK-1, HK-2, and PDK-2) in CSCs, which is increased the CSCs population in the cancer cell population.[13] Most of the studies reported the glycolytic is the main energy source for CSCs.[14] However, other studies revealed that quiescent or slow-proliferate CSCs are less glycolytic in breast cancer.[13] Further, increased the mitochondria numbers in breast cancer cells promote the stemness phenotype and enhanced the metastatic potential, as well as resistant to DNA damage, decrease the efficacy of chemotherapy agents.[14] The Myc and MCL1 crosstalk synergistically upregulate the mitochondrial oxidative phosphorylation and reactive oxygen species led to an accumulation of hypoxia inducible factor 1 subunit alpha (HIF-α) maintain the stemness phenotypes and drug-resistant in TNBC.[15] Growing evidence suggests the CSCs exhibit metabolic flexibility by switching metabolic phenotypes between glycolytic and oxidative phosphorylation based on the location and energy demanding. In the tumor region with adequate oxygen and exhibit high proliferation rate, the CSCs mainly use both glycolysis and oxidative phosphorylation to gain energy. While in the center hypoxic region, the glycolysis is the main energy source.[14] Due to these unique metabolic phenotypes of BCSCs, inhibiting glycolysis and or oxidative phosphorylation may attenuate the stemness and drug-resistant phenotype of triple BCSCs. Taken together, understanding the regulation of BCSCs as well as the potential targeting therapy for these cells is crucial for identifying a novel approach for TNBC therapy.

Epithelial-Mesenchymal Transition

The change in cancer cell phenotype from epithelial to mesenchymal is called epithelial-mesenchymal transition (EMT). This occurs through the activation of EMT transcription factors, mainly snail family transcriptional repressor 1 (SNAIL), zinc finger E-box homeobox (ZEB), and twist family bHLH transcription factor 1 (TWIST). This process generates new clones of tumor cells that are highly anchor-independent and invasive. EMT is characterized by the loss of E-cadherin and increased expression of N-cadherin and vimentin. Growing evidence indicates that EMT activation is required in the initiation of distant cancer cell metastasis. The EMT cells can invade the tissue surrounding the primary tumor site and penetrate the bloodstream. Moreover, circulating cancer cells express more EMT markers than primary tumor cells. In TNBC, EMT activation induces aggression in cancer cells compared with parental cancer cells.[12] Furthermore, it provides a resistant phenotype to TNBC cells against chemotherapy.[16] Even EMT activation decreases tumor-infiltrating lymphocytes (TILs) at the tumor sites as well as the immunotherapy response of syngeneic breast cancer mice models.[17] Other studies have reported that TNBC patients with high EMT markers have a significantly poorer prognosis than TNBC patients with low EMT markers.[18] Inhibiting EMT suppresses distant metastasis and increases the response to chemotherapy therapy in TNBC patients. Thus, targeting EMT may be beneficial for TNBC treatment.

Evading Growth Suppression and Cell Proliferation

Defects in cell cycle checkpoint regulators commonly occur in patients with cancer, including breast cancer. In the past 3 years, targeted therapy drugs inhibiting the cell cycle have been approved by the Food and Drug Administration (FDA). Abemaciclib, ribociclib, and palbociclib were approved to treat the advanced metastatic HR+/HER2− breast cancer through cyclin-dependent kinase 4/6 (CDK4/6) inhibition. However, this therapeutic approach elicits a minimal response or no response in TNBC due to molecular regulation of the cell cycle in TNBC subtypes is different from HR+/HER2− breast cancer subtypes.[19] Understanding the regulation of cell cycles, particularly TNBC, may provide a novel approach for suppressing cancer progression and providing patients with a favorable prognosis.

Potential New Targeted Therapy for Triple-Negative Breast Cancer Tumor Cells

Cadherin 11

Cadherin 11 (CDH11) is a type-II cadherin that supports the cell–cell junction through hemophilic binding. It is highly expressed in normal mesenchymal cells. CDH11 is also normally required for the migration of neuroepithelium during normal cerebral development.[20] Interestingly, CDH11 is highly expressed in invasive breast cancer cell lines. Our previous study showed that patients who express high CDH11 levels have a poorer prognosis than patients who express low CDH11 levels in breast cancer, including TNBC.[21] Studies have revealed that CDH11 promotes the progression and distant metastasis of renal cancer and prostate cancer.[22,23] In lung cancer, CDH11 depletion suppresses resistance to paclitaxel treatment.[24] In TNBC, CDH11 inhibition suppresses proliferation, migration, EMT, CSC phenotype, and spontaneous metastasis.[21,25] In the previous study, targeting CDH11 with a monospecific antibody suppressed tumorigenesis and metastasis *in vivo* xenograft mice models.[26]

CDH11 inhibition decreased inflammation in an RA mice model through suppressing the production of interleukin-6 (IL-6), a proinflammatory cytokine.[27] In several cancer types, IL-6 has different roles in terms of regulating the function of immune cells, such as T-cells and dendritic cells (DCs).[28] The blockage of IL-6 suppresses the proliferation and migration of TNBC

cells. Targeting IL-6 significantly reduces tumor growth and decreases the thoracic metastasis of TNBC in xenograft mouse models in combination with anti-C-C motif chemokine ligand 5.[29] Notably, the activation of the IL-6 signaling pathway induces EMT and increases the stemness of TNBC cells. Moreover, increasing IL-6 secretion by TNBC cells induces THP-1 cell polarization into M2-like macrophages.[30] This may provide a new mechanism of CDH11 regulation in the TNBC TME. However, the interaction between CDH11 and IL-6 in TNBC is still unclear.

Wnt/β-catenin

β-catenin is encoded by the catenin beta 1 (CTNNBB1) gene that involves in cell-cell adhesion and gene transcription. When the Wnt ligand binds to its receptor, β-catenin moves from the cytoplasm to the nucleus to activate the Wnt signaling pathway target genes. TNBC cell lines express higher β-catenin levels than the ER+ breast cancer cell line.[31] A high β-catenin expression correlates with the poor disease-free survival of TNBC patients.[32] However, another study revealed a favorable prognosis in TNBC patients with a high expression of β-catenin.[33] The prognostic value of β-catenin in TNBC patients may vary based on its location. The presence of β-catenin in the cytoplasm and nucleus is characteristic of Wnt signaling activation. Sequestering β-catenin in membrane sites inhibits its translocation to the nucleus and suppresses Wnt signaling pathways.[34] Loss of β-catenin in the membrane and cytoplasmic accumulation are significantly more frequent in TNBC.[35] β-catenin inhibition suppresses the stem cell-like phenotype and migration ability of TNBC cells. In an *in vivo* study, β-catenin knocking down reduced the size of TNBC tumors.[31] Furthermore, growing evidence indicates that β-catenin plays a crucial role in the chemoresistant phenotype regulation of TNBC. A decline in β-catenin expression sensitizes cancer cells to doxorubicin and cisplatin.[31] TNBC cell lines that highly express β-catenin possess a resistant phenotype to paclitaxel.[32]

Patients with non-T-cell-inflamed tumors obtain less benefit or no benefit from immunotherapy treatment for many cancers, including breast cancers. Understanding the mechanism behind non–T-cell-inflamed tumors may improve the efficacy of immunotherapeutic approaches to such tumors. A study of 31 solid tumor types revealed that the β-catenin signaling pathway was activated in 90% of non-T-cell-inflamed tumors. However, in melanoma, the intrinsic activity of the Wnt/β-catenin pathway induced immune exclusion.[36] The β-catenin overexpression in TNBC patients has been correlated with the high expression of stromal TIL CD8+. However, the β-catenin overexpression also positively associated with preset of regulatory T cell (FOXP3+) in the stromal sites may lead to CD8+ T-cells deactivation in TNBC patients.[37] In another study, the Wnt signaling pathway regulated T-cell activation through programmed cell death 1 ligand 1 (PD-L1) modulation. PD-L1 expression is positively correlated with the stemness of TNBC cells. The Wnt signaling pathway inhibition can suppress PD-L1 expression levels.[38] Thus, through targeting cancer cells as well as TMEs, β-catenin may be a potential targeted therapy for TNBC.

Lysine demethylase 5B (KDM5B)

Histone modification is a mechanism for regulating gene transcription. Lysine 4 on histone H3 (H3K4) demethylation by KDM5B represses the initiation of gene transcription in normal adult cells.[39] In patients with breast cancer, KDM5B is positively correlated to metastasis.[40] Migration ability and EMT initiation are required to initiate distant metastasis in breast cancers, including TNBC. Our previous study revealed that KDM5B inhibition reverses EMT, inhibiting the cell migration and proliferation of breast cancer cell lines, including TNBC cell lines.[40-42] A decrease in KDM5B suppressed the metastasis and reduced the survival rate of mice *in vivo* models.[41,42] Enforcing the normal breast cell line to express KDM5B induces it to acquire a cancer-stem-cell-like phenotype, and inhibiting KDM5B in TNBC suppresses cancer-stem-cell-like markers and enhances chemosensitivity.[41]

The presence of DCs at tumor sites is necessary to activate both innate and acquired immune cells against cancer cells. The stimulator of interferon genes (STING) signaling pathway is the main regulator in DC activation. It detects cancer cytosolic DNA as an activating signal induces type I interferon (IFN) secretion, either autocrine or paracrine, leading to the enhanced antigen presentation ability of DCs.[43] Moreover, type I IFN enhances the cytotoxic effect of cytotoxic T-lymphocyte or natural killer (NK) cells against cancer cells.[44,45] However, in most cancers, STING is commonly inactivated through an unknown mechanism. In multiple cancer types, KDM5B expression is negatively correlated with STING expression. Promoting STING expression through KDM5 inhibition dramatically increases type I IFN production in a DNA-dependent manner in breast cancer cells.[46] Thus, KDM5B may suppress immune activation through STING regulation in TNBC cells. Targeting KDM5B in TNBC may provide benefits through attenuating the cancer cells and promoting immune-activated TME.

Signal peptide CUB-EGF domain-containing protein 2

Signal peptide CUB-EGF domain-containing protein 2 (SCUBE2) is normally expressed in endothelial cells and nonendothelial cells such as fibroblasts, renal mesangial cells, and normal mammary ductal epithelial cells. Moreover, SCUBE2 is expressed in primary breast cancer cells and has prognostic value. A clinical study showed that patients with breast cancer who are positive for SCUBE2 have a better disease-free survival rate than those negative for SCUBE2.[47] SCUBE2 expression in TNBC cells was significantly downregulated relative to normal ductal cells.[48] This finding indicates that SCUBE2 acts as a crucial tumor suppressor in patients with breast cancer, such as TNBC. Forcing SCUBE2 expression in noninvasive and invasive breast cancer cell lines inhibited cell proliferation and tumor growth *in vitro* and nude mice models, respectively.[47,49]

A study on TNBC cells revealed that SCUBE2 expression reversed the transforming growth factor-beta-induced EMT to MET through an increase in E-cadherin-containing adherent junctions.[50] Conversely, the SCUBE2 was found higher in tumor sphere cells than those adherent cells in TNBC. Enforcing the SCUBE2 expression level in TNBC stem cells increases the cell motility *in vitro*. Overexpression SCUBE2 in TNBC stem cells enhanced metastasis by Notch signaling pathway activation *in vivo* study.[51] These contradictions may suggest the SCUBE2 act as either tumor suppressor or oncogene based on which cells are used. Further studies are needed to investigate the correlation of SCUBE2 expression level for each breast cancer subtypes since breast cancer is not a single disease. Each subtype exhibit distinct cell molecular pathways and clinical characteristic. The *in vitro* study need to explore the mechanism of SCUBE2 in the TNBC stem cells versus those non-CSCs to confirm the SCUBE2 acts as a tumor suppressor gene or oncogene.

CDK2

According to advanced therapeutic study for the past 3 years on patients with advanced metastatic HR+/HER2 − breast cancer through targeting CDK4/6, TNBC patients exhibited only a negligible response no response to this therapeutic approach. A study on TNBC revealed several CDK4/6 inhibitor resistance mechanisms. The overexpression of CDK2-and retinoblastoma-deficient (Rb-) phenotypes leading to CDK4/6 inhibition is insufficient to induce cell cycle arrest at the G1/G0 phase.[52] Failure of the CDK4/6 inhibition approach suppresses the TNBC cell cycle, leading to the targeting of CDK2, with promising results obtained. Using small-compound CDK2 inhibitor drugs to decrease CDK2 expression significantly suppressed TNBC cell proliferation and induced cell arrest.[53] CDK2 inhibition using this small compound drug suppressed tumor growth, with no mortality, in xenograft mouse models.[53] Combining CDK2 inhibition and the chemotherapy agent eribulin suppresses TNBC growth *in vitro* and *in vivo*.[54]

In human diseases such as cancers, unrepaired DNA damage promotes the release of DNA into cytoplasmic sites and activates the STING signaling pathway. The STING signaling pathway increases type I IFN, which leads to immune system activation in many cancers, including breast cancer.[43] CDK2 is a regulatory machine in the G1-S and S phases of the cell cycle. Most of gene targets of CDK2 pathways are regulator proteins for DNA replication and DNA damage repair. Knocking down CDK2 induces DNA damage through inhibiting DNA damage repair in breast cancers, including TNBC.[55,56] Through this mechanism, CDK2 may have a regulatory role in the immune status of TNBC patients.

Tumor Microenvironment in Triple-Negative Breast Cancer

The noncancerous surrounding of tumor sites, including the fibroblast, immune cells, and cells constituting blood vessels, is called the TME. Moreover, all proteins produced by cancer cells and noncancerous cells in tumors supporting cancer cell progression constitute the TME.[57] Cancer initiation, oncogene activation, and tumor suppressor inactivation transform normal cells into malignant cancerous cells.[58] Tumor tissue and metastasis cannot be accomplished by cancer cells alone. Cancer cells recruit stromal cells surrounding cancer cells through the secretion of cytokines and chemokines, and through other factors.[59] Through this mechanism, an environment is created comprising noncancerous cells that produce tumor growth signals and intermediate metabolites and promote metastasis.[58] This collaborative work between cancer cells and the TME accelerates the proliferation rate and metastasis capability. Moreover, TMEs determine response to therapy, such as primary tumor eradication, relapse, resistance, or metastasis to distant organs.[60] The aggressiveness of TNBC is not only caused by the high heterogeneity of the cancer cell but also TMEs. TNBC has high TIL, tumor-associated macrophage (TAM), and vascular endothelial growth factor A (VEGF) than less aggressive luminal subtypes.[61] TILs and TAM cell numbers determine whether patients benefit from advanced immune therapy.[62] In TNBC, TILs are significantly correlated with chemotherapy response as well as pCR after NAC.

Angiogenesis

New capillary formation from preexisting vasculature, termed angiogenesis, is crucial in physiological functions, including the healing of injured tissue. In cancer tissue, blood vessels are essential for nourishment and the waste metabolite disposal of cancer cells. The high proliferation of cancer cells leads to the rapid growth of tumor tissues without rapid angiogenesis lead to the generating of a hypoxic area at the tumor's center. In supporting further tumor growth, and the cancer cells secrete angiogenic factors as well as proteases. It initiates the degradation of the basalis membrane of blood vessels and promotes the movement and growth of endothelial cells to generate neovascularization of the hypoxic area of tumor tissues. This neovascularization allows the oxygen and nutrient supply to reach the rapidly proliferating cancer cells to promote tumor growth and support the distant organ metastasis of cancer cells.[63] The discovery of angiogenesis inhibitors provided a new therapeutic approach against cancers. Thousands of cancer patients received angiogenesis inhibitor therapy but did not have long-term benefits.[64,65] Conversely, antiangiogenesis therapy suppressed the neovascularization of tumor tissue, inducing the rapid growth of tumors to develop a more hypoxic area. In clonal selection in the hypoxic area, viable cancer cells show resistance to hypoxic conditions as well as chemotherapy and possess a cancer-stem-cell-like phenotype.[66] This mechanism may have led to the lack of satisfactory results from the single therapeutic approach of antiangiogenesis in patients with cancer. Targeting angiogenesis and cancer-stem-cell-like cells may improve the response and long-term survival of patients with cancer.

The microvascular density of breast cancer tissue holds prognostic value in breast cancer patients. Notably, TNBCs

have higher microvascular density than non-TNBCs. TNBC patients have significantly higher expression of VEGF and have a shorter survival time than non-TNBC breast cancer patients.[67] Small interfering RNA mediated inhibition of VEGF suppressed migration and invasion *in vitro* as well as tumor growth in an orthotopic mouse model of TNBC. However, a few phase III clinical trials of anti-VEGF antibody therapy of bevacizumab and ramucirumab failed to reveal an improved survival rate of TNBC patients.[68] Another study revealed that antiangiogenic agents increase CSCs through the generation of a hypoxic area in TNBC. The hypoxic condition activates a Wnt signaling pathway mediated by HIF1α, HIF2α, Akt, and β-catenin to induce cancer stemness. This mechanism may limit the efficacy of antiangiogenic drugs as a single therapy against TNBC.[69] Combination therapy targeting angiogenesis and cancer-stem-cell-like cells may provide a better prognosis and survival rate for TNBC patients.

Targeting Programmed Cell Death 1 Ligand 1 to Activate Immune Cells

Innate and adaptive immune cells are required to eradicate cancer cells. The immune cells recognize abnormal cells as targets. During the clonal selection of tumor progression, cancer cells improve their survival ability through evading immune cell surveillance. The cancer cells develop the immune checkpoint blockade to drive immune anticancer cells to suppress immunity, creating a tumor growth-promoting microenvironment. The most advanced immune checkpoint blockade studied is PD1/PD-L1 protein-protein interaction. PD1 is a membrane receptor protein expressed by T-cells and NK cells. PD-L1 is expressed by cancer cells as well as noncancerous tumor cells, including cancer-associated fibroblasts, T regulatory cells, myeloid cells, and endothelial cells. An interaction of PD-L1 with PD1 inhibits the T-cell activation signal as well as NK cell activation. Advances in immune therapy targeting PD-L1 improved the prognosis of patients with melanoma, nonsmall-cell lung cancer, and clear-cell kidney carcinoma; however, breast cancer was the least responsive cancer to this treatment approach.[70] In terms of improving the treatment response of breast cancer to immunotherapy, growing evidence has shown that TNBC is the breast cancer subtype with the best response to immunotherapy. TNBC patients have higher TIL expression than non-TNBC patients.[61] Moreover, they have higher PD-L1 expression than non-TNBC patients.[71] Notably, PD-L1 expression was positively and significantly correlated with the TIL number in breast cancer tissues.[72] PD-L1 expression has prognostic value for patients with stage I–III TNBC.[73] Furthermore, the pCR of TNBC patients is correlated to PD-L1 expression.[74] These findings suggest the TNBC patients are the best candidate for immunotherapy approaches. By 2019, the FDA approved the immunotherapy drug atezolizumab that targets PD-L1 as a first targeted therapy to treat advanced TNBC. In phase 3 clinical trial, the median progression-free survival rate of patients given atezolizumab combined with nab-paclitaxel was significantly higher than that of patients given a placebo with nab-paclitaxel (7.2 months vs. 5.5 months, $P = 0.002$). Moreover, atezolizumab increased the median progression-free survival of patients with PD-L1 positive tumors (7.5 months in the atezolizumab group vs. 5 months in the control group, $P < 0.001$). This finding indicates that targeting PD-L1 through atezolizumab drug therapy is beneficial for patients with advanced TNBC.[75]

Tumor-Associated Macrophages

TAMs defined as macrophage infiltrating tumor tissue or populated in the microenvironment of solid tumors. These cells were recruited and activated by signals in the tumor microenvironment. As part of tumor microenvironments, TAMs exhibit an important role in tumor progression and metastasis. After activated in the tumor microenvironment, macrophage divided into classical-activated macrophages (M1) and alternative-activated macrophages (M2). The M1 induce inflammation in response to eliminates pathogens and tumor cells. While the most macrophages profoundly polarize into M2 macrophages exhibit immune-suppressive phenotypes and promote tumor progression. The M2 secretes cytokines such as IL-4, IL-3, IL-6, CCL7, CCL8, CCL9, CCL18, and CXCL12, which are attenuate the immune response against tumor cells.[76]

The TNBC tissue patients markedly have higher infiltrating of macrophages relative to non-TNBC tissue patients.[77] Infiltrating TAMs exhibit the worst prognostic factor for TNBC patients. A large number of infiltrating TAMs in the tumor site significantly associated with a higher risk of metastasis, the lower rate of disease-free survival, and overall survival relative to lesser number infiltrating TAMs of TNBC patients.[78] Further, the TNBC had significantly higher M2 TAMs than M1 TAMs.[79] In TNBC, TAMs induce tumor growth and progression by secretion of immune inhibitory cytokines, reduce functional effector of TILs, and promoting the regulatory T cell.[80] Drive the M2 TAMs to M1 TAMs may activate the immune microenvironment against TNBC cells.

Potential Small Compound Drugs Targeting Tumor Cells and the Tumor Microenvironment

Traditional herbal medicines have been widely used to cure diseases such as cancers. An increase in cancer detection methods and therapies has led to an increase in anticancer drug development. This led to the transition from natural herbal extracts to synthetic drug production on a large scale by pharmaceutical manufacturers. However, recently, a tendency to return to natural herbal medicine has emerged, which has promoted the intense study of small natural compounds derived from herbs to cure all cancers, including TNBC. Herein, we discuss a few natural, small compounds with anticancer properties against TNBC.

Ovatodiolide

Ovatodiolide is a biologically active macrocyclic diterpenoid extracted from *Anisomeles indica*. In our previous study,

ovatodiolide treatment could sensitize TNBC cell lines to doxorubicin. Also, this small compound inhibits stem-cell-like phenotypes in TNBC cells.[81] PD-L1 expression in the cancer cells induces immune evasion by binding with an inhibitory immune checkpoint receptor, PD-1 protein, which is expressed on activated T-cells. Interestingly, the CSCs (ALDH+/CD44+) are profoundly increased PD-L1 expression levels relative to non-CSCs (ALDH+/CD44+) in TNBC cells.[38] Insight the Wnt signaling pathways inhibition attenuates the cancer stemness,[21] selective Wnt inhibitors decreased the PD-L1 expression in TNBC cells.[38] In renal cancer, ovatodiolide suppresses cancer cell viability, invasion, migration, and survival *in vitro* studies as well as tumorigenicity *in vivo* studies through targeting β-catenin.[82] Ovatodiolide treatment attenuates the malignancy of oral cancer through decreased exosomal Mir-21/STAT3/β-catenin cargo.[83] Our previous study demonstrated that Ovatodiolide treatment suppresses the canonical Wnt signaling pathway that attenuates CSC-like phenotypes in hepatocellular carcinoma.[84] Taken together, the Ovatodiolide may provide a new promising small compound drug suppress the CSCs and activates the immune cells against TNBC through Wnt/PD-L1 axis. However, the mechanism of ovatodiolide regulates the Wnt signaling pathway and PD-L1 expression level in TNBC are remaining unclear. In other studies have shown that ovatodiolide modulates colon cancer TMEs through inhibiting M2 TAM generation *in vitro* and *in vivo* studies, provide another possible mechanism of ovatodiolide against the TNBC cells.[85] Targeting CSCs, PD-L1, and M2 TAM through ovatodiolide treatment may provide improved responses and prognoses for TNBC patients. However, the effect of this small compound on the TME of TNBC is unclear, with further study required.

Antrocin

Antrocin (AC) is a sesquiterpene lactone isolated from *Antrodia cinnamomea*.[86] In prostate cancer cells, AC treatment sensitizes cells to radiotherapy through PI3K/AKT and MAPK signaling pathway suppression. Further, the AC also attenuates the type 1 insulin-like growth factor 1 receptor (IGF-1R)-mediated induction of β-catenin.[87] However, in TNBC, AC treatment inhibits cell growth and induces apoptosis through Akt/mTOR signaling pathway inhibition.[88] Furthermore, our previous study showed that AC synergistically enhanced the efficacy of paclitaxel against TNBC *in vitro* and *in vivo*. This study also revealed AC suppresses tumorigenicity and the stem-cell-like phenotype of TNBC cells through β-catenin/Notch1/Akt signaling pathways inhibition.[86] As described previously, the CSCs exhibit higher PD-L1 expression levels than non-CSCs in TNBC. The Wnt signaling pathway inhibition decreased both self-renewal ability and PD-L1 expression levels on CSCs.[38] Although the effect of AC to PD-L1 expression level is remaining unclear; currently, studies support the possibility of AC treatment on TNBC suppress the PD-L1 expression in CSCs through Wnt pathway inhibition. Moreover, a recent study revealed the Notch, MAPK/ERK, and PI3K/AKT inhibitors treatment significantly decrease PD-L1 on CSCs of TNBC.[89] This adds the possible mechanism of AC suppress the PD-L1 expression level on BCSCs via Notch/Akt signaling inhibitions.

Pterostilbene

Pterostilbene, a natural stilbene isolated from blueberries, has anticancer activity against TNBC.[90] Our previous studies have revealed that this natural small compound inhibits EMT in TNBC cells through upregulating E-cadherin and downregulating Snail, ZEB1, vimentin, and Slug. Consistently, *in vivo* results have shown that pterostilbene also inhibits tumor growth and metastasis.[91] In breast tumor, which is arising from more mesenchymal breast carcinoma cell lines expressed a low level of MHC class I, and high levels of PD-L1. Moreover, the stromal tumor invaded by immune suppressor cells regulatory T-cells, and M2 (protumor) macrophages, while the CD8+ T cells are exhausted.[17] Reversing the EMT to MET by pterostilbene may promote the immune-activating tumor microenvironment in TNBC. This approach may improve the TNBC patients to the immunotherapy approach. Furthermore, coculturing TNBC cell lines with M2 TAMs increase CSC (CD44+/CD24−) population as well as cancer cell invasion and migration ability. Pterostilbene treatment suppresses the CSC population in TNBC cells cocultured with M2 TAMs and inhibits tumorigenicity and metastasis in mouse models.[90]

Conclusion

The heterogeneity of cancer cells supported by a protumorigenic microenvironment induces TNBC to exhibit aggressive clinical characteristics, which are difficult to treat [Figure 1]. The current standard of chemotherapy confers only a low pCR in TNBC patients. The stemness, EMT, evasion of growth suppression, angiogenesis, and immune cells may provide a mechanism for cancer cell and TME interaction in TNBC patients. The dual targeting of cancer cells and inhibiting protumorigenic TMEs could provide improved treatment responses and survival in TNBC patients. Extensive studies are required to determine promising targeted therapy against TNBC.

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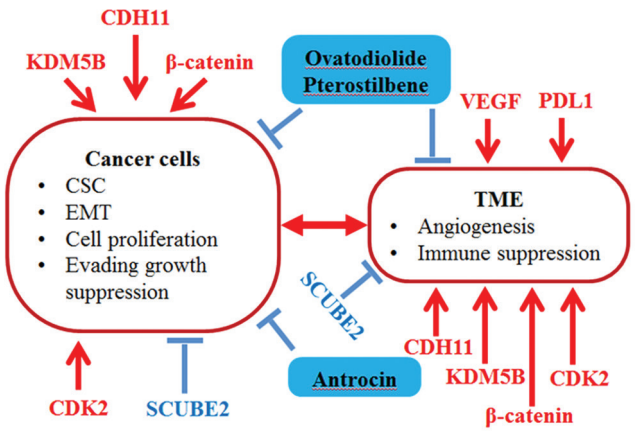

Figure 1: Potential targeted therapy, including potential natural small compounds, for triple-negative breast cancer. cancer stem cells, epithelial-mesenchymal transition, cell proliferation, and evasion of growth suppression lead to triple-negative breast cancer cell heterogeneity. Neovascularization through angiogenesis and immune cell suppression create a protumorigenic tumor microenvironment. Crosstalk between cancer cells and the tumor microenvironment are required for tumor initiation, progression, drug resistance, relapse, as well as distant metastasis. Herein, we highlight several oncogenes (red) and one tumor suppressor gene (blue) that regulate cancer cell heterogeneity as well as the tumor microenvironment as a potential new approach to triple-negative breast cancer treatment. On the basis of our previously study, ovatodiolide, pterostilbene, and antrocin are potential natural small compounds with anticancer properties against triple-negative breast cancer, achieved through targeting cancer cells and the tumor microenvironment

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Conflicts of interest

There are no conflicts of interest.

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