Targets Related to Vasculogenic Mimicry in Breast Cancer

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ABSTRACT: Vasculogenic mimicry (VM) is the release of angiogenic factors from tumor cells, leading to morphological changes, migration and proliferation of vascular endothelial cells, ultimately leading to neovascularization. The presence of VM has been identified in breast cancer, the formation of VM also gives breast cancer a poor prognosis such as drug resistance and metastasis. The authors focus on the role of the PI3K/Akt signaling pathway, hypoxia-inducible factorα(HIFα), the P38/MAPK signaling pathway, cyclooxygenase (COX2), the epithelial-mesenchymal transition (EMT) pathway, non-coding RNAs, and the tumor phenotype in the development of VM in breast cancer, thus offering new ideas for the future treatment of breast cancer.

1. INTRODUCTION

Breast cancer is the most common cancer among women worldwide. Two pathways have been shown to exist in tumors, namely angiogenesis and VM. the former consists directly of endothelial cells (ECs), or from pre-existing blood vessels. VM, however, is a tubular structure that is not dependent on endothelial cells and consists directly of invasive tumor cells. Due to the presence of VMs, breast cancer has an increased chance of metastasis and drug resistance. This has led to a decrease in the quality of survival and survival rates of breast cancer patients. In recent years, scholars at home and abroad have elucidated some mechanisms of VM formation in breast cancer mainly in terms of vascular signaling pathways, epithelial-mesenchymal transition and tumor microenvironment. In this paper, we summarize the pathways of VM generation and its impact on breast cancer and understand its latest progress, so as to provide new ideas for developing targeted drugs to block VM generation and optimize clinical treatment strategies.

2. SINGLING PATHWAYS IN VM FORMATION

2.1. Vascular signaling pathways

The main vascular signaling pathway is the regulation of the level and site of phosphorylation of erythropoietin-producing hepatocellular receptor A2 (EphA2) by vascular endothelial carotene (VE-carotene), which activates extracellular signal-regulated kinase (ERK)1/2 and focal adhesion kinase (FAK) and then PI3K centrally; PI3K can also be activated directly by EphA2.[123]. The PI3K/Akt signaling pathway can stimulate cell survival as well as promote VM production. Epidermal growth factor receptor (EGFR) is overexpressed in tumors[4], Moreover PI3K and Akt as important factors downstream of it, can induce VM expression[5]. If the EGFR/PI3K/Akt number pathway is negatively regulated, hypoxia-induced VM production will be reduced and further tumor deterioration will be avoided[6].

Hypoxia-inducible factor-α (HIF-α) is an important switch for angiogenesis, which can directly or indirectly regulate VE-cadherin and (Eph)A2, thereby promoting VM production and providing blood supply to tumors[7]. Hypoxic conditions can activate the expression of HIF-1α in tumor cells, which can upregulate the expression of vascular endothelial growth factors (VEGF)[89], In contrast, VEGF is considered to be an important factor in angiogenesis, which can promote VM formation by activating VEGFR2, VEGFR3, and thus downstream targets of PI3K [10]. And it has now been shown that HIF-2α may be associated with the activation of VE-cadherin, which subsequently promotes the MMP2 signaling pathway, leading to VM formation[11].

The P38/MAPK signaling pathway regulates cell growth and differentiation. In breast cancer, tissue polypeptide antigen (TPA) inhibits PI3K/AKT and MAPK signaling by suppressing AP-1 and NF-KB, leading to a decrease in MMP9 and inhibition of VM formation.[11]. Current studies have demonstrated that the MARK signaling pathway promotes tumor VM formation and tumor cell invasion and metastasis.

Cyclooxygenase (COX2) catalyzes the conversion of arachidonic acid to prostaglandin (PG) E2 and, by activating prostaglandin-like receptors (EP) 1-4, thereby enhancing tumor cell proliferation and angiogenesis[13]. PGE2 enables tumor stem cell (CSC) proliferation, the EMT process (transformation of epithelial tumor cells into mesenchymal tumor cells) and immune activation, generating immune resistance[14]. Furthermore, Ravi et al [15]found that in TNBC, phycocyanin could inhibit the expression of COX2 and thus VM production. Some studies have shown that COX2, VEGF-A and IL-8 can be

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used as postoperative tests to predict the risk of recurrence in TNBC patients[16].

2.2 EMT pathway

Epithelial-mesenchymal transition (EMT) is the transformation of tumor cells from an epithelial phenotype to a mesenchymal phenotype through a specific procedure that can enable tumor cells to acquire a migratory capacity. Studies targeting VM have shown that EMT allows tumor cells to acquire a mesenchymal phenotype, which mimics endothelial cells in generating vascular ducts, allowing for enhanced invasiveness, distant spread and chemoresistance of tumor cells.

Among these is the Wnt/β-catenin signaling pathway which generates EMT and promotes cancer cell invasion. Wnt protein binds to frizzled protein (Fz) on the envelope, phosphorylates and activates scattered protein (Dsh), which interacts with Axin protein and the APC complex disintegrates. This results in the inability of β-catenin to be degraded and, when levels rise to a certain level, its direct entry into the nucleus. β-catenin binds to E-cadherin to mediate intercellular adhesion, preventing cell migration and causing metastasis. β-Catenin enters the nucleus and can interact with the TCF/LEF signaling pathway, leading to overexpression of its target genes. It is associated with the development of EMT[17]. In TNBC, the Wnt signaling regulatory molecules Wnt-C59/β-catenin inhibitor Sulindac Sulfide and β-catenin-siRNA, and the ryanodine polymerase inhibitor XAV939 can cause the Wnt/β-catenin signaling pathway to be blocked at different nodes and have been found to inhibit VM formation[18].

It is well documented that transforming growth factor-β (TGF-β) is also involved in the EMT process in tumors, which falls into two main pathways. The first is a Smad-dependent pathway: TGF-β first binds to the TGF-β type II receptor (TpRII) on the tumor cell membrane and phosphorylates the TGF-β type I receptor (TpRI) via the TpRII kinase, which activates the Smad2/3 downstream chain. TGF-β can also induce EMT formation by directly activating downstream extracellular regulated kinases (Erk) via the MAPK pathway without relying on Smad[19]. It can also induce EMT through induction of PI3K, Wnt pathway[20]. There are already too many pathways that can interfere with TGF-induced VM production, and we can start with TGF to inhibit VM production.

Nodal signaling is a trait (TGF-β) expressed in various malignancies. High expression of Nodal signaling has been shown to be strongly associated with poor prognosis and metastasis in breast cancer. In vitro, Nodal signaling upregulates the level of VM-related protein expression and can promote the formation of VM. Nodal can also downregulate E-cadherin via the Smad2/3 pathway and subsequently promote the expression of Slug, Snail and C-myc. It increases breast cancer cell proliferation and induces the EMT process, which leads to the development of VM. Nodal also modulates the breast cancer stem cell phenotype, promoting the expression of ALDH (acetaldehyde dehydrogenase) 1, CD44, and CD133, followed by an increase in the multi-lineage differentiation potential of cancer cells[21].

SND1 as a multifunctional protein is usually highly expressed in tumors and cancer metastasis is often associated with its high expression. Moreover, in the breast cancer cell line MDA-MB-231 cells, SND1 can lead to epithelial-mesenchymal transformation and the formation of VM, which is inhabited mainly by transcriptional activation of DNMT3A expression and hypermethylation of the E-cadherin promoter, culminating in epithelial-mesenchymal transformation and VM formation, promoting breast cancer migration as well as metastasis[22].

Twist, a conserved helix-loop-helix transcription factor directly represses the expression of E-cadherin and upregulates vimentin and N-cadherin, while also inducing the expression of snail1 and snail2, thereby allowing EMT to occur[23]. Regarding Twist1, Drasin et al. found that Twist1-mediated elevation of miR-424 increased breast cell motility decreasing adhesion and allowing cancer cells to migrate[24]. Endocrine therapy is also a common approach to breast cancer, and some studies have developed that in estrogen-positive patients, the estrogen receptor can be regulated by Twist, eventually causing loss of estrogen receptor function and thus resistance to estrogen receptor drugs[25]. If the Twist gene can be knocked out, resistance to chemotherapeutic drugs and endocrine drugs can be avoided, thus enhancing patient outcomes.

CD133 is located at membrane protrusions such as microvilli or as a fifth transmembrane glycoprotein on the apical surface of epithelial cells, and it is also considered a universal marker for a variety of tumor CSCs such as breast cancer. Liu et al. found that VM was closely associated with CD133 positivity in TNBC whole clone MDA-MB-231[26].In TNBC xenografted mice, hypoxia-induced Twist1 made CD133 positive and after generating VM, the mice became resistant to sunitinib[27].

Prostaglandin (PE) G2 can also promote the EMT process by down-regulating E-cadherin and can also promote nuclear translocation of β-catenin, which in turn activates its target cells and ultimately induces EMT[28]. Therefore, if PEG2/COX 2 can be used as a target, VM formation can be inhibited through both EMT and vascular signaling pathways.

Notch is a conserved transmembrane protein that promotes the invasive process of EMT by upregulating Slug[29]. It has been shown that exogenous IL-6 can inhibit miR-204 expression in MCF-7 cells, thereby activating the Notch pathway and generating VM[30]. CHOI et al. found that bone morphogenetic protein (BMP-4) is associated with the production of EMT. Upregulation of Smad4 activates the BMP-4-induced Notch signaling pathway. This suggests that BMP-4 is dependent on Smad4 and thus activates Notch, ultimately leading to EMT production [31].

Sphingosine 1-phosphate receptor 1 (S1PR1) is also implicated in the formation of VM, a biologically active signaling lipid that regulates vascular development, function and maturation, and deletion of S1PR1 contributes to the formation of VM. S1PR1 allows VE-cadherin to separate from -catenin and prevent the EMT process from occurring thereby producing VM[32].

3. REGULATION OF VM IN BREAST CANCER BY NON-CODING RNAs

Non-coding RNAs and long non-coding RNAs are also involved in the regulation of VM and breast tumor formation. Cancer cells have been shown to gain the ability to invade and proliferate by expressing miRNAs, thus contributing to EMT. Ma et al. showed that miRNA-9 upregulates E-cadherin expression and induces activation of the Wnt/β-catenin signaling pathway in the breast cancer cell line SUM149, and then translocation of β-catenin into the nucleus induces breast cancer EMT. This suggests that miRNA level is closely associated with VM formation.

4. VM AND TOMOR PHENOTYPE

Breast cancer can be classified into different phenotypes based on estrogen receptor (ER), progesterone receptor (PR) and overexpression of epidermal factor receptor (Her-2). If none of these three receptors are expressed, then it is called triple-negative breast cancer (TNBC).

Human chorionic gonadotropin(hCG) contributes to VM formation. hCG can be inhibited by breast cancer 1(BRCA1), and in breast cancer, overexpression of hCG leads to mutations in BRCA1, when hCG can signal through the TGFR, leading to an abnormal increase in cells34. hCG also depends on the VM phenotype in inducing VM formation, in luminal-A (ER+, PR+, Her-), hCG inhibits cell proliferation and tumor growth35, but in HER2-positive patients, hCG promotes growth and metastasis in vivo36.

LR1G1 is a tumor suppressor that negatively regulates TKRs signaling through ubiquitination-induced degradation of tyrosine kinase receptor TKRs or by blocking TKRs heterodimeric conformation, which leads to inhibition of PI3K/AKT and ERK1/2 signaling pathways37. It has been shown that HER2 positivity significantly suppresses LRIG1 protein levels, leading to enhanced AKT/ERK signaling pathways38. However, the LRIG1 gene was expressed in ER-positive breast cancer39, so the incidence of VM was lower in ER-positive patients than in TNBC and HER2-positive patients.

5. CONCLUSION

The formation of VM in breast cancer is mainly divided into the vascular signaling pathway and the EMT process to form VM. peg2 and COX can block VM formation through both pathways, and blocking their pathways can rapidly inhibit VM formation. twist, PI3K/Akt, MAPK and Nodal can promote VM formation, leading to metastasis and drug resistance in cancer cells. Changes in non-coding RNA levels can also affect VM formation. And the incidence of VM varies between tumor phenotypes, with HER2-positive patients having a greater chance of VM generation compared to luminal-A. In conclusion, the many determinants of VM and the in-depth research of targeted drugs in the appeal process will allow optimizing the treatment of VM in breast cancer and further improve the survival and quality of life of breast cancer patients.

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