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Clinical application and research progress of lymphovascular markers for breast cancer

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Abstract: Breast cancer has become the most common malignant tumor in women, and its diagnosis and treatment principles mainly depend on the traditional histological and molecular pathological features. However, the systemic metastasis of breast cancer begins when cancer cells invade the primary tumor site as well as surrounding blood and lymphatic vessels. Pathologists have been able to distinguish between lymphatic and vascular invasion through a combination of immunohistochemical and vascular endothelial labelling techniques, and have recognized the role of and vascular endothelial cells in tumor progression and metastasis. This paper summarizes the relevant clinical research progress and application of specific lymphovascular endothelial markers in breast cancer in recent years, in order to provide ideas for further revealing the progress mechanism of breast cancer and exploring new prognostic indicators and therapeutic targets for breast cancer.

Keywords: Breast cancer; Vascular endothelial marker; Lymph vascular invasion; Lymphangiogenesis; Angiogenesis; Podoplanin; Platelet/endothelial cell adhesion molecule; Endoglin; Vascular epidermal growth factor receptor; Chemokine receptor

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Global Cancer Statistics for the year 2022 [1] revealed that breast cancer accounted for 31% of new cancer cases among women, making it the most prevalent malignant tumor in terms of incidence. The diagnosis and treatment of breast cancer are primarily based on traditional histopathological and molecular pathological features according to various authoritative guidelines [2]. Currently, the focus of breast cancer diagnosis and treatment is on tumor recurrence and metastasis. As a systemic disease, the systemic metastasis of breast cancer begins with the invasion of cancer cells into the primary tumor site and its surrounding tissues, specifically within the primary tumor or the surrounding "lymphovascular invasion." In recent years, pathological researchers have used endothelial cells with different molecular characteristics to distinguish lymphatic and vascular invasion through immunohistochemistry (IHC) and vascular endothelial biomarkers. Then the role of lymphatic endothelial cells (LEC) in tumor progression and metastasis was recognized gradually [3-4]. On the other hand, since the principle of anti-angiogenesis treatment for solid tumors was proposed at 1972 [5], targeted anti-angiogenesis drugs combined with chemotherapy and radiotherapy, as an anti-tumor treatment strategy, have had a positive impact on tumor treatment, and research on angiogenesis in breast cancer has also made great breakthroughs. In recent years, a large number of breast cancer specific vascular

endothelial markers have emerged, some of which have become routine diagnostic methods for breast cancer vascular tumor thrombus. This article reviews the clinical application and research progress of specific lymphovascular endothelial markers (including lymphatic endothelial markers and vascular endothelial markers) in breast cancer in recent years.

1 Lymphatic endothelial marker

Lymphatic endothelial markers are specific markers expressed by LEC during during lymphatic vessel formation. They can be distinguished from surrounding tissues and are easily detectable at the histological level. Representative lymphatic endothelial markers include D2-40, podoplanin (PDPN), and vascular endothelial growth factor receptor-3 (VEGFR-3).

1.1 D2-40

Monoclonal antibody D2-40 is a selective marker of lymphatic endothelium discovered by Kahn *et al* in 2002. In paraffin sections of breast cancer, colon cancer, prostate cancer and other primary tumors, D2-40 can clearly distinguish tumor embolism in the lymphatic vessels around the tumor. Subsequent research showed that D2-40 not only improve the detection rate of lymphatic invasion in breast cancer [6], but also play a role in predicting the survival of breast cancer patients [7]. However, the use of D2-40 as a prognostic indicator for assessing breast cancer recurrence and metastasis is still debated. A study by Britto *et al*. [8] found no correlation between the D2-40 parameter measured by IHC and the risk of lymph node metastasis as well as cancer progression, and D2-40 parameter was not been recommended to use as an indicator to change the risk category. However, Vosough *et al*. [9] showed that D2-40 IHC marker was helpful in the diagnosis of lymphatic invasion in invasive breast cancer. D2-40, a selective monoclonal IHC marker for LEC, is currently used for the diagnosis of lymphovascular invasion in tissue samples fixed with formalin and paraffin, and serves as a potential predictive indicator of cancer invasiveness by improving the accuracy of lymphatic invasion detection.

1.2 PDPN

PDPN was discovered by Nose *et al*. in 1990. Current research showed that PDPN was mainly expressed on the surface of human LEC, promoting lymphatic endothelial cell adhesion and lymphangiogenesis. Therefore, PDPN is used as a marker for LEC in pathological diagnosis and related research [10]. Chen *et al*. [11] used gene recognition and control to deplete tumor LEC overexpressing PDPN in animal experiments. The results showed that induced lymphatic vessel lesions in PDPN-tk mice successfully inhibited lymphatic vessel formation, and also validated the role of primary tumor lymphatic vessel formation in controlling axillary lymph node and parenchymal organ metastasis. Schoppmann *et al.* [12] proved that the cancer related fibroblasts expressing PDPN were highly invasive and were subtypes of invasive breast cancer, which was further verified in the research of Du *et al.* [13]. Recent studies have confirmed that the expression of PDPN is related to the occurrence, development and prognosis of breast cancer. The role of PDPN in breast cancer will become a potential target for treatment.

1.3 VEGFR-3

VEGFR-3, a receptor tyrosine kinase, is mainly distributed in LEC of adult tissues after embryonic development. VEGFR-3 is exclusively expressed in LEC and promotes tumor cell adhesion to the corresponding lymphatic vessels by binding to the highly-expressed ligand vascular endothelial growth factor-C (VEGF-C) in tumor cells [14]. Ding *et al*. [15] proved the value of VEGFR-3 in evaluating lymphatic angiogenesis and microvessel density in breast cancer. In 2023, Torres Ruiz *et al*. [16] detected the expression of VEGFR-3 in two triple negative breast cancer models *in vitro*, and found that elevated VEGFR-3 levels correlated with poor survival and reduced doxorubicin treatment efficacy, indicating that the expression level of this receptor could be a potential marker of meager doxorubicin response, suggesting that the combination of chemotherapy and VEGFR-3 blockage could be a potentially useful therapeutic strategy for the treatment of triple-negative breast cancer.

1.4 Chemokine receptor (CCR) 7

CCR7 is 7-transmembrane G protein-coupled receptor and is a member of the CC subfamily CCR. In 2001, Müller *et al*. [17] proposed a "chemokine receptor" model to explain the biological behavior of tumor cells homing to specific organs for the first time. And they found that malignant tumor cells produced CCRs, which reacted with their homologous ligands and migrate along chemokine gradients, ultimately promoting corresponding organ metastasis. Liu *et al*. [18] found that among all known CCRs, breast cancer cells specifically expressed activities CXCR4 and CCR7, and their ligands were CXCL12 and CCL21, respectively. The CCR7-CCL21 axis is a key factor in tumor lymph node metastasis. This view was further confirmed in the study of Deng *et al*. [19] in 2022. Hayasaka *et al*. [20] showed that CXCL12/CXCR4 signal pathway promoted the migration and invasion of human breast cancer cells MDA-MB-231 to tumor lymphatic vessels expressing CCR7 ligands, and supported the CCR7 signal related to lymph node metastasis, providing a basis for the theory that CCR7 participates in tumor cell invasion, and further consolidating the role of CCR7 in tumor cell invasion. CCR7, as a crucial link in the development of cancer, can further improve the prediction of the presence and extent of lymph node involvement.

2 Vascular endothelial markers

Vascular endothelial markers of breast cancer include platelet/endothelial cell adhesion molecule (PECAM-1, also known as CD31), CD34 and endoglin (also known as CD105), which have been proved to be specific markers of neovascularization during the occurrence and development of breast cancer.

2.1 CD31

CD31 is a member of the adhesion molecule immunoglobulin superfamily, involved in the process of tumor cell adhesion to endothelial cells and promoting tumor angiogenesis. Zhang *et al*. [21] found that CD31 mediates tumor cell adhesion to endothelial cells. When platelets bound to tumor cells, they could promote tumor cell contact and adhesion to endothelial cells through the connection between CD31 and endothelial cells. Pospelova *et al*. [22] used the serum level of CD31 as a potential biomarker for long-term follow-up of breast cancer patients after treatment. The results showed that the serum level of CD31 significantly increased in patients receiving radical breast cancer treatment, especially those with neurological symptoms. Since CD31 is considered to be a marker of endothelial dysfunction caused by microvascular injury, this result confirms the hypothesis that endothelial dysfunction plays a leading role in central nervous system injury, suggesting that CD31 can provide strategies for determining central nervous system injury in breast cancer patients after treatment, which may help to improve the management of such patients. In summary, the expression of CD31 is related to many clinicopathological characteristics of breast cancer, and its diagnostic evaluation and prognostic guiding value for breast cancer patients still needs further research.

2.2 CD34

CD34, a single chain highly glycosylated transmembrane protein, exists in endothelial cells of microvessels and is recognized as a sensitive marker for tumor neovascularization and lymphatic vessels. CD34 can be used to detect tumor tissue microvascular density through IHC technology, evaluating tumor proliferation activity [23]. The study by Pan *et al*. [24] showed that positive CD34 in IHC suggested scattered positive blood vessels within the tumor mostly. New blood vessels can promote the metabolism of cancer cells, and accelerate tumor growth. In addition, the increase contact between cancer cells and new blood vessels with weak walls and incomplete stromal membranes could promote the adhesion of cancer cells into blood vessels, forming metastatic lesions through blood circulation. The high expression of CD34 suggests an increase in microvascular density and the number of microvessels in cancer tissue, indicating an increased possibility of tumor infiltration and metastasis. Therefore, CD34 detection will provide a new potential tumor marker for the diagnosis and prognosis evaluation of breast cancer.

2.3 CD105

CD105 is a transmembrane glycoprotein expressed on active endothelial cells, and is a specific neovascular endothelial marker. Compared to conventional pan vascular endothelial cell markers such as CD31 and CD34, CD105 is highly sensitive to staining proliferating endothelial cells and does not show significant specificity for mature endothelial cells. Therefore, CD105 is more suitable for labeling tumor tissue microvessels [25]. Jadhao *et al*. [26] found that knocking down the expression of CD105 in endothelial cells can reverse the activation of TGFβ/SMAD3/VEGF signaling axis, MAPK/p38 signaling pathway, and cytokine regulation, limiting the potential for angiogenesis. Therefore, targeting CD105 may serve as a potential therapy for controlling cancer angiogenesis and limiting cancer progression. Abbas *et al*. [25] detected 200 breast cancer and 100 normal breast tissue paraffin blocks, and found that CD105 marker was highly specific for newly developed tumor blood vessels and could be a useful predictor of angiogenesis and breast cancer metastasis. In the study of Vo *et al*. [27], patients with metastatic breast cancer who received hormone therapy had higher CD105 levels before treatment, indicating lower efficacy and shorter overall survival. Therefore, compared with other endothelial markers, CD105 has a potential role in the diagnosis and treatment of breast cancer.

3 Application of vascular endothelial markers in breast cancer

In the era of precision medicine, the correct cancer risk stratification is crucial for developing effective treatment strategies. The treatment strategies for cancer aims to maximize survival rates while minimizing unnecessary interventions. Traditional prognostic indicators for breast cancer have limited prognostic value in early-stage breast cancer without lymph node metastasis or distant metastasis. While the characteristics of tumor microenvironment, such as tumor lymphangiogenesis and angiogenesis, contribute to the evaluation and prediction of cancer prognosis, supplementing the TNM staging system and tumor molecular typing.

LEC play a crucial role in tumor lymphangiogenesis. LEC are the main cells responsible for establishing the lymphatic system, forming the lymphatic vessel walls and transporting migrating cells into the lymphatic vessels. During the lymphatic metastasis process in breast cancer, malignant tumor cells compress the LEC junctions and migrate along LECs towards lymph nodes and distant organs. This indicates that tumor cells exploit LECs to facilitate cancer invasion [3]. In highly active tumor tissues, infiltration of LECs and lymphatic vessel

formation known as tumor-associated lymphangiogenesis can frequently be observed, indicating that the formation of new lymphatic vessels is a key initial step in tumor spread [28-29]. Compared with normal lymphatic vessels, tumor associated lymphatic vessels exhibit highly heterogeneous morphology and functionality. The traditional concept of lymphatic metastasis is that tumor cells passively transport under the pressure of lymphatic fluid, but it has been proven that it is more likely the result of the interaction between tumor cells and the tumor microenvironment (such as LEC) [30]. Therefore, a deep understanding of tumor lymphangiogenesis and LEC markers contributes to exploring the mechanisms underlying breast cancer initiation and development, facilitating the discovery of new potential predictive markers and treatment targets.

In the process of breast cancer progression, tumor angiogenesis is an independent and key prognostic factor for invasive breast cancer, regardless of lymph node metastasis [31]. However, it is also reported that angiogenesis could not predict the recurrence risk of primary breast cancer patients [32]. So, the significance of tumor related angiogenesis remains controversial. A meta-analysis suggested that blood vessel invasion was an independent predictor of poor prognosis of operable breast cancer, and was related to invasive clinicopathological characteristics [33]. IHC of endothelial markers has also tried to detect vascular invasion, but the selection of antibodies will significantly affect the detection rate, leading to a lack of consensus on the prognostic value of blood vessel invasion in breast cancer. However, Klingen Thor *et al*. [36] used CD31 to detect the blood vessel invasion of specific breast cancer patients, and the results showed that blood vessel invasion was significantly related to the recurrence free survival rate and specific survival rate of cancer, and was an independent prognostic factor.

The emergence of vascular endothelial markers has also advanced the study of vascular emboli (lymphovascular invasion) in breast cancer. Lymphovascular invasion means that tumor cells break through the epithelial basement membrane, invade the stroma, reach the lower vascular space, and ultimately invade the vascular system [35], which is a prerequisite for lymph node metastasis. The College of American Pathologists (CAP) recommends lymphovascular invasion should be evaluated and reported in Cancer Protocols [36]. Although some patients with positive vascular invasion in clinical practice did not experience lymph node metastasis, it suggests a risk of recurrence and metastasis. Screening out such patients and formulating corresponding treatment plans will help to improve the survival rate of breast cancer [37]. In recent years, many studies have shown that lymphovascular invasion is associated with local recurrence, distant metastasis and poor prognosis of breast cancer patients [38]. However, conventional HE staining cannot distinguish the contraction gap caused by tumor cell thrombus in lymphatic or vascular and tissue fixation, and cannot specifically identify lymphovascular invasion or blood vessel invasion. Meanwhile, IHC technology is currently not commonly used as an initial screening method for vascular invasion. Therefore, although the negative prognostic value of vascular invasion is recognized, its inclusion in the standard treatment decision-making process for breast cancer has not been fully defined, and has not been recognized in most guidelines [39]. But the application of vascular endothelial specific markers and the study of lymphatic vessels and blood vessels through IHC technology can further explore the relationship between vascular invasion and the progress of breast cancer, and provide theoretical basis for the study of tumor metastasis mechanism from different angles.

4 Conclusion

In conclusion, with the clinical application of specific vascular endothelial markers, the role of tumor related lymphatic vessels, angiogenesis, vascular invasion and other important tumor microenvironment characteristics in the occurrence, development and metastasis of breast cancer are gradually being elucidated, which will provide new ideas for revealing the progress mechanism of breast cancer and exploring new prognostic indicators and therapeutic targets for breast cancer.

Conflict of interest None

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· 学术前沿 ·

乳腺癌脉管标记物的临床应用与研究进展

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摘要：乳腺癌已成为目前女性发病率最高的恶性肿瘤，其诊疗原则主要依赖于传统的组织学和分子学病理特征。然而，乳腺癌的全身性转移始于癌细胞侵入原发肿瘤部位及其周围的血管和淋巴管。病理学家通过免疫组织化学技术和脉管内皮标记技术的结合，得以区分淋巴管与血管侵袭，并认识到淋巴管内皮细胞及血管内皮细胞在肿瘤进展及转移中的作用。本文归纳了近年乳腺癌特异性脉管内皮标记物的相关临床研究进展及应用，以期为进一步揭示乳腺癌的进展机制、探索其新的预后指标和治疗靶点提供思路。

关键词：乳腺癌；脉管内皮标记物；脉管癌栓；淋巴管生成；血管生成；平足蛋白；血小板内皮细胞黏附分子；内皮糖蛋白；血管内皮生长因子受体；趋化因子受体

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2022 年全球癌症统计结果显示,乳腺癌以 31%的新增癌症病例占比成为目前女性发病率最高的恶性肿瘤[1]。在各类权威指南中,TNM 肿瘤分期及相关激素受体状态为当前乳腺癌诊疗的主要参照因素[2]。目前,肿瘤的复发和转移一直是乳腺癌诊治的重点。乳腺癌作为一种全身性疾病,其全身性转移始于癌细胞侵入原发肿瘤部位及其周围组织,即原发性肿瘤内部或周围的“淋巴血管侵袭(lymphovascular invasion)”。近年来,病理学研究者利用不同分子特征的内皮细胞,通过免疫组织化学(immunohistochemistry, IHC)技术和脉管内皮标记技术来区分淋巴管与血管侵袭。由此,研究者逐渐认识到淋巴管内皮细胞在肿瘤进展及转移中的作用[3-4]。另一方面,自 1972 年,实体肿瘤的抗血管生成治疗原则被提出后[5],靶向抗血管生成药物联合化疗及放疗,作为一种抗肿瘤治疗策略对肿瘤的治疗产生了积极影响,乳腺癌血管生成的相关研究也取得很大突破。近年来,大量乳腺癌特异性脉管内皮标记物出现,其中部分标记物已成为常规乳腺癌脉管癌栓的诊断方法。本文对近年乳腺癌特异性脉管内皮标记物(包括淋巴管内皮标记物和血管内皮标记物)的临床应用及研究进展作一综述。

1　淋巴管内皮标记物

淋巴管内皮标记物是在淋巴管生成评估过程中,由淋巴管内皮细胞特异性表达,可区分于周围组织的特定标记物。具有代表性的淋巴管内皮标记物,包括 D2-40、平足蛋白(podoplanin,PDPN)及血管内皮生长因子受体-3(vascular epidermal growth factor receptor-3, VEGFR-3)等。

1.1　D2-40　单克隆抗体 D2-40 是由 Kahn 等在 2002 年发现的淋巴管内皮选择性标记物,在乳腺癌、结肠癌、前列腺癌等原发肿瘤的石蜡切片中,D2-40 在肿瘤周围的淋巴管中能清楚地划分出肿瘤栓塞。之后的研究结果进一步显示,D2-40 不仅能提高乳腺癌淋巴管浸润的检出率[6],还能在乳腺癌患者的生存预测中起到一定作用[7]。尽管 D2-40 展现出较高的检测效能与预测价值,但其能否作为评估乳腺癌复发转移的预后指标目前仍存在争议。在 Britto 等[8]的研究中,IHC 测定的 D2-40 参数与淋巴结转移及肿瘤进展风险之间没有关联,不主张用该参数作为改变风险类别的指标。但 Vosough 等[9]研究显示,D2-40 IHC 标记物有助于乳腺浸润性癌淋巴管浸润的诊断。D2-40 作为一种淋巴内皮细胞选择性单克隆 IHC 标记物,目前应用于常规处理福尔马林固定和石蜡固定的组织样本中淋巴管癌栓的诊断,并通过提高淋巴管侵袭检测的准确性而作为肿瘤侵袭性的潜在预测指标。

1.2　PDPN　PDPN 是 1990 年由 Nose 等发现,目前研究显示 PDPN 主要表达于人体的淋巴管内皮细胞表面,促进淋巴管内皮细胞黏附和淋巴管生成,因而作为淋巴管内皮标志物应用于病理诊断和有关研究[10]。Chen 等[11]在动物实验中通过基因识别与控制来消耗 PDPN 过表达的肿瘤淋巴管内皮细胞,结果显示,PDPN-tk 小鼠中诱导的淋巴管病变成功实现了对淋巴管生成的抑制,同时还验证了原发性肿瘤淋巴管生成在控制腋窝淋巴结和实质脏器转移中的作用。Schoppmann 等[12]在研究中证明 PDPN 表达的癌症相关成纤维细胞是具备高度侵袭性的浸润性乳腺癌预测亚型,该观点在 Du 等[13]的研究中被进一步验证。近年研究已证实,PDPN 的表达与乳腺癌的发生、发展及预后有关。PDPN 在乳腺癌中的作用将成为治疗的潜在靶点。

1.3　VEGFR-3　VEGFR-3 是一种受体酪氨酸激酶,在胚胎发育后主要分布于成人组织中的淋巴内皮细胞。VEGFR-3 仅表达于淋巴管内皮细胞,通过与在肿瘤细胞中高度表达的配体血管内皮生长因子-C(vascular epidermal growth factor-C, VEGF-C)结合,促进肿瘤细胞黏附于相应的淋巴管[14]。2012 年 Ding 等[15]证明了 VEGFR-3 对乳腺癌淋巴管生成和微血管密度的评估价值。2023 年 Torres-Ruiz 等[16]检测了 VEGFR-3 在两种三阴性乳腺癌体外模型中的表达,发现 VEGFR-3 的高表达与化疗患者较差的生存率和体外多柔比星疗效的降低相关,说明该受体的表达水平可能是阿霉素反应微弱的潜在标志,提示化疗和 VEGFR-3 阻断相结合可能是治疗三阴性乳腺癌的一种潜在有用的治疗策略。

1.4　趋化因子受体(chemokine receptor, CCR)7

CCR7 为 7 次跨膜的 G 蛋白偶联受体,是 CC 亚家族 CCR 的成员之一,2001 年 Müller 等[17]首次报道并提出了“CCR”模型来解释肿瘤细胞向特定器官归巢的生物学行为;恶性肿瘤细胞产生 CCR,这些受体与其同源配体反应,沿着趋化因子梯度迁移,最终促使相应的器官转移。Liu 等[18]的研究发现,在所有已知的 CCR 中,乳腺癌细胞特异性表达活性 CXCR4 和 CCR7,其配体分别为 CXCL12 和 CCL21,CCR7-CCL21 轴是肿瘤淋巴结转移的关键因素,此观点在 Deng 等[19]的研究中被进一步证实。Hayasaka 等[20]的实验表明,CXCL12/CXCR4 信号促进 MDA-MB-231 人乳腺癌细胞向表达 CCR7 配体的肿瘤内淋巴管迁移和侵袭,并支持与淋巴结转移相关的 CCR7 信号,为 CCR7 参与肿瘤细胞侵袭的理论提供了依据。CCR7 作为肿瘤发展过程的关键一环,能进一步提高对淋巴结受累的存在及程度的预测。

2　血管内皮标记物

乳腺癌血管内皮标记物包括血小板内皮细胞黏附分子-1(platelet/endothelial cell adhesion molecule-1,PECAM-1,亦称 CD31)、CD34 和内皮糖蛋白(endoglin,亦称 CD105)等,经研究证明它们是乳腺癌发生、发展过程中新生血管的特异性标记物。

2.1　CD31　CD31 是黏附分子免疫球蛋白超家族成员,参与肿瘤细胞黏附于内皮细胞并促进肿瘤血管形成的过程。张文进等[21]在研究中发现,CD31 介导肿瘤细胞黏附于内皮细胞,当血小板与肿瘤细胞结合时,可通过 CD31 与内皮细胞的连接促进肿瘤细胞对内皮细胞的接触与黏附。Pospelova 等[22]将 CD31 血清水平作为乳腺癌患者治疗后长期随访的潜在生物标志物,结果显示,接受根治性乳腺癌治疗、特别是有神经系统症状的患者,其 CD31 血清水平显著升高。由于 CD31 被认为是微血管损伤导致的内皮细胞功能障碍的标志物,该结果证实内皮细胞功能障碍在中枢神经系统损伤中起主导作用的假设,提示 CD31 可以为确定乳腺癌患者治疗后中枢神经系统损伤提供策略,而有助于改善此类患者的管理。综合来看,CD31 的表达与多项乳腺癌临床病理特征有关,其对乳腺癌患者的诊断评估和预后指导价值仍需进一步研究。

2.2　CD34　CD34 是一种单链高度糖基化型跨膜蛋白,存在于微血管的内皮细胞中,是目前公认的肿瘤新生血管内皮、淋巴管敏感标记物,可以通过 IHC 技术检测肿瘤组织微血管密度,来评价肿瘤增殖活跃度[23]。潘鑫源等[24]的研究显示在大多数情况下,CD34 IHC 染色阳性提示肿瘤内存在散在的阳性血管。新生血管能促进癌细胞的新陈代谢,加速瘤体生长,同时癌细胞与管壁薄弱、基质膜不完整的新生血管接触的增多,会进一步促使癌细胞黏附进入血管,通过血液循环形成转移病灶。而 CD34 的高表达提示肿瘤组织内的微血管密度及数量的增加,预示肿瘤浸润和转移的可能性增加。因此,CD34 检测将为乳腺癌的诊断和预后评估提供一个新的潜在肿瘤标志物。

2.3　CD105　CD105 是一种表达于有活性的血管内皮细胞上的跨膜糖蛋白。相较于常规作为肿瘤组织微血管标记物的 CD31、CD34 等泛血管内皮细胞标记物,CD105 对增殖的血管内皮细胞染色高度敏感,同时对成熟的血管内皮细胞未见明显的特异性,因此 CD105 更适用于标记肿瘤组织微血管[25]。Jadhao 等[26]的研究则发现敲低内皮细胞中 CD105 的表达可以逆转邻苯二甲酸酯诱导的 TGFβ/SMAD3/VEGF 信号轴、MAPK/p38 信号传导和细胞因子调节的激活,从而限制血管生成潜力。因此,靶向 CD105 可能作为控制肿瘤血管生成和限制肿瘤进展的潜在疗法。Abbas 等[25]在 200 个乳腺癌和 100 个正常乳腺组织石蜡块的检测中发现,CD105 标记物对新发展的肿瘤血管具有高度特异性,可作为血管生成和肿瘤转移的预测因子。在 Vo 等[27]的研究中,接受激素治疗的转移性乳腺癌患者治疗前 CD105 水平升高预示疗效降低和总生存期缩短。因此,CD105 相较其他内皮标记物表现出对乳腺癌诊疗评估的潜在作用。

3　脉管内皮标记物在乳腺癌中的应用

在精准医学时代,正确的肿瘤风险分层是制定有效治疗方案的基础,肿瘤的治疗策略需要最大限度地提高生存率,同时尽量减少不必要的干预。传统的乳腺癌预后指标,在淋巴结阴性或无远处转移的早期乳腺癌中未能显示出较高的预后价值。而肿瘤微环境的特征有助于肿瘤预后的评估与预测,对 TNM 分期系统和肿瘤分子分型进行补充。

在肿瘤淋巴管生成中,淋巴管内皮细胞起到了至关重要的作用。淋巴管内皮细胞是建立淋巴管系统的主要细胞,构成淋巴管壁并将迁移的细胞输送到淋巴管内。在乳腺癌的淋巴转移过程中,恶性肿瘤细胞挤压淋巴管内皮细胞连接处,并沿着淋巴管内皮细胞向下一站淋巴结和远处器官迁移[3],从而导致肿瘤的侵袭。在高度活跃的肿瘤组织中,经常可以观察到淋巴管内皮细胞浸润和淋巴管形成,即肿瘤相关淋巴

管生成[28]是肿瘤扩散的关键起始步骤[29]。与正常淋巴管相比,肿瘤相关淋巴管表现出高度异质性的形态和功能。淋巴转移的传统观念是肿瘤细胞在淋巴液的压力下进行被动运输,但现已证实,它更可能是肿瘤细胞和肿瘤微环境(如淋巴管内皮细胞)之间相互作用的结果[30]。因此,对肿瘤淋巴管生成和淋巴管内皮细胞标记物的深入理解有助于探索乳腺癌发生发展的机制,促进寻找新的潜在预测性标记物和治疗靶点。

在乳腺癌的进展过程中,不论淋巴结有无转移,肿瘤血管生成是侵袭性乳腺癌独立且关键的预后因素[31],但也有报道称血管生成不能预测原发性乳腺癌患者的复发风险[32],因此,肿瘤相关血管生成的意义仍然存在争议。一篇荟萃分析认为,血管侵犯是可手术乳腺癌预后不良的独立预测因素,并与侵袭性临床病理特征相关[33]。内皮标记物的 IHC 染色也曾尝试检测血管侵犯,但抗体的选择会显著影响检测率,导致血管侵犯在乳腺癌中的预后价值无法达成共识。不过,Klingen Tor 等[34]利用 CD31 检测特定乳腺癌患者的血管侵犯情况,结果提示血管侵犯与肿瘤的无复发生存率和特异性生存率显著相关,是一个独立的预后因素。

脉管内皮标记物的出现还推动了脉管癌栓(即淋巴血管侵袭)的研究进展。脉管癌栓意味着肿瘤细胞突破上皮基底膜,侵入间质,到达下层血管间隙,最终侵犯脉管系统[35],是淋巴结转移的先决条件。美国病理学家学会(College of American Pathologists, CAP)建议在 Cancer Protocol 中评估和报告脉管癌栓[36]。尽管临床上部分脉管癌栓阳性的患者未出现淋巴结转移,但也预示着可能存在复发转移的风险。将此类患者筛选出来并制定相应的治疗方案,有助于提高乳腺癌的生存率[37]。近年来,多项研究结果显示淋巴血管侵袭与乳腺癌患者的局部复发、远处转移和较差的预后相关[38]。然而,由于常规 HE 染色无法区分淋巴管/血管癌栓与组织固定所导致的收缩间隙,且不能特异性识别淋巴管癌栓和血管癌栓的局限性,以及 IHC 技术目前并不常规用于脉管癌栓的初始筛查,因此,尽管脉管癌栓的负面预后价值被认可,但其在乳腺癌标准治疗决策过程中的纳入尚无定论,在大多数指南中也尚未得到承认[39]。不过,结合脉管内皮特异性标记物的 IHC 技术显著提高了乳腺癌脉管癌栓的检出率,相关的特异性脉管内皮标记物也能客观而标准化地区分淋巴管和血管癌栓。由此可见,脉管内皮特异性标记物的应用结合 IHC 技术开展淋巴管和血管的有关研究,可进一步探讨脉管癌栓与乳腺癌进展的关系,从不同的角度为研究肿瘤转移机制提供理论依据。

4　结　语

综上所述,随着特异性脉管内皮标记物的临床应用,肿瘤相关淋巴管、血管生成及脉管癌栓等重要肿瘤微环境特征在乳腺癌的发生、发展、转移过程中的作用逐渐阐明,将为揭示乳腺癌的进展机制、探索乳腺癌新的预后指标和治疗靶点提供新的思路。

利益冲突　无

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