

The Mechanism of Tumor Metastasis

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Abstract. Nowadays, cancer is the leading cause of human health problems, and the vast majority of deaths are caused by metastatic cancer. The disease is not easy to detect in the early stage, in a latent state, after a period of time, is found to be in an advanced stage, difficult to cure. The current treatment methods include chemotherapy, radiotherapy, surgery, anti-cancer drugs, etc., after clinical application, there is a little basis but also has its limitations. In particular, there are many studies on the mechanism of action of a series of factors that cause tumor metastasis, the specific cause of metastasis is still unclear, which brings challenges to the treatment of metastatic tumors. This article reviews the basic definition and characteristics of cancer, the process of tumor metastasis, describes the known influencing factors, and explores possible targeted therapies. It is hoped that this will help and inspire future research into cancer treatments.

Keywords: Angiogenesis, EMT, invasion, intravasation, extravasation.

1. Introduction

Cancer is a malignant tumor developed from epithelial tissue, characterized by abnormal proliferation and differentiation of tumor cells, unstoppable growth, and biological characteristics such as infiltration and metastasis. Its formation is a complex process that can be mainly divided into three steps: carcinogenesis, cancer promotion, and progression. According to data from the WHO, there were about 20 million new cases of cancer worldwide in 2022, with approximately 9.96 million deaths, making it one of the main causes of death in humans.

There's more than 91% of cancer-related deaths are caused by metastatic diseases. The 5-year survival rate of patients with metastatic cancer is also significantly lower than that of patients with localized cancer [1]. Metastasis is a process influenced by multiple factors, that is when normal cells mutate into carcinogenic cells, which then proliferate uncontrollably, inhibit programmed cell death, evade the immune system, and stimulate angiogenesis. Epithelial mesenchymal transformation (EMT) happened and increases its self-invasiveness, invades surrounding tissues and migrates to nearby blood vessels or lymphatic vessels, penetrating blood vessels and entering the circulatory system, Becoming a circulating tumor cell system (CTCs) [2]. Next, platelets will adhere to the surface of circulating tumor cells, and the secondary structure can reduce recognition and clearance by the immune system, helping tumor cells survive in the bloodstream. Next, circulating tumor cells undergo mesenchymal epithelial transformation (MET) and colonize the "pre metastatic foci" of distant organs (an inflammatory environment with immunosuppressive characteristics formed by the secretion of cytokines or exosomes in situ tumor tissue that is conducive to tumor cell colonization), continuing to establish cancerous growth in distant organs.

There are currently multiple mature treatment methods for primary cancer lesions, and their toxicity is usually lower than that of treatment for metastatic cancer patients. The existing treatment strategies usually only intervene at a certain stage of tumor metastasis, lacking effective treatment strategies for the overall process of tumor metastasis. Previously, the treatment method for metastatic cancer was generally systemic chemotherapy, but patients often experienced serious effects like organ failure and increased disease infection rates. The efficacy of this strategy is currently being questioned [3]. Meanwhile, because of the acquired resistance of metastatic tumors to existing medicines, clinical metastasis of tumors is still largely incurable, with only a few exceptions. The latest advances in cancer treatment have provided treatment methods for metastatic cancer that have minimal impact on other systems in the human body, including epigenomic modifications or drug conjugates. However,

the impact of the above treatment methods on metastatic cancer is not significant, and in most cases, the cancer will eventually recur. One of the main obstacles to researching and developing new treatment strategies is our insufficient understanding of tumor metastasis. Therefore, current research aims to gain a deeper understanding of the invasion, infiltration, exosmosis, circulation, or colonization processes and other regulatory factors in cancer metastasis, hoping to provide some help for the treatment of metastatic cancer. This article will provide a review of our current understanding of cancer metastasis, introducing the known processes and influencing factors during tumor metastasis, hoping to provide inspiration or assistance for the treatment of metastatic tumors in the future.

2. Tumor formation

Tumor is a non-hereditary genetic disease. It is abnormal proliferation caused by gene changes in cells under the influence of tumorigenic factors, resulting in the loss of normal regulation of their growth. About 80% of human tumors are caused by contact with external carcinogens, which can be divided into initiators, promoters, and complete carcinogens based on their functions; There are also endogenous factors, such as genetic factors, endocrine factors, immune factors, etc. Malignant tumors have the characteristics of immortality, migration, loss of contact inhibition, rapid growth, blurred boundaries with surrounding cells, and can metastasize to other parts of the body, disrupt normal organ structures, cause dysfunction of the body, and threaten life.

Cancer cells vary in size and morphology, usually larger than their source cells, with a faster growth rate and a significantly higher nuclear to cytoplasmic ratio than normal cells, up to 1:1. They may also exhibit megakaryosis, binucleation, or multinucleation. The chromosomes in the nucleus are aneuploidy, and some staining may be missing or increased. Abnormal changes in normal cell chromosomes can initiate the process of cell apoptosis. However, in cancer cells, the signaling pathways related to cell apoptosis are obstructed, making them immortal, just like microorganisms that parasitize within the cell. They are not controlled by normal growth regulatory systems and can continue to divide and proliferate. The mitochondria of tumor cells exhibit different polytypes, swelling, and proliferation; The cytoskeleton is disordered, some components are reduced, and the assembly of the cytoskeleton is abnormal; The surface characteristics of cells change, producing tumor related antigens.

The formation of malignant tumors is often related to the gradual accumulation of mutations in oncogenes and tumor suppressors. Proto oncogenes are related to cell proliferation and are essential for maintaining normal life activities in the body. Their gene sequences are highly conserved in evolution. When the structure or regulatory region of oncogenes undergoes mutations, gene products increase or activity increases, it may cause to excessive cell proliferation and the development of tumors. Protooncogenes and their products mainly include: (1) transcription factors such as MYC, FOS, JUN; (2) Protein kinases and other signal transduction components, like SRC, RAS, RAF; (3) Growth factor receptors, for example the FMS and the ERB-B; (4) Growth factors, such as SIS; (5) Cell cycle proteins, like BCL-1; (6) Cell apoptosis regulatory factors, such as BCL-2 [4].

The products of tumor suppressor genes inhibit cell proliferation, can help cells to differentiation, and can inhibit cell migration, that act as a negative regulation role. It is generally believed that mutations in tumor suppressor genes are recessive. The products of tumor suppressor genes mainly include: (1) transcription regulatory factors, like Rb and p53; (2) Negative regulatory transcription factors, such as WT; (3) DNA repair factors, like BRCA1 and BRCA2; (4) Inhibitors of signaling pathways, such as RAS-GTP-enzyme-activating protein (NF-1); (5) Cyclin dependent kinase inhibitor (CKI), p15, p16, p21 are examples; (6) Signal pathway components related to development and stem cell proliferation, such as APC, AXIN, etc.

When the oncogene is activated and the function of the tumor suppressor gene is lost, malignant tumors will occur. There are four main mechanisms for the activation of oncogenes: point mutation, chromosomal translocation (when chromosomal translocation or inversion occurs, the oncogene is

downstream of the active transcriptional gene strong promoter, which may lead to overexpression), gene amplification (specific genome amplifies to a normal 8-32 fold, commonly found in malignant tumors of the hematopoietic system), Obtaining strong promoters and enhancers (inserting certain components containing promoters and enhancers into the genome, resulting in gene activation and overexpression). Or it can lead to overexpression of genes or increase the activity of product proteins, leading to excessive cell proliferation and the formation of tumors.

Cancer suppressor genes can lose their cancer inhibitory function when mutated or lacking for certain reasons, while mutations in individual alleles cannot inhibit gene function. Only when two alleles undergo mutations simultaneously can the gene lose its normal anti-tumor function. There are three main pathways that can inactivate tumor suppressor genes: allelic recessive effect, dominant negative effect of tumor suppressor genes, and haploid deficiency hypothesis.

3. Angiogenesis

After the formation of cancer, the continuous division and proliferation of tumor cells consume a large amount of oxygen and nutrients. When the volume of a solid tumor is less than 2mm, oxygen and nutrients can be obtained through diffusion. As tumor tissue gradually grows, new blood vessels need to be formed to obtain nutrients and oxygen to ensure exponential growth of the tumor. The angiogenesis of tumors originates from existing capillaries or small veins behind capillaries in the tumor. Firstly, perivascular cells shed and blood vessels dilated; Next, endothelial cells migrate to the surrounding space of blood vessels and migrate towards the direction of angiogenesis stimulation generated by tumor cells or host cells; Then endothelial cells proliferate in the direction of angiogenesis, which may be guided by pericytes; endothelial cells adhere to each other and form lumens, while also forming the basement membrane and peripheral cell attachment. Finally, the granulation buds fuse with each other and build a new circulatory system (Fig 1).

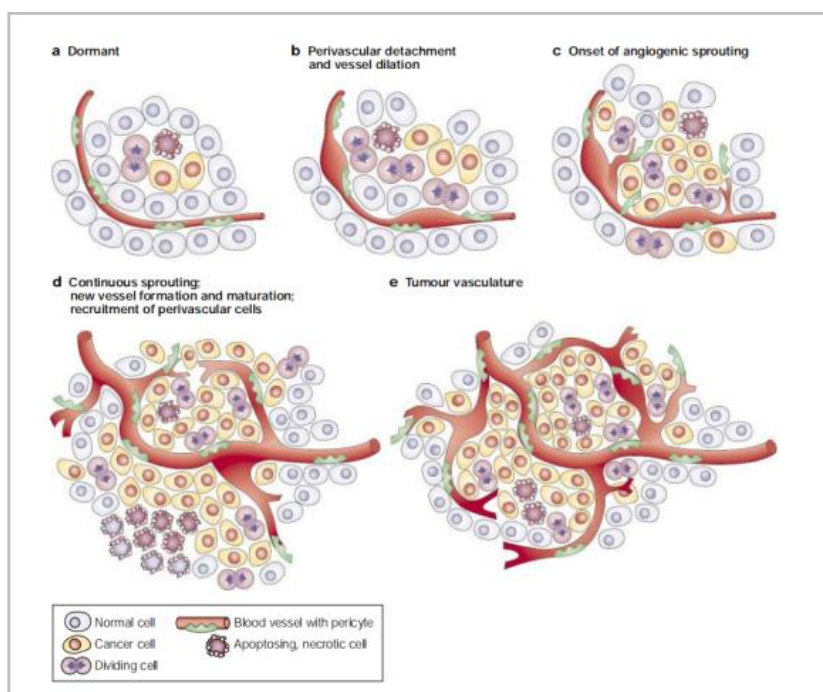


Figure 1. Angiogenesis process [5]

The factors have known to affect angiogenesis include: (1) Hypoxia inducible factor-1, HIF-1 under normoxic conditions α and HIF-2 α Hydroxylated by FIH-1 and PDH, and degraded through proteasome mediated degradation. Under hypoxia, FIH-1 and PHDs are inactivated, and HIF-1 expression is upregulated, which can directly promote the production of various angiogenic factors, cytokines, chemokines, and matrix metalloproteinases by tumor cells [6]. It acts on adjacent tissue endothelial cells, inducing their proliferation, migration, and maturation; (2) Angiogenic factor, the

most important factor affecting angiogenesis, can be secreted by tumor cells. The hypoxic microenvironment is the main regulatory factor for VEGF secretion. When VEGF increases, it can stimulate the upregulation of receptor expression in endothelial cells, maximizing the effect of VEGF. Anti angiogenic factors, such as thrombin-1 and endostatin, can inhibit the transcription factor HIF-1 and participate in the components of the extracellular matrix; (4) Extracellular matrix plays a role in supporting, nourishing cells, and providing an external environment for survival. It can combine with various growth factors to promote angiogenesis [7].

The main pathway for cancer cells to spread from primary cancer to other organs of creature's body is called angiogenesis, but they can also spread through lymphatic and peripheral pathways. In lymphatic diffusion, overexpression of VEGF-C or VEGF-D can promote the development of cancer related lymphatic vessels, thereby aiding tumor cell dissemination. The cells of primary cancer flow along with lymph nodes, metastasizing from near as far to various lymph nodes, and may also metastasize; Or reverse metastasis may occur due to cancer obstructing antegrade lymphatic drainage. These lymphatic dendritic cells recruit to tumors, forming an immunosuppressive environment. In perineural metastasis, tumor grow between nerve axons and the surrounding neural, and other organs like blood vessels or lymphatic vessels form a peripheral environment to initiate metastasis. The above methods can be carried out without angiogenesis, and the multiple transmission pathways of tumor cells make the targeting of this critical step in metastasis more complex [8].

4. Epithelial-mesenchymal transition

After angiogenesis, to successfully metastasize, tumor cells undergo epithelial mesenchymal transformation to obtain invasive and stem cell like characteristics, enabling them to enter the vascular system and survive. Epithelial mesenchymal transition refers to the biological process in which epithelial cells lose epithelial markers and acquire mesenchymal characteristics.

During EMT, the expression of epithelial-related genes, for example, E-cadherin, ZO-1, and occlusion decrease, while the expression levels of mesenchymal genes, such as vimentin, and fibronectin increase. The expression of cell adhesion molecules such as E-cadherin decreases, and its cytoskeleton changes from keratin to vimentin dominated, giving it both mesenchymal and epithelial morphological features. Through EMT, epithelial cells lose apical basal polarity and cell-cell connectivity, as well as epithelial phenotypes such as loss of connectivity to the basement membrane, resulting in higher mesenchymal phenotypes such as migration and invasion, resistance to apoptosis, and degradation of extracellular matrix (Fig 2).

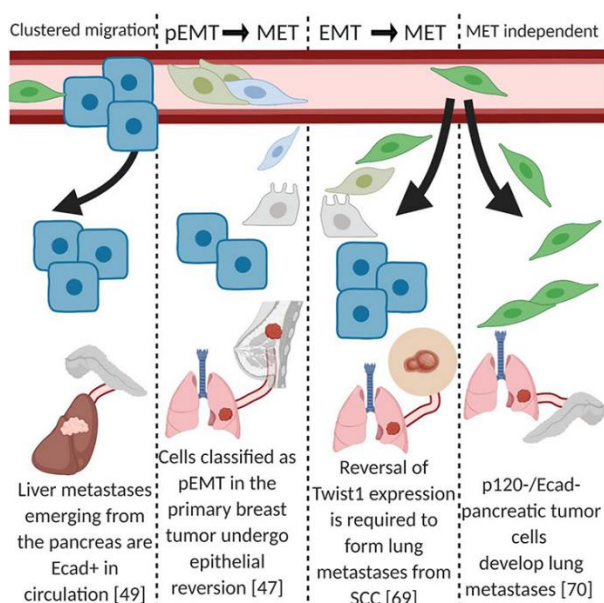


Figure 2. Primary tumor cells with extravasation and metastasis colonization

From many existing models, we can see that large metastatic tumors exhibit strong expression of epithelial markers (blue in the figure), so we can consider this as a condition for the expansion of the metastatic site. Research has also found that cells that previously received EMT restored epithelial markers and lost mesenchymal features (green). In a mixed population of metastatic cells, epithelial cell clusters may be the only or main group capable of expansion and metastasis [9].

Compared with other EMTs *in vivo*, EMTs in tumor development mainly have five signaling pathways: the tyrosine kinase receptor, Wnts, integrin, NF- κ B, and TGF- β pathway, and also with some specific transcription factors like Snail factor (snail 1 and snail 2) and Twist factor [10].

EMT is an indispensable step in most tumor metastasis processes, therefore, diagnostic methods based on EMT key molecules and therapeutic methods targeting EMT key molecules are key to the study of EMT mechanisms in tumor metastasis. However, with the continuous expansion of the field of modern molecular mechanism research, it has been found that there are more and more complex biological mechanisms that can regulate epithelial mesenchymal transition. This also leads to low sensitivity of intervention strategies specifically targeting EMT, but on the other hand, it provides more options for intervention in EMT.

Due to the widespread presence of EMT as a biological process in organisms and the extremely difficult monitoring of the entire process of EMT in tumor cells, targeted therapeutic drugs still need further research. Although some of these drugs have been proven to effectively improve chemotherapy efficacy, the side effects of EMT inhibitors on biological processes are still an issue that cannot be ignored. Small molecule inhibitors (TGFB) have shown serious harm to the heart in clinical trials, with only few types of small molecule inhibitors, like the galunisertib, allowed to enter clinical research. Right now, the consequences of using EMT inhibitors in long term are still unclear. Of particular importance, if EMT inhibitors begin to promote the MET process, they may further accelerate tumor metastasis.

5. Invasion

The infiltration of tumor cells into blood vessels or lymphatic vessels is a necessary process that triggers tumor cell escape from the primary lesion and distant metastasis. Before undergoing infiltration, the tumor will also undergo a stage of interaction that alters the adhesion between cells and matrix: invasion (infiltration). Tumor invasion refers to the extension and radiation growth of tumor cells towards the surrounding area, without a complete envelope, and causing damage to surrounding tissues and organs. It is a phenomenon of abnormal distribution of tumor cells in tissue gaps, including a series of processes such as tumor cell adhesion and breakthrough of the basement membrane, as well as movement and proliferation within the matrix. The types of infiltration are generally divided into four types: (1) tissue infiltration, which refers to direct dissemination or direct spread. Tumor cells proliferate in the surrounding stroma to form irregular masses, which can further invade adjacent lymphatic vessels, blood vessels, or infiltrate along the gaps around nerves; (2) Vascular infiltration refers to the phenomenon of tumor cells invading local blood vessels or small veins and spreading along the blood vessels. Some may form tumor thrombi, which can lead to hematogenous metastasis of the tumor after detachment; (3) Lymphatic infiltration refers to the growth and spread of tumor cells along local lymphatic vessels, which can lead to lymphatic metastasis; (4) Serous membrane and mucosal tendrils refer to the continuous growth of tumor cells on the serous membrane and mucosal surface after infiltration along the serous membrane and submucosal space. And it can be divided into single cell migration and group migration, among which collective migration seems to be more invasive and metastatic than single cell dissemination, and may also prevent chemotherapy intervention [11].

The newly formed vascular malformations, proliferation, excessive branching, and high permeability and leakage in tumors allow tumor cells to escape from the primary site and enter the human circulatory system. There are two types of infiltration: (1) in the passive infiltration, cells shed cell apoptosis due to reduced nutrient supply caused by the hypoxic environment and vascular system

leakage of the tumor; (2) During the process of active infiltration, cells will migrate towards blood vessels through the growth factor and nutrient gradients due to the regulation of biological factors. These cells lack ECM and basement membrane and actively penetrate into blood vessels [12].

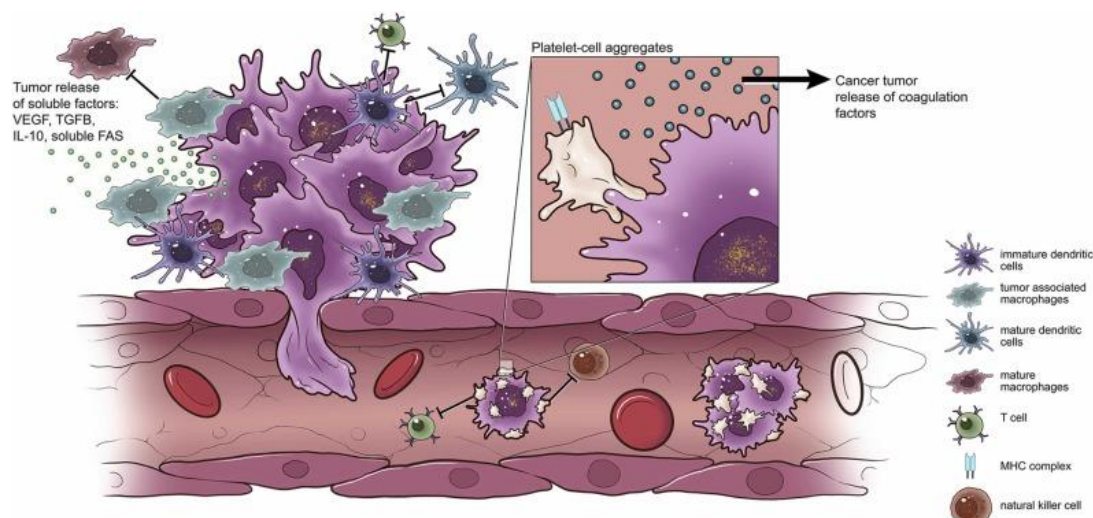


Figure 3. Invasion [7]

After entering the vascular system, they are often disrupted by shear stress or immune monitoring (Fig 3), so the likelihood of tumor cells leaving the primary tumor successfully colonizing the anterior metastatic focus of the distal organ is low. Cancer cells have multiple mechanisms to evade damage to the immune system. Tumor cells release soluble factors like VEGF, IL-10 and Fas contribute to the formation of an immunosuppressive environment. Also, in the circulatory system, these cancer cells bind to platelets, forming a microtubule structure that prevents them from being invaded by natural killer cells or T cells, as well as allowing tumor cells to bear the mechanical force [2]. The epithelial-mesenchymal transition induction within these CTCs make intermediate filaments can recombine to withstand this force [13].

6. Extravasation

To complete tumor metastasis, cancer cells also need to undergo an extravasation process. During this process, tumor cells undergo MET to restore the characteristics of epithelial cells. They then migrate to the endothelial cells to the site of metastasis, regulate their own endothelial barrier, then migrate to the lower tissue of the organ through the endothelium. Most kind for extravasation is pericellular migration, where cancer cells move between endothelial cells. During the extravasation process, many ligands and receptors (such as cadherin and integrin) help cancer cells adhere to endothelial cells. The degree of extravasation and its success also depend on the interactions between tumor cells and cells in the circulatory system, including platelets, MDSC, and TAM [14].

After tumor cells colonize the anterior metastatic lesion of the distal organ, they will form an inflammatory environment with immunosuppressive characteristics that is conducive to tumor cell colonization under the action of cytokines or exosomes secreted by the in-situ tumor tissue, namely the tumor microenvironment (TME). Its composition and selectivity are crucial for the growth and progression of tumors [15]. The main components of TME are innate and adaptive immune system components, as well as stromal cells: they originate from macrophages in tissues and bone marrow, polarized into cancer related components such as immunosuppressive TAM, T cells, NK cells, blood vessels, lymphatic vessels, etc. The situation of immune cells in the tumor microenvironment and the expression of immune regulatory receptors are further suppressed in metastatic tumors, enabling further tumor development [16].

7. Conclusion

All in all, metastasis of tumors depends on multiple steps and stages, which enable them to withstand constantly changing conditions, EMT, cellular stress, survival outside tissue microbiota, diffusion, immune evasion, and TME co selection, ultimately achieving colonization of other organs. With the continuous development of complex preclinical and in vitro models such as single cell profiling and lineage tracking, the goal now is to safely assess the transfer dependence of patient cohorts targeting heterogeneous patients or biomarkers. Significant progress in transfer research and clinical drug development is expected to improve the limitations and clinical treatment of current metastatic cancer patients. This article aims to summarize and organize the entire process of tumor metastasis, enhance public awareness of tumor related issues, and provide inspiration and assistance for tumor related research.

References

- [1] Mattiuzzi C, Lippi G. Current Cancer Epidemiology. *J Epidemiol Glob Health*. 2019; 9 (4): 217-222.
- [2] Pereira-Veiga T, Schneegans S, Pantel K, Wikman H. Circulating tumor cell-blood cell crosstalk: Biology and clinical relevance. *Cell Rep*. 2022; 40 (9): 111298.
- [3] Vinod SK, Hau E. Radiotherapy treatment for lung cancer: Current status and future directions. *Respirology*. 2020; 25 Suppl 2: 61-71.
- [4] Dhanasekaran R, Deutzmann A, Mahauad-Fernandez WD, Hansen AS, Gouw AM, Felsher DW. The MYC oncogene - the grand orchestrator of cancer growth and immune evasion. *Nat Rev Clin Oncol*. 2022; 19 (1): 23-36.
- [5] Aibixin, Mechanism and Research of Tumor Angiogenesis, 2022-05-162023-09-15, <https://baijiahao.baidu.com/s?id=1732950875824503323&wfr=spider&for=pc>.
- [6] Bhat SM, Badiger VA, Vasishta S, Chakraborty J, Prasad S, Ghosh S, Joshi MB. 3D tumor angiogenesis models: recent advances and challenges. *J Cancer Res Clin Oncol*. 2021 Dec; 147 (12): 3477-3494.
- [7] Castaneda M, den Hollander P, Kuburich NA, Rosen JM, Mani SA. Mechanisms of cancer metastasis. *Semin Cancer Biol*. 2022; 87: 17-31.
- [8] Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell Mol Life Sci*. 2020; 77 (9): 1745-1770.
- [9] Bakir B, Chiarella AM, Pitarresi JR, Rustgi AK. EMT, MET, Plasticity, and Tumor Metastasis. *Trends Cell Biol*. 2020 Oct; 30 (10): 764-776.
- [10] Huang Y, Hong W, Wei X. The molecular mechanisms and therapeutic strategies of EMT in tumor progression and metastasis. *J Hematol Oncol*. 2022; 15(1): 129. Published 2022 Sep 8.
- [11] Liu Q, Ma Z, Cao Q, et al. Perineural invasion-associated biomarkers for tumor development. *Biomed Pharmacother*. 2022; 155: 113691.
- [12] Chiang SP, Cabrera RM, Segall JE. Tumor cell intravasation. *Am J Physiol Cell Physiol*. 2016; 311 (1): C1-C14.
- [13] Lin D, Shen L, Luo M, et al. Circulating tumor cells: biology and clinical significance. *Signal Transduct Target Ther*. 2021; 6 (1): 404. Published 2021 Nov 22.
- [14] Kim S, Wan Z, Jeon JS, Kamm RD. Microfluidic vascular models of tumor cell extravasation. *Front Oncol*. 2022; 12: 1052192. Published 2022 Nov 11.
- [15] Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med*. 2013; 19 (11): 1423-1437.
- [16] Elia I, Haigis MC. Metabolites and the tumour microenvironment: from cellular mechanisms to systemic metabolism. *Nat Metab*. 2021; 3 (1): 21-32.