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Research progress on the correlation between platelet aggregation and tumor progression

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Summary. Platelets are generally considered as the main functional unit of the coagulation system. However, more and more studies have confirmed that platelets also have an important relationship with tumor progression. Tumor cells can utilize platelets to promote their own infiltration and hematogenous metastasis, and platelets are activated and aggregated in this process. Therefore, platelet aggregation may be a concomitant marker of tumor progression. This is of great significance for predicting tumor metastasis before timely treatments.

Key words: Platelet, Aggregation, Tumor progression, Metastasis, Growth, Prediction, Molecular mechanism

Platelets promote tumor metastasis

Platelets are small, nuclei-free cytoplasmic components separated from the cytoplasm of mature megakaryocytes in bone marrow. They have endoplasmic reticulum, Golgi apparatus, mitochondria and dense granular materials, and can translate mRNA into proteins. Platelets have many functions, such as adhesion, aggregation and release. In the past, it was believed that platelets were mainly involved in blood coagulation. Later, it was found that platelets also play important roles in immunity, signal regulation and tumor metastasis. In 1865, the phenomenon that tumor cells can cause platelet aggregation was discovered. For example, platelets are normally distributed in normal ileum and colon, while platelets gather along the intestinal epithelium in adenocarcinoma (Cariello et al., 2021). This phenomenon is called tumor cell-induced platelet aggregation (TCIPA). TCIPA has attracted the attention of researchers, who have found that platelets

play important roles in tumor metastasis.

The mechanism of tumor metastasis has been widely studied. Its main steps include infiltration, immune evasion, and exosmosis (Massagué and Obenauf, 2016).

During infiltration, tumors grow and release tumor cells that pass through interstitial connective tissues, penetrate vascular basement membrane, and enter blood circulation directly or through the lymphatic system, thus becoming circulating tumor cells (CTC) (Chaffer and Weinberg, 2011). This process includes several important changes. The first change is epithelial mesenchymal transformation (EMT) (Thiery et al., 2009). EMT refers to the process in which polar epithelial cells are transformed into active interstitial cells with free movement within the cell matrix. At the same time, the extracellular matrix is also decomposed by matrix metalloproteinases (MMP) secreted by tumor cells (Kessenbrock et al., 2010). This process enables tumor cells to infiltrate. The second change is that tumor cells adhere to the basement membrane of vascular endothelium, enhancing endothelial permeability.

Regarding immune evasion, CTCs lose the protection of the tumor microenvironment in the blood and most CTCs can be cleared by attacks from the immune system. Only a few CTCs can survive these attacks, which is called immune evasion.

For exosmosis, tumor cells enter blood vessels, quickly pass through the circulation, and stagnate in the vascular system of secondary organs. This process usually takes only a few minutes (Plantureux et al., 2020). It is considered that the mechanical capture that involves platelets is the main mechanism of CTC stagnation in the vascular system (Massagué and Obenauf, 2016). Then, CTCs leave the blood vessel and transfers to target organs. Before this, tumor cells secrete specific factors and exosomes to establish a microenvironment suitable for tumor cell growth in distant metastasis target organs, which is called pre-metastatic niche (Kaplan et al., 2006).

A variety of studies have shown that platelets play important roles in tumor metastasis. They are involved in tumor metastasis in the following aspects:

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i) Platelets participate in tumor angiogenesis, which is involved in the whole process of tumor formation, growth, and metastasis. Neovascularization can provide nutrition and oxygen for tumor growth and metastasis, and avoid apoptosis induced by hypoxia. Platelets can secrete both anti-angiogenic factors such as 5-hydroxytryptamine and platelet reactive protein-1 and angiogenic factors such as VEGF, EGF, and PDGFRs. Under normal circumstances, there is a balance between angiogenic factors and anti-angiogenic factors, and among multiple signal pathways. Of note, tumor cells can control the balance between angiogenic factors and anti-angiogenic factors to shape tumor blood vessels (Manzat Saplacan et al., 2017).

ii) Platelets help tumor cells adhere to blood vessels. In the capillary network, the diameter of tumor cells is larger than that of capillaries, so they stagnate; in vessels with larger diameter, tumor cells can still adhere to platelets and vascular endothelium through P-selectin (Kim et al., 1999; Cariello et al., 2021). Later, platelets can also expand the endothelial cell gaps, and tumor cells are easier to spread through the endothelium. It is reported that the direct interaction between cancer cells and platelets will promote platelets to release ATP, making it easier for cancer cells to cross the vascular barrier (Schumacher et al., 2013).

iii) CTCs in the blood are easily damaged by the rheological shear stress of the blood, while platelets, as the adhesion medium, make tumor cells and other cells form cell clusters that provide a physical barrier. Tumor cell aggregation and adhesion to other cell types contribute to the successful survival of tumor cells in circulation (Egan et al., 2014). In the meantime, platelet aggregation can slow down blood flow and weaken rheological shear stress.

iv) It is believed that platelets transfer their own major histocompatibility complex I to the surface of tumor cells, which can thus avoid the killing by natural killer cells (Placke et al., 2012). In addition, *in vitro* experiments show that platelet-derived TGF-β down-regulates the activated immune receptor NKG2D on natural killer cells (Kopp et al., 2009). Platelet-derived growth factor released by platelets can also inhibit natural killer cell function (Gersuk et al., 1991; Manzat Saplacan et al., 2017). This is also a possible mechanism by which platelets help tumor cells achieve immune evasion.

v) Platelets can induce and maintain the EMT phenotype of tumor cells in a variety of cancers, which is characterized by E-cadherin expression decrease and morphological changes, as well as metabolic changes to adapt to the metastatic environment (Spillane et al., 2021). TGF-β secreted by platelets plays important roles in this process (Spillane et al., 2021).

vi) Platelets participate in the composition of pre-metastatic niche. Labelle et al. revealed how platelets help colorectal cancer metastasize to the lungs after injecting mouse colon cancer MC38GFP cells into mice, using immunofluorescence staining and cytokine determination of lung sections (Labelle et al., 2014). Their results show that platelets gathered around the tumor cells only one minute after the tumor cells entered the blood circulation, and then the tumor cell clusters stagnated in the pulmonary capillary network. After 30 minutes, granulocytes were recruited by CXCL5 and CXCL7 chemokines secreted by platelets and promoted the survival and metastasis of CTCs (Labelle et al., 2014). Gil-Bernabé et al. demonstrated that platelets activated by CTC tissue factor can also recruit monocytes / macrophages, which are a necessary condition for the construction of pre-metastatic niche and the survival of CTCs (Gil-Bernabé et al., 2012).

vii) Progress in colon cancer research: *in vitro* and animal models. Many studies have shown both correlation and lack of correlation of tumor progression with *in vitro* and animal models. Simulating human colon cancer has long been a hot topic for researchers and oncologists, with the goal of better replicating disease progression and treatment responses. Lannagan et al. have made the following progress in research on genetics, stem cell biology, tumor microenvironment, and immunology: use of patient-derived organoids (isolated and co-cultured) as predictors of treatment responses; molecular stratification of tumor to predict results and treatment responses; mouse model of metastatic disease; transplantation models that can be used to reduce the risk of clinical trials (Lannagan et al., 2021).

viii) Three-dimensional gastrointestinal model and organoid study on metabolism of human colon cancer. Recent advances in the field of tumor metabolism have raised awareness of the importance of the tumor microenvironment in tumor growth and progression. Heinrich Warburg's original theory is that cancer cells lack oxidative respiration, and therefore have to undergo aerobic glycolysis to generate energy. However, further research has shown that there is metabolic repro-gramming in the tumor microenvironment, controlled by communication between the tumor and stromal cells. The importance of this communication exposes the need to use complex models in cancer research. Although these models have contributed to many discoveries, they also have many limitations. Improved models are currently being developed using 3D cell culture technology. These models are more physiologically relevant, allowing different types of cells to co-culture and establishing gradient concentrations of solutes. The recent development of organ-like technology has greatly promoted the development of 3D cell technology. Organoids can develop from different tissues, including tumors, representing the spatial organization of cell populations and originating tissues. In the field of cancer

metabolism, interactions between different cell types, matrices, and different solutes and oxygen concentrations are crucial parameters. Current models for studying metabolism either include only one cell population, cannot represent solute/oxygen gradients, or cannot skillfully collect samples. Therefore, the characteristics of organ-like culture make it a powerful model for metabolic research, drug development, disease modeling, and even personalized medicine (Silva-Almeida et al., 2020).

ix) Exocrine miRNA confers chemoresistance in ovarian cancer by targeting the Cav1/p-gp/M2-type macrophage axis. Many *in vitro* functional assays have been conducted in ovarian cancer cells and the therapeutic effects of miR-1246 inhibitors have been tested in animal models of ovarian cancer. The results show that the direct target gene Cav1 of miR-1246 is involved in the exocrine transfer process (Kanlikilicer et al., 2018).

Interaction mechanism between tumor cells and platelets, as well as the related molecules

Mechanism of platelet activation and aggregation induced by tumor cells

Tumor cells can induce platelet activation and aggregation in the circulatory system not only by direct contact, but also by secreting signal molecules into the circulatory system. Integrin αIIbβ3 (or GPIIb/IIIa) is a classical pathway by which tumor cells directly induce platelets (Amirkhosravi et al., 2003). It is reported that platelet aggregation and tumor metastasis can be reduced by oral intake of GPIIb/IIIa inhibitors (Blair and Frelinger, 2020). Other platelet membrane glycoproteins such as GPIb are also involved in this process (Oleksowicz et al., 1995). P-selectin is a class of cell adhesion molecules on the surface of platelets, and tumor cells bind to P-selectin ligands on the platelet surface to induce platelet activation and aggregation (Egan et al., 2011; Qi et al., 2015). Tsuruo et al. found that a transmembrane sialic mucin-like glycoprotein Aggrus is expressed on the surface of tumor cells and can cause platelet aggregation, and discovered by comparing NL-14, NL-17 and NL-44 in mouse colon cancer cell lines that the ability of tumor cells to induce platelet aggregation is positively correlated with the metastatic potential (Tsuruo and Fujita, 2008). The specific mechanism is binding to platelet surface receptor CLEC-2, which promotes megakaryocyte growth and pre-platelet production, and drives platelet activation (Tamura et al., 2016).

Ovarian cancer cells can secrete ADP and TXA2, promote platelet activation and recruit them to participate in TCIPA (Egan et al., 2011). Tissue factors exposed by small extracellular vesicles shed by tumor cells can also initiate exogenous coagulation cascade, leading to local production of thrombin and inducing platelet activation and aggregation (Borsig et al., 2001).

The induction process often depends on multiple pathways at the same time. A study using Caco-2 colorectal cancer cell line showed that tumor cells can induce platelet aggregation. Besides direct contact mediated by the binding between fibrinogen and integrin αIIbβ3, tumor cells can also produce a small amount of thrombin, which can indirectly lead to platelet activation and aggregation (Zarà et al., 2018). TCIPA process induced by MCF-7 may be dependent on ADP, GPIIb/IIIa and GPIb-IX (negative regulation) activation pathways at the same time (Oleksowicz et al., 1995).

Tumor cells have the ability to induce platelet activation and aggregation. This has been proved to be involved in the progression of several types of cancer, such as lung cancer, colon cancer, breast cancer, pancreatic cancer, ovarian cancer and brain cancer. In this process, platelets protect circulating tumor cells from the harmful effects of shear forces, protect tumor cells from the invasion of the immune system, and provide growth factors to promote the metastasis, diffusion, and growth of tumors at the primary and metastatic sites. Here, we present a broader perspective on platelet aggregation induced by specific factors that primarily contribute to cancer development, including coagulation factors, adhesion receptors, growth factors, cysteine proteases, matrix metalloproteinases, glycoproteins, soluble mediators, and selectins. These factors may exist on the surface of tumor cells and in the microenvironment of tumor cells, and some of them may trigger more than one simple receptor-ligand mechanisms such as p-selectin. Strasenburg et al. briefly discussed the physiological role of these factors in platelet activation, followed by providing scientific evidence and discussing their potential role in specific cancer progression. Targeting tumor cells with antiplatelet drugs to induce platelet aggregation (TCIPA) may open the way for the development of new therapeutic methods. On the one hand, it may affect the prognosis of patients by enhancing known therapies for advanced tumors. On the other hand, the use of drugs that are easily available and widely used in general practice may provide an unparalleled opportunity for tumor prevention (Strasenburg et al., 2022).

There is a correlation between the ability of tumor cells to aggregate platelets and their tendency to metastasize. TCIPA promotes tumor cells to embolize blood vessels and form metastatic foci. Matrix metalloproteinases (MMPs) play an indispensable role in the cascade of tumor proliferation and metastasis. Therefore, Juraz et al. studied the role of MMPs in the TCIPA process and the regulation of nitric oxide using *in vitro* experiments. Human HT-1080 fibrosarcoma and A549 lung epithelial cancer cells induce TCIPA in a concentration dependent manner, which is monitored by aggregates. This aggregation leads to the release of MMIP-2 from platelets and cancer cells, which is measured by zymogram (Jurasz et al., 2001).

Cancer-related RNA biomarkers EGFRVIII and PCA3 are detected in the platelets of patients with

glioma and prostate cancer, respectively. Furthermore, the cancer cell-specific fusion gene EML4-ALK is detected in the platelets of patients with lung cancer. Therefore, the concept of tumor cell-educated platelets (TEPs) is proposed, which means that tumor cells transport mRNA to platelets in the form of exosomes and guide their expression (Tjon-Kon-Fat et al., 2018). Platelets have long been known to play important roles beyond hemostasis and thrombosis. Now recognized as a bona fide mediator of malignant disease, platelets influence various aspects of cancer progression, most notably tumor cell metastasis. Interestingly, platelets isolated from cancer patients often display distinct RNA and protein profiles, with no clear alterations in hemostatic activity.

This phenotypically distinct population, termed tumor-educated platelets, now receive significant attention for their potential use as a readily available liquid biopsy for early cancer detection. Although the mechanisms underpinning platelet education are still being defined, direct uptake and storage of tumor-derived factors, signal-dependent changes in platelet RNA processing, and differential platelet production by tumor-educated megakaryocytes are the most prominent scenarios (Roweth and Battinelli, 2021). It further reveals the mechanism of platelet activation and aggregation induced by tumor cells at the level of gene expression (Table 1). TEPs have the same biological functions as normal platelets, and can be used for the earlier detection of hepatocellular carcinoma (Best and Wurdinger, 2020).

High frequency CD8 T cell-platelet aggregates are associated with T cell suppression in patients with myeloproliferative tumors. Activated platelet (act-PLT) can release a wide range of molecules, promote tumor cell proliferation and maintain tumor integrity. It is reported that transforming growth factor-β (TGF-β) released from platelet and lactate can inhibit T cell function and promote drug resistance of mouse models to adoptive T cell therapy (Carnaz Simões et al., 2022).

Tumor-related platelets as a non-invasive biomarker source for cancer detection and progression monitoring. As a non-invasive method for detecting and monitoring diseases, liquid biopsy represents a potential revolution in cancer diagnosis, which can supplement or even replace current tissue biopsy methods. Some blood-based biological sources and biomolecules, such as cellular free DNA and RNA, proteins, circulating tumor cells, and extracellular vesicles, have been explored for molecular testing development. TEPs are involved in the progression and spread of several solid tumors, and spliced TEP RNA proxy markers can provide specific information about the presence, location, and molecular characteristics of cancers. So far, TEP samples from patients with different tumor types (including lung cancer, brain cancer and breast cancer) have been tested. The results show that TEPs in cancer patients are different from those in patients with inflammation and other non-cancer diseases. How platelets are "educated", what mechanisms lead to RNA splicing within platelets, and whether the relative contribution of specific platelet subsets in cancer patients has changed remain to be studied. Ultimately, TEP RNA may complement the biological sources and biomolecules currently used for liquid biopsy diagnosis, potentially enhancing the detection of early cancer, and promoting noninvasive disease monitoring (Best et al., 2018).

Mechanism of platelet reaction on tumor cells

Researchers finding that the relationship between tumor cells and platelets is not one-way, put forward the corresponding concept of platelet-educated tumor cell, and point out that platelets can also react on tumor cells in the following ways. First, platelets release their content α granules into tumor cells (Plantureux et al., 2020). For example, platelets secrete cytokine Gas6, which regulates the phosphorylation of signal transducer and activator of transcription (STAT6) in tumor cells through Mer receptor (Ren et al., 2021). It is

Table 1. TCIPA-associated molecules and their mechanisms of action.

Molecules	Mechanisms	Distribution
Aggrus-CLEC-2	Promotes megakaryocyte growth and pro-platelet production	Tumor cell membrane surface protein, platelet membrane surface protein
GPIIb/IIIa	Exposure of fibrinogen receptor after activation	Resting platelet open tubule, activated platelet membrane surface
GPIb-IX	Containing sialic acid that makes platelets repel each other	Resting platelet membrane surface; transfer to open tubule system upon activation
ADP	Regulation of the secretion of open tubule system and dense granules through Ca2+ signaling pathway	Tumor cells or induced platelet release
TXA2	Act on platelet TXA2 receptor	Tumor cell and activated platelet secretion
MMPs	Tumor diffusion and metastasis cascade	Tumor cell secretion; aggregation of platelets
P-selectin (CD62P)	Ligands bind to tumor cells to activate platelets	Platelet intimal sugar particles are activated and fused to the platelet membrane through an open conduit system

TCIPA, tumor cell-induced platelet aggregation; CLEC-2, C-type lectin-like receptor 2; GPIIb/IIIa, glycoprotein IIb/IIIa complex; GPIb-IX, glycoprotein Ib-IX complex; ADP, adenosine diphosphate; TXA2, thromboxane A2; MMPs, matrix metalloproteinases.

demonstrated that knockout of Gas6 gene or Mer gene can inhibit STAT6 phosphorylation (Ren et al., 2021). STAT6 has been shown to be associated with tumor cell apoptosis and growth in non-small cell lung cancer (Linger et al., 2013). Second, transfer of adhesion proteins such as β3-integrin subunits is dependent on microbubbles. Third, platelets can induce the expression or inhibition of different RNAs in cancer cells (Dovizio et al., 2013; Tjon-Kon-Fat et al., 2018). These studies suggest that the interaction between platelets and tumors and the interwoven reticular regulatory structures play important roles in tumor metastasis.

TGF-β secreted by platelets down-regulates the activity of natural killer cell surface active receptor NKG2D, thereby weakening its scavenging effect on tumor cells (Lazarova and Steinle, 2019). Macrophages can promote metastasis of tumor cells and kill tumor cells. Platelets regulate the transcription level of macrophages through miR-126-3p, and can make macrophages not release lysosomal enzymes after phagocytizing tumor cells, but carry tumor cells for metastasis (Joshi et al., 2014).

Platelets have organ specificity in promoting metastasis

Coupland et al. discovered that the effect of platelets on lung metastasis is significantly stronger than that of liver metastasis in a mouse B16F1 melanoma model and 4T1.2 breast cancer model (Coupland et al., 2012). The explanation is that in the lungs, platelets play important roles in anchoring tumor cells to the endothelium against relatively high intrinsic blood flow velocity. By contrast, in the liver, due to the relatively slow blood flow in hepatic vessels, the interaction between endothelium and tumor cell adhesion protein is enough to prevent tumor cells from making subsequent extravasation, and its dependence on platelets is low. Another comparison of mouse heterotopic tumor implantation models constructed from 9 human tumor cell lines proves this conclusion again (Pang et al., 2015).

Research progress of platelets on tumor proliferation

The role of platelets in promoting tumor metastasis has become a consensus, but there is no conclusion on the role of platelets in tumor growth. An *in vitro* study of pancreatic cancer indicates that inhibition of platelet activation by clopidogrel hinders tumor growth (Mezouar et al., 2015). Consistently, another study on pancreatic tumors using mouse models also points out that platelets can infiltrate into the tumor micro-environment from tumor blood vessels, and adhere to tumor cells under the mediation by P-selectin to promote proliferation (Qi et al., 2015). However, a new study shows an opposite conclusion using a mouse colorectal cancer model (Plantureux et al., 2020). This study using a mouse colorectal cancer model suggests that platelets in tumor blood vessels account for about 80%, while

platelets in tumor microenvironment account for about 20%, and they were mainly distributed around the tumor rather than in the tumor center (Plantureux et al., 2020). The study further shows that platelets outside the tumor blood vessels only adhere to the tumor cells one by one and do not aggregate. Therefore, this does not belong to the category of TCIPA, and depends on cadherin-6 rather than classical integrin αIIbβ3-fibrinogen platelet aggregation pathway (Plantureux et al., 2020). The way by which platelets inhibit the proliferation of tumor cells may be that the particles secreted by platelets include CXCL12, CCL2, IFN-γ and IL-4 cytokines, which recruit macrophages to initiate tumor cell P21-mediated apoptosis. Whether platelets have a unified effect on tumor growth or change with tumor types, and their specific pathway of action still need more studies to draw conclusions.

Progress in related clinical application and research

Based on the above conclusions, it is a possible research direction to detect platelet-related indicators to predict tumor prognosis. In a study on 783 patients with colorectal cancer, 463 patients with colorectal adenoma and 689 healthy subjects, how colorectal cancer is related with the indicators such as platelet count (PC), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT) was investigated (Zhu et al., 2018). The results show that MPV and PCT levels are related to vascular invasion, rather than metastasis. The study excluded the influence of gender and dealt with the influence of age simply. The patients were only divided into two groups: over 60 years old and under 60 years old (Zhu et al., 2018). Another research group has also studied the correlation between colorectal cancer and platelet indexes such as platelet count, MPV, PDW and large platelet ratio, which has certain significance in the diagnosis, staging and prognosis of colorectal cancer (Copija et al., 2020).

There have been many basic studies on the relationship between platelet aggregation and tumor metastasis, but the index of platelet aggregation rate has not been directly used to predict cancer metastasis. In addition to the traditional platelet aggregation-based testing technologies, a new technique using TEPs mRNA as a tumor marker has been proposed, and it performs gene sequencing of tumor cell exosomes or platelet particles in circulating blood (Best et al., 2015). But theoretically, the effect of platelets on tumors is mainly manifested in the metastatic stage, so the expected result of this technology should be the ability to distinguish non-metastatic tumors from metastatic tumors, rather than the ability to diagnose tumors at all stages. Another study that uses integrin αIIb mRNA as a tumor marker concludes that the level of TEPs mRNA has no correlation with the stage of non-small cell lung cancer (Xing et al., 2019). However, integrin αIIb is an important surface antigen for platelet aggregation. Nevertheless, the researchers put forward a conjecture

that only mRNA is overexpressed in the sample, and protein synthesis has not yet started, so tumor metastasis is still in a lagging state, and correlation cannot be observed. This explanation confirms the predictive value of TEPs mRNA technology, which still needs further confirmation by patient follow-ups.

Until recently, it was believed that the nucleic acid content of platelets was entirely determined by their progenitor megakaryocytes. However, it is now clear that other mediators, such as cancer cells, can interfere to affect the RNA pool of platelets. Platelets are highly dynamic cells that communicate and affect the environment. For example, platelets participate in various stages of cancer development and progression by supporting tumor growth, survival, and proliferation. Cancer cells can directly and/or indirectly affect platelet RNA content, leading to educated platelets that are mediated by tumors. Changes in tumor induced platelet RNA profiles have been described as a new source of potential biomarkers. Single platelet RNA biomarkers and complex RNA markers can be used for early detection and treatment monitoring of cancers. Platelet and tumor-related RNA transfer elucidated the potential use of platelet RNA biomarkers as biological sources for liquid biopsy, and showed methods for evaluating platelet transcriptome content (D'Ambrosi et al., 2021).

There is increasing evidence that platelets play an important role in cancer metastasis. The interaction between platelets and circulating tumor cells (CTCs) promotes tumor metastasis. CTCs induce platelet activation and aggregation, which aggregates and protects CTCs from shear stress and natural killer cells. Finally, platelets stimulate CTC resistance, epithelial cells transition to mesenchymal cells, angiogenesis, exosmosis, and ultimately metastasis. Cell adhesion molecules (CAMs) have been identified as active participants in the interaction between CTC and platelets, but the specific mechanism of the contribution of platelet-related CAMs to CTC metastasis is unclear (Liu et al., 2021). Compared with the commonly used tumor markers, platelet detection has the advantages of low cost, non-invasiveness, and convenience. However, it is still unclear which parameters can better reflect the functional status of platelets. From the action of platelets in the process of tumor metastasis, platelet aggregation rate is a potential indicator that has not been fully studied. According to the "organ specificity" theory proposed by some studies, it may be necessary to take the tumor type and its metastatic organs into consideration for predicting its performance.

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