REVIEW

Manipulation of immune‒vascular crosstalk: new strategies towards cancer treatment

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Abstract Tumor vasculature is characterized by aberrant structure and function, resulting in immune suppressive profiles of tumor microenvironment through limiting immune cell infiltration into tumors, endogenous immune surveillance and immune cell function. Vascular normalization as a novel therapeutic strategy tends to prune some of the immature blood vessels and fortify the structure and function of the remaining vessels, thus improving immune stimulation and the efficacy of immunotherapy. Interestingly, the presence of “immune‒vascular crosstalk” enables the formation of a positive feedback loop between vascular normalization and immune reprogramming, providing the possibility to develop new cancer therapeutic strategies. The applications of nanomedicine in vascular-targeting therapy in cancer have gained increasing attention due to its specific physical and chemical properties. Here, we reviewed the recent advances of effective routes, especially nanomedicine, for normalizing tumor vasculature. We also summarized the development of enhancing nanoparticle-based anticancer drug delivery via the employment of transcytosis and mimicking immune cell extravasation. This review explores the potential to optimize nanomedicine-based therapeutic strategies as an alternative option for cancer treatment.

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1. Introduction

Tumor vascularization plays an important role in promoting tumor growth and metastasis, owing to the fact that tumor blood vessels provide nutrients and oxygen required for rapid development of cancer cells. Tumor vascularization is characterized by aberrant structural and functional dynamics, where blood vessels are immature, tortuous and hyperpermeable. These features result in heterogeneous and ineffective blood flow in tumors, which may mitigate the delivery of chemotherapeutic drug and the infiltration of immune effector T cells into tumor. Vascular normalization as a novel therapeutic strategy to inhibit angiogenesis has gained increasing attention over the past years. Instead of inhibiting the growth of tumor blood vessels, vascular normalization tends to prune some of the immature blood vessels and fortify the remaining vessels by recruiting and integrating mature pericytes in the vessel wall. Consequently, it produces a homogeneous distribution of perfused and less permeable blood vessels and generates an ameliorated hypoxic microenvironment. More importantly, normalization of the tumor vasculature has been elucidated to promote the effectiveness of immunotherapeutics and improve immune stimulation, which in turn further intensifies tumor vascular normalization. Therefore, this new paradigm of “immune-vascular crosstalk” for potentiating the interaction between vascular normalization and immune reprogramming offers the opportunity to develop new cancer therapeutic strategies that target both vasculature and immunotherapy.

Nanoparticles (NPs) can be tailored to meet a broad range of specific application requirements. As a result, the ever-growing usage of NP-based nanomedicine has opened exciting possibilities to treat cancer, through either normalizing vasculature or enhancing drug delivery into tumors. Nanomedicine is normally regarded to take advantage of the enhanced permeability and retention (EPR) effect found in tumor blood vessels to deliver anticancer agents across the vasculature into tumor. Recently, a growing body of evidence has illustrated that the penetration of NPs into tumors is majorly through transcytosis, an active route to deliver therapeutics into tumors.

This review highlights the central role played by the immune-vascular crosstalk for cancer therapy and summarizes the current strategies to promote this crosstalk by targeting both angiogenic factors and immune regulator. On the basis of the properties of tumor vasculature, unique NPs can be functionalized to cater to the therapeutic purpose. Herein, we also review the potential strategies for developing novel therapeutics, in particular NP-based nanomedicine, targeting tumor vasculature to facilitate anticancer drug delivery to better fulfill their potential.

2. Tumor vasculature: a key element of the tumor microenvironment

The tumor microenvironment (TME) has been well accepted as a major determinant of tumor progression and resistance to anti-cancer therapy. In particular, the effectiveness of immunotherapy has been linked to the immunosuppressive microenvironment curtailing the penetration of T cells into the tumor parenchyma and inhibiting their function once there. It has also been increasingly recognized that the immunosuppressive microenvironment is strongly attributed to abnormalities, both structurally and functionally, in angiogenic vessels of solid tumors. Despite tumor angiogenesis is meant to supply nutrients and blood to the tumor required for energy demand and growth, the angiogenic vessels of tumors show chaotic architecture and disturbed barrier function resulting in a hyperpermeable state, and lack of perfusion etc. Further, abnormal tumor blood vessels also lead to the immune suppressive profiles of the TME through their impacts on immune cell delivery into tumors, endogenous immune surveillance, and immune cell function.

2.1. Tumor vasculature is structurally abnormal

In contrast to normal, usually quiescent vasculature, the pathological induction of angiogenesis is dysregulated temporally and spatially in the tumor vascular system. As such, a newly formed tumor vasculature is highly disorganized where the blood vessels are extremely mal-shaped, dilated and tortuous, with uneven diameter, excessive branching and shunts. The endothelium that lines tumor vessels is also distinct from that of normal vessels. Tumor endothelial cells show an aberrant morphology, with absent or widened inter-endothelial junctions that sometimes may appear to project into the vessel lumen. A number of tumor blood vessels are not evenly composed of endothelial cells, rather are instead mosaic in nature and are formed by mural-like cancer cells that mimic the function of endothelial cells. Furthermore, vessel walls with uneven diameters include plentiful trans-cellular openings and fenestrations owing in part to compression of the immature walls. Notably, vascular density and fractal dimension can be used to analyze the tumor vascular structure, and it has been demonstrated that the fractal dimension is higher for tumors than for normal vasculature. To visualize the structure and architecture of tumor vasculature, magnetic resonance imaging (MRI) as a noninvasive imaging technique and scanning electron microscopy (SEM) have been both well employed in various studies. Nevertheless, two-photon microscopy is regarded as a better option in terms of cellular and extracellular imaging due to its superior spatial resolution.

The structure integrity of the blood vessel network is dependent on reciprocal interactions between endothelial cells and associated perivascular cells (mural cells), which mainly contain pericytes and smooth muscle cells (SMC). In the tumor vascular system, there is a defective pool of recruited mural cells, and this depleted coverage contributes to the hyperactivated state and atypical properties of endothelial and mural cells. As a result, mural cells in tumors are remarkably aberrant in shape, less abundant in quantity and are frequently connected loosely to endothelial cells, even extending cytoplasmic processes away from the vessel wall and into the tumor parenchyma, posing a challenge for drug delivery. In addition, loss of mural cells in tumors grants the blood vessels vulnerable to antiangiogenic therapies. Vascular endothelial growth factor (VEGF) signaling not only initiates the formation of new blood vessels, but also stabilizes the blood vessels via recruiting mural cells. At least four signaling pathways participate in the recruitment of mural cells, they include (a) platelet-derived growth factor B (PDGFB) and PDGF receptor β (PDGFR-β) signaling; (b) sphingosine-1-phosphate-1 (SIP1) and endothelial differentiation sphingolipid G-protein-coupled receptor 1 (EDG1) signaling; (c) Ang1 and Tie2 signaling; and (d) transforming growth factor (TGF)-β signaling.

Basement membranes as another critical component of vascular structure and thin sheets of specialized extracellular matrix (ECM) are predominantly made of collagen IV and laminins that are generated mainly by endothelial cells during the
process of angiogenesis. It has also been revealed that the basement membrane of tumor vessels rather loosely interacts with endothelial cells and pericytes and comprises multiple layers and presents an abnormal thickness. Various studies have illustrated that the basement membrane of tumor vasculature is discontinuous or absent, while other studies show that it exists but is morphologically aberrant. The basement membrane of the tumor vessels may also be focally disrupted with holes and project wide extensions deep into the perivascular interstitium, and is composed of other types of extracellular matrix ingredients. All of these characteristics are in agreement with the unstable or dynamic traits of tumor vasculature.

2.2. Tumor vasculature is functionally abnormal

The structural abnormalities in tumor vasculature give rise to disrupted vascular function. Tumor endothelial cell are highly heterogeneous and thereby vascular function of respective tumor blood vessels vary based on the type of tumor and progression stage. In general, tumors desperately require an elevated flow of oxygen and nutrients from the blood, thus a central hallmark of solid tumors is the heterogeneous distribution of blood flow. It has been found that abnormalities in both blood vessels and viscosity increase the resistance to blood flow in tumors. To this end, the overall perfusion rate in tumors in much lower than in other normal tissues and the average red blood cell velocity in tumor vessels can also be dramatically lower than in normal vessels. Additionally, blood flow in tumors is also unevenly distributed, fluctuates with time and space and may even alter its direction in tumor vasculature. As a consequence, regions with poor perfusion are frequently seen in tumors. Reduced perfusion can prevent the delivery and penetration of anti-tumor drugs into tumors. Thus, therapeutic strategies enhancing drug penetration via improving the vascular perfusion are of important experimental and clinical significance.

In addition to poor vascular function, increase in vascular permeability is another fundamental trait of tumor angiogenesis. Tumor vasculature is extremely permeable, with typical pore sizes ranging from 100 nm to 2 μm. The newly formed blood vessels in tumors are comprised of endothelial cells that fail originally to form proper junctions, leaving gaps between endothelial cells that facilitate plasma proteins to escape. The gap openings may be further aggravated ascribed to immune damage or ischemic injury to individual endothelial cells in the tumors. Moreover, a unique endothelial cell permeability structure, named the vesiculo vacuolar organelle (VVO), has been identified in the vasculature. VVOs are grape-like clusters that open to the interendothelial cell cleft, either below or above sites of specific junctional attachment, posing permeability in tumor blood vessels. In TME, VEGF and other inflammatory mediators such as histamine and serotonin trigger vascular permeability by disrupting the expression of membrane junctional proteins, which regulate the binding between endothelial cell membranes.

Tumor hypoxia is a consequence of inadequate oxygen supply induced by extensive abnormalities in the vascular network. Further, the leakage of tumor vessels contributes to high interstitial fluid pressure (IFP), and the immature tumor vessels are prone to collapse owing to this high IFP than normal mature vasculature. The tumor environment turns to hypoxic due to vascular collapse that can be transient in nature.

2.3. Tumor vasculature contributes to immunosuppressive tumor microenvironment

An increasing number of studies have illustrated that unproductive and abnormal tumor-associated blood vasculature is a major influencer of the tumor immunosuppressive microenvironment, and markedly affects how cancer cells escape the anticancer immunosurveillance, metastasis, and respond to immunotherapy. Additionally, pro-angiogenic factors not only repress the function of numerous immune cells, but also reduce leukocyte—endothelial interactions and impede the infiltration of immune effector cells into the tumor parenchyma. More interestingly, hypoxia and acidosis microenvironment caused by aberrant tumor vasculature decrease the infiltration of immune-supportive cell populations (e.g., T cells, natural killer cells, M1-like macrophages and dendritic cells), impede delivery of chemotherapy and immunotherapeutic entities, but facilitate attraction of immune-suppressive immune cells (myeloid-derived suppressor cells, neutrophils, Tregs, etc.). In addition, some underlying mechanisms have been revealed for tumor endothelial cells lining the blood vessels that specifically suppress tumor immunity. For instance, immune effector cells cannot pass across the endothelial cells into the tumor bed via downregulation of intracellular adhesion molecule 1 (ICAM1) and vasculature cell adhesion molecule 1 (VCAM1), which are required for their extravasation. Therefore, targeting tumor vasculature has been regarded as an effective strategy to enhance immunotherapy (Fig. 1).

3. Immune–vascular crosstalk in tumors

The recent advancement of cancer immunotherapy especially immune-checkpoint blockade has revoked the significance of the immune system in impeding the development of cancer. Cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), programmed cell death receptor-1 (PD-1), and programmed cell death ligand-1 (PD-L1) have been regarded as the most broadly studied and recognized inhibitory checkpoint pathways. Of note, anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) are both approved by the Food and Drug Administration (FDA) for the treatment of advanced melanoma, which not only provides great opportunities for rapid development in the clinical and scientific understanding of new immune and targeted therapies but also brings in remarkable benefit in the treatment of a variety of cancers. Also, chimeric antigen receptor (CAR) T-cell therapy, which implies that a patient’s T cells are reprogramed to express a synthetic receptor that binds a tumor antigen, is accepted as a significant development in personalized cancer treatment. More interestingly, vascular endothelial cells exert a critical effect on limiting acquisition and transmigration of effector T cells into tumors, for example, through hindering activation of blood vessels and increasing the expression of immune checkpoint molecules. To this end, angiogenic blood vessels can be important therapeutic
target for modulating immunotherapy especially the achievement of vascular normalization gives rise to increased T cell infiltration into tumor parenchyma. On the other hand, interferon γ (IFNγ) secreted by T cells was able to enhance the expression of some key chemokines including chemokine (C–X–C motif) ligand 9 (CXCL9), CXCL10 and CXCL11. These chemokines play important roles in stimulating pericyte recruitment that results in tumor vascular normalization. As a consequence, vascular normalization and immune reprogramming can establish a feedback loop, which means enhancement in one side will lead to the reinforcement of the other’s effects. Taking advantage of immune–vascular crosstalk would be benefit for the treatment of cancers. The molecules and signaling pathways involved in immune–vascular crosstalk in tumors are summarized in Table 1.31–59.

3.1. Conception of tumor vascular normalization

Vascular normalization is thought to be reversal of the angiogenic vessels back towards a mature and stabilized vasculature. The vascular normalization is mainly presented as elevated pericyte coverage and a more normal basement membrane support.60 These structural alterations possibly influence to diminish tumor hypoxia and IFP, to reduce the extent of hyperpermeability and to enhance the tumor vascular perfusion. With the correction of abnormal vasculature, the TME can be normalized and ultimately tumor progression is controlled and response to other therapies is improved. Of note, the requirement for vascular normalization has been further emphasized with the recent advances in tumor immunotherapy. An important driver for the failure of tumor immunotherapy for the non-responding patients might be the incapability of the immune cells to efficiently penetrate into the tumor mass. Thereby, tumor vascular normalization appears to be a promising route (Fig. 2).

3.2. Tumor vascular normalization affects immune reprogramming

With the advancement of cancer immunotherapy, how to strengthen the effectiveness of cancer immunotherapy has been thought to be a pivotal question to obtain answers. In this situation, the strategies to convert a “cold” tumor that is lack of immune effector T cells into a “hot” tumor with elevated infiltration of T cells have been received much attention.62 Aberrant tumor blood vessels lined by vascular endothelial cells have been accepted to exert an essential effect on restricting immune effector cell penetration into the tumors.63,64,65 Conversely, tumor vascular normalization is thought to provide a suitable microenvironment for immune effector cell mobilization and recruitment into the tumors.66–68

Figure 1 Abnormal tumor vasculature creates immune-suppressive microenvironment. Tumor blood vessels are frequently structurally disorganized and tortuous with poor pericyte coverage and abnormal basement membrane support. Functionally, the blood flow appears to be erratic with heterogeneous distribution and the blood vessels possess disrupted barrier function, resulting in a hyperpermeable state and a hypoxic microenvironment. The abnormal tumor vasculature tends to create an immune-suppressive microenvironment via influencing the infiltration of a series of immune-associated cell populations (e.g., CD8+ T cells, CD4+ T cells, dendritic cells, MDSCs, Tregs, macrophages).

Table 1 Summary of immune–vascular crosstalk in tumors.

| Strategy/mechanism | Associated molecule/signaling | Ref. |
|-------------------|-----------------------------|------|
| Tumor vascular normalization affects immune reprogramming | | |
| Formation of tumor-associated HEVs | LIGHT | 51 |
| | CCL19, CCL21, CXCL13, CCR7, LSEL, et al. | 52 |
| Inhibition of endothelial anergy | LTβR | 53 |
| | Effector T cell-derived TNFα and LTα | 53 |
| Suppression of VEGF-A and b-FGF | VCAM1, ICAM1, SELE | 54 |
| Blocking endothelin-1/endothelin-B receptor | Suppression of VEGF-A and b-FGF | 55 |
| Tumor immune reprogramming influences vascular normalization | Anti-CTLA4 or anti-PD1 IFNγ | 56 |
| Presence of CD8+ T cells | STING | 57 |
| | TCR | 58 |
| Presence of CD4+ T cells | CXCL9, CXCL10 and CXCL11 | 59 |
| | ICAM and SELE | 60 |
T cell entry into tumors in a process that involves a list of critical adhesion molecules and chemokines. Vascular normalization as an endothelium-targeting therapy can promote immune effector T cells in proficient numbers across the vascular barrier through enhancing vascular perfusion and decreasing hypoxia. Apart from genetic manipulations, the therapeutic blockade of proangiogenic factors at proper low-dose is also able to normalize tumor vasculature, which results in improved cancer immunotherapy.

In addition, it has been well illustrated that high endothelial venules (HEVs) are anatomically different post-capillary venules that work as major portals of entry for lymphocytes into lymph nodes and other secondary lymphoid organs. The amount of HEVs was closely linked to the density of tumor-infiltrating CD3⁺ and CD8⁺ cytotoxic T cells, suggesting that tumor-associated HEVs may be critical for T cell infiltration and thereby preventing tumor progression. It was reported that depletion of regulatory T cells (Tregs) contributed to augmented amount of HEV and a related elevation in T cell infiltration in murine tumor model. It was shown that high density of tumor HEV independently rendered a low risk of relapse and was substantially associated with longer overall survival rates. More surprisingly, combining vascular normalization with a vascular targeting peptide coupled to LIGHT (also known as TNF superfamily member 14; TNFSF14), a ligand for the lymphotoxin β receptor, promoted the formation of HEVs and tertiary lymphoid structures in pancreatic neuroendocrine tumors. When gene profiles were analyzed with the presence of HEV, it was characterized that genes encoding lymphoid chemokines including chemokine (C–C motif) ligand (CCL) 19 (CCL19), CCL21 and CXCL13 as well as T-cell homing receptors (CCR7 and LSEL) were significantly upregulated in HEVhigh tumors than HEVlow tumors. In terms of the underlying mechanisms that HEV induces T lymphocyte infiltration, it has been revealed that HEV may be triggered by lymphotoxin (LT)α/β −LTβ receptor (LTβR) signaling, or by effector T cell-derived tumor necrosis factor α (TNFα) and LTα, which bind to stromal TNF receptors, thereby bypassing LTβR signaling.

Moreover, tumor-associated endothelial cells have low expression of cell adhesion molecules including E-selectin (SELE), VCAM1 and ICAM1, which can give rise to unresponsiveness of tumor endothelial cells to inflammatory signals. As such, endothelial anergy (inflammatory activation) can be promoted whereas immune effector cell trafficking into tumors may be hampered. Interestingly, the downregulation of ICAM-1 by the proangiogenic VEGF-A and basic fibroblast growth factor (b-FGF), as well as overexpression of the endothelin-1/endothelin B-receptor signaling axis, enable tumor cells to evade T-cell attack. It was also demonstrated that anti-angiogenic therapy mediated vascular normalization was capable of reversing endothelial cell anergy leading to (re)sensitising tumor blood vessels to inflammatory stimuli by triggering the expression of homing molecules and thereby an enhanced T-cell-dependent anticancer immunity. In addition, anti-angiogenic agents in combination with...
immunotherapy are also able to relieve endothelial anergy and trigger T lymphocyte infiltration into tumors that prior to treatment were of an immune-excluded phenotype.

3.3. Tumor immune reprogramming influences vascular normalization

Indeed, immune reprogramming can lead to the shift from immunosuppressive TME to an immune-supportive microenvironment. It is very surprising that immune reprogramming, in particular achieved by immune checkpoint inhibitors, is able to activate T cells to normalize tumor vasculature. To some extent, the essential parameters of tumor vascular normalization may be recognized as reliable and noninvasive biomarkers for predicting immune checkpoint blockade. For instance, it was shown that anti-CTLA4 or anti-PD1 resulted in improved vascular perfusion, which was associated with anti-tumor activity. More importantly, depletion of CD8+ T cells, neutralization of IFN-γ, or implantation of tumors in IFN-γ receptor depleted mice abolished the enhanced vessel perfusion, implying the fundamental role of immune reprogramming in promoting vascular normalization. In addition to CD8+ T cells, it has been reported that the penetration of CD4+ T cells is also capable of triggering tumor vascular normalization. Tian et al. showed that adoptive transfer of CD4+ T cells was linked to diminished vascular permeability and hypoxia, but increased perfusion. On the other side, depletion or inactivation of CD4+ T cells reduced vascular normalization with less pericyte coverage. However, Tregs repressed INFγ-expressing CD4+ cells and produced VEGF via hypoxia-induced CCL28, which both contribute to a proangiogenic tumor environment that is related to abnormal tumor vasculature. Further, Yang et al. demonstrated that the activation of the stimulator of IFN genes (STING) signaling, an important bridge between innate and adaptive immunity that induces immune responses, with STING agonists could normalize tumor blood vessels in both implanted and spontaneous cancers. The vascular normalizing effect of STING activation was strongly dependent on type I IFN signaling and presence of CD8+ T cells, again indicating that stimulation of immune supportive microenvironment paves the way for achievement of tumor vascular normalization. To this end, the elevated penetration and impaired immune suppressive signals could further enhance immune checkpoint inhibitors to augment the activation of T cells. Apart from eliminating tumor cells, the activated T cells can also further induce normalization of tumor vasculature, thereby generating a positive reinforcement loop that results in tumor regression.

Based on the fact that immune reprogramming exerts a striking effect on inducing tumor vascular normalization, an increasing number of studies have paid attention to exploring the potential combination strategy of vascular normalization and immunotherapy. For example, anti-PD-L1 monoclonal antibody in combination with VEGF-R2 small molecule inhibitor can dramatically alleviate the levels of PD-1 and PD-L1, enhance tumor-infiltrating T lymphocytes, and suppress tumor progression by decreasing Tregs and myeloid-derived suppressor cells (MDSCs). Also, the combination of immune checkpoint anti-CTLA4-4 blockade (ipi-limumab) with anti-VEGF blockade (bevacizumab) in a phase I study revealed the activation of tumor endothelial cells and the infiltration of immune cells that were linked to favourable clinical outcomes in patients with metastatic melanoma. More interestingly, treatment with dual anti-CTLA4 and anti-PD1 therapy that was regarded to predominantly influence T cells would be able to give rise to tumor vascular normalization, contributing to decrease tumor progression.

Of note, despite these results are very interestingly, the profiles of immunotherapy-mediated tumor vascular normalization need further identification and clarification in clinical settings. For example, the situation suitable for immunotherapy-mediated vascular normalization, the duration of the response, and the differences from antiangiogenic therapy-induced vascular normalization maintain unknown.

3.4. The underlying mechanisms of immune–vascular crosstalk

3.4.1. How does vascular normalization promote immune response?

In terms of the underlying mechanisms of immune–vascular crosstalk, the reciprocal feedback loop between vascular normalization and immune activation is dependent on dual-functional signaling of a wealth of endothelium-associated factors especially proangiogenic factors and immune modulator proteins (Fig. 3). Also, the interconnection between endothelium-associated factors and immune modulator proteins has been regarded as the hub of positive feedback loop. For instance, it was reported that IFNγ released by endothelial cells could increase the expression of PD-L1 to reinforce adaptive resistance to the inhibition of VEGF-A and Ang2. Employing an anti-PD1 antibody to this therapeutic strategy would further increase the antitumor responses.

In fact, immune cells in the circulation are dependent on the blood vessel network to get to the tumor and kill tumor cells. Nevertheless, structural and functional abnormalities of tumor vasculature as well as excessive angiogenesis set up difficult hurdles for leukocyte recruitment. A series of proangiogenic factors such as VEGF-A cannot only inhibit the production of T cells, but also decrease effector functions (proliferation and cytotoxicity) of T cells by interfering with the capability of thymocytes to produce functional rearrangements of T-cell–receptor (TCR) genes. More interestingly, it was shown that VEGF-A secreted in the tumors upregulated the expression of PD-1 and other inhibitory checkpoints involved in CD8+ T cell exhaustion, which could be rescued by antiangiogenic agents targeting VEGF-A/VEGFR signaling. These data suggest that proangiogenic factors can have impact on the presence of T cells as well as the activity. However, downregulation or deactivate the aggregation of adhesion molecules on endothelial cells hampers T cell infiltration and represses anti-tumor immunity. In this regard, a number of essential molecules expressed on endothelial cells confer tumor endothelial barrier that suppresses T cell arrest. For example, tumor endothelial cells upregulated the expression of Fas ligand (FasL) in response to the stimulation of tumor-derived VEGF, IL-10 and prostaglandin E2, which has been demonstrated to selectively kill effector CD8+ T cells but not Tregs.

Since molecules contributing to abnormal tumor blood vessels play pivotal roles in restricting the infiltration and function of T cells, it is conceivable that routes to achieve vascular normalization may reverse this tumor suppressive microenvironment via restoring T cell behaviours. This is substantiated by effectively targeting VEGF/VEGFR signaling, which not only impedes the sprouting of new blood vessels but also results in vascular normalization. The extravasation of T cells into the tumor tissue counts on the expression levels and clustering patterns of ICAM-1 and VCAM-1. VEGF is able to diminish the expressions or repress
the clustering of these essential adhesion molecules to hinder the interactions between leukocytes and endothelial cells, all of which could be rescued with VEGF antibodies or inhibitors. Moreover, it was shown that sunitinib as the multi-targeted receptor tyrosine kinase (RTK) inhibitor for VEGF receptors suppressed the levels of interleukin 10 (IL-10), FOXP3, PD-1, CTLA4, whereas increased IFN-γ in the MCA26 colon cancer mouse model. The proportions of CD4+ and CD8+ T cells were significantly enhanced but the inhibitory molecules PD-1 and CTLA4 were dramatically hampered after sunitinib treatment.

3.4.2. How does immune reprogramming enhance vascular normalization?

In light of the other half of the feedback loop, immune cells are regarded as a source for a list of soluble factors that affect angiogenesis and endothelial cell behaviour. T cells are capable of improving angiogenesis through secretion of some proangiogenic factors, such as FGF-2 and heparin-binding epidermal-like growth factor (HB-EGF). Nevertheless, the most fundamental T cell derived factors including TNF, TGF-β, and IFNs, on the opposite, present anti-angiogenic functions. Of note, T cells especially Th1 cells secrete IFN-γ, whose presence in the tumors is often linked to good prognosis. Interferon-mediated CXC family chemokines repressed the proliferation of endothelial cells but contributed to the increased infiltration of T cells. NK cells and dendritic cells. CXCL9, CXCL10 and CXCL11 are thought to be interferon-inducible angiostatic chemokines that can directly impede angiogenesis by binding CXCR3 on endothelial cells. More interestingly, IFN-γ and surface molecule CD40L could upregulate the endothelial adhesion molecules including ICAM1 and SELE, which induce the infiltration of T cells. IFN-γ also diminished the expression of VEGF-A, but enhanced the levels of CXCL9, CXCL10 and CXCL11 that expressed in endothelial cells, resulting in the recruitment of pericyte that is associated with vascular maturation and stabilization.

3.5. Abnormal tumor vasculature produces major hurdles for cancer therapy

To fulfill the therapeutic effects, drugs must first reach tumor region through blood vessels, followed by extravasation, and next penetrate through tumor parenchyma to reach all of the cancer cells. Therefore, the responsiveness by tumors to anticancer therapeutics is impacted by the vasculature both directly and indirectly. Given the fact that tumor vasculature is extremely heterogeneous and tortuous with disrupted endothelial cell lining in the inner layer and the blood flow in tumors is resistant and variable, the delivery of systemically administered drugs is compromised and the distribution of therapeutic drugs is non-uniform. In addition, the presence of intratumor vascular shunts is another mechanism leading to compromised drug delivery. This is owing to that the major portion of a systemic drug dose is thus shunted around and away from the primary tumor. Abnormal tumor vasculature also creates large barrier for the transport of therapeutics from blood vessels to tumor tissues. Diffusion and convection are thought to be the crucial driving force behind the transport of anticancer drugs across the vascular wall into tumors. Drug diffusion is highly dependent on concentration gradients and convection is reported to lie mainly upon gradients of pressure.

Figure 3: Vascular normalization and immune reprogramming form a positive feedback loop. Tumor vascular normalization mediated by inhibition of some critical angiogenic factors (e.g., VEGF-A and ANG2) or regulation of various pivotal molecules (e.g., PHD2 and RGS5) enhances the infiltration of T cells into tumor parenchyma, promoting immune reprogramming. On the other hand, IFNγ secreted by T cells reinforces the expression of some key chemokines including CXCL9, CXCL10 and CXCL11, which play important roles in stimulating pericyte recruitment that leads to tumor vascular normalization. Therefore, vascular normalization and immune reprogramming can form a positive feedback loop, resulting in the increased efficacy of cancer therapy via their reciprocal enhancement effects.

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between the vascular space and the interstitial space. In tumors, the gradient of oncotic pressure is nearly zero and the IFP is frequently augmented and similar to the microvascular pressure. In this situation, the extravasation of therapeutics is significantly alleviated, especially in the central part of tumors, where the IFP is comparable to the microvascular pressure\(^\text{87,89}\).

Notably, hypoxic tumor cells, largely due to the abnormal tumor blood vessels with limited diffusion and perfusion, are resistant to chemotherapy\(^\text{80}\). Another pivotal component of the tumor niche that partially associated with abnormal blood vessels is ECM. ECM anomalies such as stiffness, which may be caused by the deposition of ECM and increased cross-linking of ECM constituents, would tend to mitigate the amount of space in the tissue available for movement of drugs. It is undoubtedly recognized that dense tissues seem to be stiffer and possess reduced drug diffusion rates. To this end, ECM stiffness has been closely linked to impaired delivery and resistance of conventional drugs\(^\text{91,92}\). Consequently, targeting abnormal ECM may provide an alternative effective route to combat cancer.

4. Novel therapeutics towards tumor vascular normalization

Tumor microenvironment-targeted therapeutics that normalize tumor vasculature or degrade ECM could prime the TME to generate favourable immune responses. Although conventional chemotherapeutic agents possess extensive tumoricidal capability, they may also cause severe adverse effects including haematologic, hepatic and renal toxicity in cancer patients, which sets up an assignable hurdle for the safety and effectiveness of the anticancer drugs in clinical applications. NPs have the potential to direct drugs to specific tissues that leads to improved drug efficacy and less toxicity. Thanks to its unique physical and chemical properties, nanomedicine is emerging as a promising therapeutic strategy to efficiently deliver sufficient anticancer agents into tumors\(^\text{93}\).

However, clinical translation of cancer nanomedicine has not succeeded as expected. Only ten nanomedicines have received regulatory approval in Europe and U.S., seven of which are liposome-based formulations. Despite improvement in pharmacokinetics and amelioration of the side effects, poor delivery efficiency, claimed to be around 1\%, has been one of the key hurdles in translating nanomedicine for cancer therapy. Extravasation is the first step in a nanomedicine’s road into a tumor when administered systemically. Therefore, tumor vasculature imposes a major impact on the efficacy of nanomedicine. Vascular permeability to nanomedicine depends on not only the physicochemical properties of NPs but also the physiological characteristics of the vasculature. Here we reviewed the strategies to enhance delivery efficiency and efficacy of nanomedicine by focusing on normalization of vasculature and the enhancement of extravasation of nanomedicine into tumors. By presenting insights into the basics of nanomedicine—vasculature interactions in tumors, we hope it would provide novel strategies to engineer cancer nanomedicine to accelerate their translation and fulfill the promises of this field (Table 2\(^\text{94–110}\)).

4.1. Strategies to develop nanomedicine to normalize tumor vasculature

4.1.1. Shut-off of VEGF and VEGF-associated signaling pathways

Inhibition of VEGF or its associated signaling pathways destroys pre-existing vascular networks and hampers growth of new blood vessels. In addition, a growing number of studies have demonstrated that suppression of VEGF can induce vascular normalization through inhibiting immature vasculature, increasing the coverage of mural cells and enhancing vascular maturation\(^\text{111,112}\). Consequently, it results in diminished vascular permeability and IFP with enhanced vascular perfusion, restored oxygen in tumor regions and hydrostatic pressure gradient across the vascular wall\(^\text{112}\). For example, Huang et al.\(^\text{12}\) reported that a low dose of anti-angiogenic therapy normalized tumor vasculature and shifted the tumor immunosuppressive microenvironment to an unsupportive microenvironment. This study suggests that in order to achieve vascular normalization efficiently, the

Table 2: Strategies to develop nanomedicine to normalize tumor vasculature and improve tumor-targeted delivery

| Target/strategy | Cargo/engineered nanoparticle | Ref. |
|----------------|-------------------------------|------|
| Nanomedicine to normalize tumor vasculature | | |
| Angiogenesis signalling pathway | | |
| VEGFA-VEGFR2 | Knockdown of VEGFA by: siRNA/PLCP | 94 |
| | siRNA/PEI-SWNTs | 95 |
| | shRNA/dtACPP-modified PEG-DGL | 96 |
| | Inhibition of VEGFR2 by: Antibody/MSV | 97 |
| Ang2 | Knockdown of Ang2 by: siRNA/chitosan magnetic nanoparticles | 98 |
| | Blockage of Ang2-Tie2 interaction by: T4 peptide (K(DEAP)-AAN-NLLMAAS)/PEG | 99 |
| PDGF | PDGF/PLGA–pSi-ES | 100 |
| Immune-associated cell populations and signalling: TAMs | Targeting TAMs-specific ligands by: MTX/FOLR2 conjugated G5-dendrimer | 101 |
| | HA/Mannose receptor conjugated MnO2 NPs | 102 |
| | siVEGFM2 peptide conjugated AuNPs | 103 |
| Enhance extravasation of nanomedicine into tumor | | |
| Transcytosis-based delivery | | |
| Adsortive-mediated transcytosis (AMT) | CPT/PBEAGA zwitterionic polymer | 104 |
| Receptor-mediated transcytosis (RMT) | Targeting iRGD/integrins αv by: siPLK1 and miR-200c/iRGD conjugated MSN | 105 |
| | CA4 and DOX/iRGD conjugated MSN | 106 |
| | Targeting GD1/II4 by: PTX/GD16 conjugated aldehyde-PEG-PLA and MPEG-PLA block copolymers | 107 |
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administration of antiangiogenic agents was required to be confined to a certain therapeutic window. Furthermore, Willett et al.\textsuperscript{113} showed that, in clinical settings, a single infusion of the VEGF-specific antibody bevacizumab fortified the structure and function of tumor vasculature among adenocarcinoma patients. Moreover, bevacizumab in combination with first-line oxaliplatin-based chemotherapy significantly improved the progression-free survival in the patients with metastatic colorectal cancer\textsuperscript{114}. Thus, an overwhelming majority of clinical evidence has demonstrated the existence of tumor vascular normalization in cancer patients who have received anti-VEGF drugs.

In fact, the tumor vasculature provides a facile target for RNAi therapeutics. Silencing VEGF with siRNA (siVEGF) has been proven as an effective therapeutic tool and one of the first potential candidates to suppress tumor growth. Biocompatible NP-based delivery vehicles allow for highly potent, specific delivery. A number of NP-based delivery systems have been used to deliver siVEGF into tumors. For instance, systemic administration of polycation liposome-encapsulated calcium phosphate NPs (PLCP) carrying siVEGF significant inhibited angiogenesis and reduced tumor growth in the MCF-7 xenografts mice\textsuperscript{64}. Targeting VEGF can also be used combined with other therapeutics to achieve synergistic effect. Polyethylenimine (PEI) modified single-walled carbon nanotubes (SWNTs) conjugates linked with candesartan (CD) were developed to deliver siVEGF, showing good biocompatibility, excellent safety, enhanced cellular uptake and angiogenesis inhibition in PANC-1 xenografted nude mice\textsuperscript{95}. Therapies targeting VEGF solely generated mixed results in cancer therapy. Current trend is to develop combination therapies targeting both angiogenesis and tumor cells. Cell-penetrating peptides (dtACPPs) have demonstrated their potential with a high tumor cell-targeting specificity\textsuperscript{115}. dtACPP-modified Polyethylene glycol (PEG)-dendrigraft poly-l-lysine (DGL) NPs have been developed to co-deliver plasmid expressing interfering RNA targeting VEGF (shVEGF) and doxorubicin (DOX), leading to effective shutdown of blood vessels and cell apoptosis within the tumors\textsuperscript{96}. Alternatively, as VEGFR2 is overexpressed in the tumor vasculature, multistage nanovectors (MSV) that preferentially target VEGFR2-overexpressing tumor blood vessels have been designed to deliver VEGFR2 antibody into tumors\textsuperscript{97}.

4.1.2. Activating angiopoietin-tie signaling axis

Angiopoietin (Ang)–Tie (tunica interna endothelial cell kinase) signaling axis is considered as another potential molecular signaling pathway that efficaciously triggers vascular normalization\textsuperscript{116}. Ang2 binds to its receptor (Tie2) on endothelial cells, resulting in further destabilization of blood vessels and increasing in vascular permeability. In contrast, Ang1, mainly secreted by pericytes, enhances the recruitment of mature pericytes to blood vessels and limits excessive endothelial sprouting, leading to tighter endothelial junctions and reduced permeability\textsuperscript{117}. Goel et al.\textsuperscript{118} showed that AKB-9778, a specific vascular-endothelial protein tyrosine phosphatase (VE-PTP) inhibitor, is able to normalize the structure and function of tumor vasculature through activation of Ang1/Tie-2 signaling, thus preventing tumor progression and improving anticancer treatment. In addition, combination therapies targeting both Ang2 and VEGF overcome the limitations of anti-VEGF monotherapy, leading to better vessel normalization and strengthened inhibition of tumor growth compared with VEGF inhibition alone\textsuperscript{119}.

As Ang2 destabilizes established blood vessels through interrupting Tie2 signaling, inhibition of Ang2 or its interaction with Tie2 have been used to develop nanomedicine targeting Ang2/Tie2 signaling pathway. siRNA targeting Ang2 has been delivered by chemically modified dendrimer NPs to the targeted sites, such as Tie2-expressing lung vasculature, in mice without detectable toxicity and chronic inflammation\textsuperscript{120}. This showed the feasibility to silence Ang2 in vivo. However, due to the wide expression of Tie2, targeted delivery of Ang2 siRNA into tumors remains a challenge. Chitosan magnetic NPs have been used to deliver Ang2 siRNA to target cells\textsuperscript{98}. Under the action of external magnetic field, this NP accumulated in tumor cells. It will be interesting to see whether it can inhibit Ang2 in vivo and suppress tumor growth. Alternatively, a tumor-responsive delivery system can be designed to hold therapeutics until it reaches tumor site, as exemplified by P-T4 NPs. T4 peptide (NLLMAAS) could block Ang2-Tie2 interaction and disrupt the downstream signaling pathway. P-T4 consisted of a modified T4 peptide (K(DEAP)-AAN-NLLMAAS) that self-assembled into PEG NPs at physiological pH. In bloodstream, hydrophobic molecule diethylaminopropyl isothiocyanate (DEAP) could protect the T4 peptide from clearance. When P-T4 reached acidic TME, DEAP was protonated to expose modified T4 to legumain, which was commonly overexpressed in tumor tissue, leading to cleavage and release of T4 peptide from the P-T4 and subsequent blocking of the Tie2 receptor on macrophages and endothelial cells. Treatment of P-T4 in a triple-negative breast cancer mouse model significantly prevented tumor recurrence\textsuperscript{99}.

4.1.3. Targeting other angiogenesis-associated signaling

Platelet-derived growth factor receptor-β (PDGFRβ), expressed by endothelial cells, is a well-characterized recruitment signal for pericytes that mainly produce PDGF receptor-β (PDGFRβ)\textsuperscript{121}. It has been reported that silencing of PDGF-B signaling decreases pericyte recruitment, which further contributes to vascular permeability\textsuperscript{122}. Therefore, restoration of the PDGF-B signaling axis is a pivotal means to fulfill tumor vascular normalization. Furthermore, in VEGF-positive tumors, placental growth factor (PIGF) may emerge as a vascular remodelling factor that significantly normalizes the structure and function of tumor blood vessels. To this end, PIGF could strongly reverse the tumor vasculature toward uniform and organized blood vessels that are basically all covered with mural cells in both genetically modified and normal occurring tumors\textsuperscript{20}. Recently, a PLGA–pSi NP-based delivery system composed of poly(D,L-lactide-co-glycolide) acid (PLGA) and porous silica NPs (pSi) has been developed to deliver PDGF to regulate angiogenesis. Further conjugation of PLGA–pSi to electrospray (ES) gelatin created a combined ES patch, which allowed for spatiotemporal release of PDGF. This system may be tailored to deliver PDGF-B to normalize tumor vasculature\textsuperscript{20}. Additionally, a growing body of evidence has shown that Notch, a receptor of delta-like ligand 4 (Dll4) in endothelial cells is recognized as another potential critical modulator for controlling vessel quiescence\textsuperscript{124}. It has been shown that Dll4/Notch signaling hinders vascular sprouting by limiting the formation of tip cells that cause the sprout at the forefront of the vessel branches, and thereby inhibition of Dll4/Notch signaling pathway generates more but poor perfused vessels. On the contrary, increased expression of Dll4 leads to impaired vascular density while elevated lumen size and vascular perfusion, and restoration of oxygenation in tumors\textsuperscript{20}.

4.1.4. Modulation of critical molecules

Apart from the signaling pathways that associate with angiogenesis, there are a series of pivotal molecules that play important roles in regulating tumor vascular normalization. Prolyl
hydroxylase domain-containing protein 2 (PHD2) exerts great effects on oxygen-sensing through the regulation of the α-subunit of HIF, a hypoxic responsive transcription factor. It was reported that haplodeficiency of PHD2 could result in normalized endothelial lining and vascular maturation in tumors, which enhanced vascular perfusion and restored oxygenation. In this situation, tumor cell invasion and metastasis were repressed. Moreover, endothelial nitric oxide synthase (eNOS) is a well-known candidate that improves the formation of stabilized vessels, however, suppression of neuronal NOS (nNOS) in tumors produces a NO gradient, propelling NO preferentially around blood vessels and showing a more mature vascular phenotype. Another well-accepted mediator for vascular normalization is the regulator of G-protein signaling 5 (RGS5) that is produced by activated pericytes and hypoxic endothelial cells. Deficiency of RGS5 gives rise to tumor vascular normalization with less permeable vasculature, vascular density is augmented but blood vessels are more uniformly distributed in Rgs5 knockout tumors. It was also revealed that R-Ras, a small GTPase of the Ras family, reinforced the tumor vascular structure and function and mitigated plasma leakage through improved endothelial integrity and pericyte association with nascent blood vessels. In addition, Magrini et al. demonstrated that CD171, a transmembrane glycoprotein, was able to modulate the function of endothelial cells and drive endothelial to mesenchymal transition (EndMT) that related to neovascularization. Thus blockade of CD171 resulted in impaired angiogenesis and promoted vascular stabilization. Further, it was also reported that CL1-R2, an IgG1 mAb directed against human CD160 that recognizes both human and murine CD160 receptor, could normalize the structure and function of tumor blood vessels. In this regard, CD160 was deemed as a potential novel target for cancer treatment and inhibition of CD160 enabled better delivery of a chemotherapeutic agent to tumors via vascular normalization. Notably, restoration of endothelial barrier integrity especially inhibiting vascular permeability has been regarded as an effective strategy to achieve vascular normalization. Zhao et al. and Li et al. reported that specific upregulation of VE-cadherin led to normalization of tumor vascular structure and function, which enhanced the tumor immunotherapy.

Gold NPs (AuNPs) are a widely used nanomaterial in drug delivery. A recent study uncovered that a polymer and folic acid-modified gold NPs (AuNPP-FA) inhibited tumor growth and metastasis even without therapeutic drugs. Further study suggested that AuNPP-FA inhibited tumor angiogenesis and normalized tumor vascular structure and function by increasing pericyte coverage and strengthening tight junctions through upregulating VE-cadherin levels on endothelial cells. Similar to what Huang et al. and Zhao et al. reported about the effect of VE-cadherin upregulation in tumor vasculature, AuNPP-FA increased infiltration of CD3⁺CD8⁺ T lymphocytes to enhance immunotherapeutic response.

4.1.5. Regulation of immune-associated cell populations and signaling

Hypoxia microenvironment caused by abnormal tumor blood vessels is reported to potentiate the recruitment of immunosuppressive Tregs. It has been revealed that extensive Treg depletion contributed to the normalized tumor blood vessels. Li et al. uncovered that around 90% depletion of Tregs in Foxp3.LuciDTR-4 mice led to normalization of the tumor vasculature, as reflected by impaired numbers of large dilated blood vessels and elevation in the number of vessels with small diameter, as well as decreased hemorrhagic areas. Of note, depletion of Tregs would also trigger the formation of HEVs outside of lymph nodes and the spleen, where they normally exist. When HEVs are present at tumors, they would be capable of strengthening the infiltration lymphocytes. In this regard, Tregs serve important regulatory roles in influencing the crosstalk between vascular normalization and immune reprograming and targeting Tregs has provided great opportunities for improving immunotherapy. Moreover, Bauer et al. clearly demonstrated that MDSC-secreted S100A8 acted as a resistance-mediating factor produced by antiangiogenic therapy. S100A8 was responsible for the destabilization of the tumor vasculature via increasing blood vessel leakiness and disrupting EC integrity, which can be

Figure 4  Effects of gold nanoparticles (AuNPs) on tumor vascular normalization. AuNPP-FA normalized tumor vasculature in both 4T1 and BGC823 tumor models. Reprinted with the permission from Ref. 134. Copyright © 2020 American Chemical Society.
abrogated by combined therapy of antiangiogenic therapy with all
trans-retinoic acid (ATRA). On the other hand, lower “vascular
normalizing” dose of angiogenesis inhibitor DC101 treatment
diminished the number of tumor-infiltrating MDSC in the
MCAp0008 breast tumors.

In addition to various immune cell populations, some crucial
immune-associated signaling pathways have also been reported to
play vital roles in modulating tumor vascular normalization. For
instance, CD4+ Th1 cell-derived IFNγ was likely to prune tumor
blood vessels via its potent antiproliferative effects on ECs. It
has also been revealed that the concentration of IFNγ exerted
significant effect on tumor vascular permeability, in particular,
block of immune CD4+ T cells and CD8+ T cells resulted in a
decrease in IFNγ level, which might give rise to the increase of
pore size in vessel wall and lead to dramatic augmented vascular
permeability. A dual-sensitive NP delivery system was developed
to co-encapsulate DOX and IFNγ against melanoma. In this
system, anticancer drug DOX was conjugated to thermosensitive
NPs (TSNs) by a pH-sensitive chemical bond, whereas IFNγ was
absorbed into TSNs through the thermosensitivity and electro-
statics of NPs. This co-delivery of DOX and IFNγ resulted excellent synergistic antitumor efficiency against B16F10 tumor
bearing mice.

Also, Johansson et al. found that low-dose TNFα treatment
stabilized the vascular network and enhanced vascular perfusion in
the RIP1-Tag5 pancreatic neuroendocrine tumors. More impor-
tantly, this inflammatory vascular remodelling induced by low-
dose TNFα tremendously boosted the therapy of antitumor
vaccination or adoptive T-cell transfer. Aminopropyltriethoxy-
silane (APTS) and/or protamine sulfate (PRO) modified superparamagnetic iron oxide NPs (SPIONs) showed high transfection efficiency for TNFα gene with no obvious cytotoxicity, resulting in reduced cell variability of Hep G2 cells and tumor size transplanted in nude mice.

A growing body of evidence has uncovered that tumor asso-
ciated macrophages (TAMs) with pro-angiogenic and immune-
suppressive (M2-like) phenotypes are indications of malignant
progression. TAM induces tumor vascularization and over-
expression of angiogenic VEGF while promoting immunosuppres-
sion within the TME. Rolny et al. demonstrated a fundamental role of TAM polarization (away from M2-like phenotype to a tumor-inhibiting M1-like phenotype) in vascular normalization due to the fact that vascular branching is more sensitive to alterations in TAM accumulation. Mechanistically, skewing of TAM polarization in tumors relies remarkably on downregulation of PI3K. Furthermore, eosinophils are also thought to be necessary components of the immune microen-
vironment that regulates tumor initiation and progression. It has
been shown that activated tumor-associated eosinophils were
essential for tumor rejection through secreting chemotactractors
that guided CD8+ T cells into the tumors. To this end, activated
eosinophils were able to trigger dramatic changes in the TME,
including macrophage polarization and normalization of the tumor
vasculature.

Despite that TAMs represent an appealing target for cancer
therapy, targeting TAMs, such as inhibition of macrophage
recruitment, or enhancement of tumoricidal activity of macro-
phages approach has been far from clinic. One of the major ob-
stacles is the nonselective targeting of TAMs, which can otherwise
compromise the immune system in general. NPs have been used to
overcome the issue of specificity. By conjugating TAMs-specific
ligands, NP can be delivered specifically to TAMs. Ovarian TAMs
express high levels of the folate receptor-2 (FOLR2). G5-
dendrimer NPs coupled with the chemotherapeutic methotrexate
(G5-MTX NPs), which targets the FOLR2 on TAMs, have been
employed to selectively deplete ovarian cancer TAMs, leading
to restriction in tumor growth as effective as cisplatin. TAMs are
highly accumulated in hypoxic regions of tumors. Attenuating

hypoxia can be used as a strategy to prime TAMs toward
M1-like phenotype, leading to inhibition of tumor growth.
Mannose receptor is highly expressed in TAM-M2 macrophages.
Conjugation of mannann guide manganese dioxide NPs (MnO2
NPs) to the M2-like TAMs in hypoxic area and reacting with
hydrogen peroxide (H2O2) to produce O2, led to the alleviation of
hypoxia and regulation of pH in the hypoxic region. Further
modification of NPs with hyaluronic acid (HA) program anti-
inflammatory, pro-tumoral M2-like TAMs to pro-inflammatory,
antitumor M1-like TAMs to further enhance the ability of MnO2
NPs to lessen tumor hypoxia and modulate chemoresistance. The
HA-coated, mannann-conjugated MnO2 particle (Man-HA-MnO2)
treatment significantly increased tumor oxygenation and down-
regulated HIF-1α and VEGF in tumors. Combination treatment
of the tumors with Man-HA-MnO2 NPs and DOX significantly
increased apparent diffusion coefficient (ADC) values of the
breast tumors, inhibited tumor growth and tumor cell proliferation
as compared with chemotherapy alone.

Although the results from above studies are intriguing, re-
ceptors for folate and mannann also expressed in other cells such as
normal epithelial cells and dendritic cells, which may lead non-
specific targeting to TAMs and cause side effects. A peptide, M2
macrophage-targeting peptide (M2pep), has been identified by
phage display to selectively and preferentially bind to murine
TAMs-specific M2 macrophages. Conjugating M2pep to gold NPs
(AuNPs) guild vehicles to M2-like TAMs without affecting other
leukocytes. Considering TAMs also express VEGF to promote
cancer progression and metastasis, further conjugating siVEGF to
M2pep-AuNPs silenced VEGF mRNA in both inflammatory
malignant M2-like TAMs and lung cancer cells at the same time to
enhance tumor inhibition, leading to complete regression of
human lung adenocarcinoma in a murine xenograft model (Fig. 5).

4.2. Normalization of tumor microenvironment facilitates
targeted delivery of nanomedicine

Compared with free small molecule drugs, nanomedicines depend
more on vascular perfusion and permeability. However, as we
discussed above, the blood supply in tumors is spatially and
temporally heterogeneous. Blood flow in some regions of tumors
is quite brisk, while impaired in other regions because of vascular
compression and excessive leakiness. As few as 20% of the blood
vessels in a tumor may actually carry blood flow, making nano-
medicine hard to reach cancer cells deep in the tumor.

Vascular normalization repairs abnormalities in vascular
structure, making tumor vessels homogeneous, mature and less
leaky with reduced IFP, as exemplified by the effect of anti-
angiogenic agents. This can lead to transient improvement in
vascular perfusion and increase in blood flow, which may ultimately
increase nanomedicine penetration into tumors. Indeed, vascular
normalization via anti-angiogenesis enhances the efficacy of
nanomedicines in a size-dependent manner, preferentially benefits
smaller rather than larger nanomedicines.

In addition, remodelling of tumor extracellular matrix also
promotes tumor-targeted efficiency of NP drug carriers. In
tumor interstitial space, elevated expression of cytokines such as TGF-β by both tumor and stromal cells promotes collagen synthesis, leading to the formation of a fibrous extracellular space. Several lines of evidence suggested that targeting TGF-β1/TGF-β receptor to normalize tumor ECM benefited NPs penetration into solid tumors. Tranilast (Rizaben) is a clinically approved antihistamine and anti-fibrotic TGF-β inhibitor. It can normalize TME as shown by a pronounced reduction in extracellular matrix components and an increase in the intratumoral vessel diameter and pericyte coverage. Combination of tranilast with a low dose of Doxil nanomedicine that could not lead to primary tumor regression, significantly improved blood vascular functionality and oxygenation and enhanced treatment efficacy as indicated by the notable reduction in tumor size. Furthermore, the tranilast–Doxil treatment favoured the accumulation of M1-like TAMs, presumably due to the increase in tumor oxygenation. Targeting TGF-β receptor also benefited nanomedicine delivery. Inhibition of the TGF-β signaling pathway with LY364947, an inhibitor of TGF-β type I receptor, promoted the penetration of siPlk1 containing NPs in mice with breast cancer showed remarkable tumor regression in the tumor tissue, significantly ameliorating the intratumoral distribution of NPs in MDA-MB-231 xenografts and further leading to enhanced internalisation of NPs into tumor cells. Dense collagen network in ECM could also be depleted by losartan. Pretreatment with losartan significantly decreased the collagen levels and improved the tumor penetration of NPs. Compared with sole NPs, DOX containing NPs combined with losartan exhibited enhanced drug delivery efficiency, striking penetration efficiency and best 4T1 breast tumor inhibition effect.

Combination of normalization of tumor vasculature and ECM enhanced the efficacy of nanomedicine delivery. By using an anti-VEGFR antibody, DC101, and an anti-TGF-β1 antibody, significant normalization of the tumor vasculature and reduction in collagen density was achieved, leading to improved tissue perfusion and enhanced delivery of NP within tumors. Taking together, vascular and ECM normalization strategies can be utilized to remodel the TME, resulting in improvement in nanomedicine delivery into solid tumors.

4.3. Strategies to enhance extravasation of nanomedicine into tumors

Crossing the tumor vasculature and penetrating into the TME remain major challenges in tumor treatment including NP-based therapies. The current view is that NPs cross the tumor vascular barrier through intercellular gaps and are retained in the tumor owing to pressure created by poor lymphatic drainage—a process termed “EPR”. Over the last decades, tremendous efforts have been directed towards enhance EPR effect to improve extravasation of NPs into the TME by optimizing NPs’ characteristics, including size, shape, electrical charge and chemical composition. However, NP-based nanomedicine that utilizing EPR effect failed to significantly enhance survival of cancer patients, which might be due to highly heterogeneous, both inter- and intra-individually, EPR in patients.

Recent report suggested that NPs penetrated into several different types of tumor mainly through transcytosis, and not via inter-endothelial gaps, which were found to be far less abundant within the tumor vasculature than was previously believed. Rational design of nanomedicines that harness transcytosis can potentially overcome various barriers to reach tumor cells located far from blood vessels with enhanced drug efficacy. In addition, immune cells have been found to enrich in solid tumors, either promote or inhibit tumor development. Therefore, immune cells can act as a “trojan horse” to deliver NP-based therapeutics into the targeted region in tumors. Here, we focus on the principles to engineer NPs to achieve deeper tumor penetration through transcytosis and immune cell-mediated infiltration.

4.3.1. Transcytosis-mediated delivery

Transcytosis refers to the transcellular transport of macromolecules and cells within membrane-bound vesicles between apical and basolateral plasma membranes. It has been suggested that up to 97% of NPs enter tumours using an active process, majorly transcytosis, through endothelial cells. Depending on the process of endocytosis, transcytosis can be categorized to adsorptive-mediated and receptor-mediated transcytosis. Compared with targeting the EPR effect, which is through the leaky vasculature, transcytosis is an active process, contributing to the enhancement of NP/drug extravasation and penetration into TME.

4.3.1.1. Adsorptive-mediated transcytosis (AMT). Adsorptive-mediated transcytosis (AMT) facilitates the pass of cationic molecules, which interact with anionic microdomains on the cytoplasm membrane of tumor endothelial cells or tumor cells through electrostatic interaction. AMT normally starts with caveola-mediated endocytosis. Recently, a tumor-targeted nanomedicine has been developed to efficiently reach even distal tumor cells through AMT in endothelial and tumor cells. γ-Glutamyl transpeptidase (GGT) is overexpressed on the external surface of...
endothelial cells and metabolically active tumor cells at the periphery of blood vessels in a variety of human tumors. Zhou et al. conjugated γ-glutamyl transpeptidase-responsive camptothecin (CPT), a common anticancer chemotherapeutic, to zwitterionic polymer to form PBEAGA-CPT conjugates. In the blood, PBEAGA-CPT was neutrally charged. When reaching the tumor endothelial cells, it was rapidly converted into a positively charged state by GGT-catalyzed γ-glutamylamide hydrolysis. Cationic charged conjugate underwent fast AMT, followed by fast AMT into cells. In cells, γ-glutamylation-based polymer—drug conjugates were mostly localized in the Golgi apparatus and avoided digestive lysosomal trapping. This enabled continuous cycles of transcytosis of the nanomedicine through multiple cells until it reached distal tumor cells and released its drug, leading to significant inhibition of tumor progression and complete tumor elimination without relapse.

4.3.1.2. Receptor-mediated transcytosis (RMT). In RMT, NPs incorporate ligands that facilitate binding to endothelial cells or tumor cells through corresponding receptors expressed on their surface. This targeting provides a synergistic effect when ligands target both tumor endothelial cells and tumor cells within the tumors.

Tumor vasculature expresses certain receptors higher than the normal vasculature. NPs conjugated with ligands that target these receptors can be led to bind to tumor vasculature and be transported to TME through RMT. Integrins αν family, including ανβ3, ανβ5, and α5β1, are preferentially expressed on tumor vasculature. RGD (Arg–Gly–Asp) is a peptide that specifically binds to αν receptors. Therefore, ligands containing the RGD integrin recognition motif can lead ligands-NPs conjugates to tumor vasculature. However, RGD-integrin αν interaction can only accumulate therapeutics in the tumor vasculature without penetration into TME. To overcome this, iRGD, the sequence of which is CRGDKGPDPC, has been developed. iRGD could be recruited to cell surfaces through the RGD-integrins interactions, followed by proteolytic cleavage to generate a C-terminal RGDK/R sequence, which was able to bind to neuropilin-1 (NRP-1), expressed by tumor vasculature and tumor cells to trigger the subsequent NRP-1 dependent transcytosis and tumor penetration. Conjugation of the iRGD peptide to the NPs surface facilitated the extravasation and penetration of the delivery system. iRGD peptide-modified mesoporous silica NPs (iMSNs), which encapsulate siP1k1 and miR-200c, showed deep tumor penetration and a significant suppression of the primary tumor growth and marked reduction of metastasis. iRGD peptide conjugated MSN have also been used to deliver combination therapy, which consisted of an antiangiogenic agent (CA4) and a chemotherapeutic drug (DOX), leading to a differentiated drug release process. CA4 was released majorly at the tumor vasculature while DOX was released mostly within the tumor cells at a lower pH value. iRGD induced tumor penetration did not require the NPs to be chemically conjugated to the iRGD. Instead, co-administration was enough to enhance NP’s penetration to TME and increase efficacy of anticancer drugs while reducing their side effects. It has been reported that NPs combined with anti-PD-L1 and coadministered iRGD, which conferred augmented tumor-targeting efficiency and strong breast cancer suppression. Moreover, Hu et al. demonstrated that coadministration of iRGD with multistage-responsive NPs led to improved drug delivery efficiency and inhibited 4T1 breast cancer progression. Surprisingly, in certain tumor models, such as orthotopic tumor model, co-delivery administration, in which iRGD peptide was not conjugated to irinotecan-loaded MSN achieved better efficacy, as shown by the number of NPs and travel distance in tumors, than iRGD-conjugated MSN delivery. One possible explanation is that conjugated NPs are limited by the relatively small and finite number of target receptors on the vasculature, while separate injection of the unconjugated peptide triggers bulk transfer of bystander NPs (in greater number) at the tumor site.

In tumor vasculature, Dil4 is over-expressed to regulate tumor angiogenesis. The highly up-regulated Dil4 on the membrane of tumor vasculature makes Dil4 an attractive target for the NP-based delivery system to achieve targeted therapy. GD16 peptide (H2N–GRCTNFHNIYICFPD–CONH2) specifically binds to Dil4 in tumor vasculature with high affinity. In this example, NPs consist of aldehyde poly (ethylene glycol)-poly (lactide–aldehyde-PEG-PLA) and MPEG-PLA block copolymers. Attaching GD16 to NPs loading an anticancer drug, paclitaxel (PTX), to form GD16–PTX-NP conjugates, which could specifically target the Dil4, lead to active transcytosis mediated by GD16-Dil4 interaction and accurate in vivo tumor neovasculature targeting property.

4.3.2. Immune cell-based delivery

Immune cell infiltration into tumor is a key prognostic marker for tumor progression. Immune cells in blood extravasate and penetrate TME through a highly orchestrated process of diapedesis, which can be either transcellular or paracellular route. Due to their intrinsic trafficking ability to infiltrate TME, a range of immune cells, such as monocytes, neutrophils, macrophages and lymphocytes, have been used as “trojan horse” for tumor-targeted delivery of anticancer therapeutics. In addition, stem cells have also shown potentials to act as carriers for tumor-targeted delivery of NP-based therapeutics. Neutrophils are one of the most abundant immune cells in a wide variety of cancer types, contributing to progression in solid tumors, especially in those with advanced-stage diseases, and serving as a robust biomarker of poor clinical outcome in various cancers. Neutrophils have been used to carry anticancer drugs containing liposomes NPs to treat glioblastoma. PTX was first encapsulated into cationic liposomes (PTX-CL), which have then been merged with NEs to form PTX-CL/NEs. Tumor-associated inflammation promoted PTX-CL/NEs to extravasate brain blood vessels and infiltrate into glioblastoma, leading to inhibition of tumor recurrence.

Migrating throughout tumor tissues in search of antigen is a normal function of lymphocytes. Engineered lymphocytes, such as T cells, with tissue-homing receptors could serve as an effective vehicle for delivering chemotherapy drugs to tumor-ridden organs. By homing into these tumor sanctuary sites and distributing throughout the tissue, each NP-carrying cell would serve as a local micro-depot of drug to dose surrounding tumor cells. In an interesting example of this, lymphocytes were used as carriers to target drug-loaded NPs to the lymphoid tissue sites where lymphomas home. Topoisomerase I poison SN-38 is a potent cytotoxic agent against Eμ–myc lymphoma. However, free or even liposome-formulated SN-38 failed to effectively reach the sites of lymphoma when administered systemically. NP-decorated T cells have been developed to deliver SN-38 to lymphoid tissue—homing lymphomas, leading to improvement in the therapeutic efficacy of...
SN-38 without toxicity\textsuperscript{109}.

Platelets also play a crucial role in tumor angiogenesis and carcinogenesis through recognizing exposed ECM and inflammation. Instead of using platelets, a platelet membrane (PM)-coated NPs (PM-NPs) have been accordingly developed\textsuperscript{172,173}. The core NPs were made by a single emulsion method and crosslinked by an acid-degradable crosslinker. The platelet membranes were derived from platelets and purified to coat the surface of NPs. Further functionalizing PM-NPs with an RGD peptide enhanced its specificity to target integrins (i.e., αvβ3) within tumor blood vessels. This system has successfully delivered antitumor drugs including DOX and PXL into multiple within tumor blood vessels. This system has successfully delivered antitumor drugs including DOX and PXL into multiple mouse tumor models, and showed high tumor penetration and targeting, with reduced off-target effects\textsuperscript{109}.

Some critical components in the cells can also be employed as effective drug delivery strategies. It has been recognized that phospholipid bilayer-enclosed extracellular vesicles (EVs) serve as a competent carrier for numerous biomolecules including proteins, lipids, DNA and a list of RNA species, owing to that EVs are able to efficiently transverse distinct extents of biological barriers, deliver their cargo, and trigger a response in their recipient cells\textsuperscript{174}. Exosomes as a subgroup of EVs are regarded as ideal natural nanocarriers for clinical applications as a result of their naturally biocompatible properties. For example, Kamerkar et al.\textsuperscript{174} showed the capability of exosomes to efficiently deliver KrasG12D siRNA to target undruggable oncogenic Kras in pancreatic tumor cells \textit{in vivo}.

5. Conclusions

In the past few years, a large body of preclinical evidence and some clinical studies have uncovered the presence of the “normalized” vessel phenotype as well as its relevance to tumor progression and cancer therapy\textsuperscript{22,24}. It has been well accepted that vascular normalization occurs under the circumstance that the balance between pro- and anti-angiogenic signaling is restored, which can be achieved either through repression of angiogenic factors or reinforcement of antiangiogenic activity\textsuperscript{17}. In particular, numerous studies have demonstrated that antiangiogenic drugs commonly render a “normalization window”, which represents a time frame of the presence of normalized vessel phenotype. To this end, prolonged inhibition of angiogenesis contributes to the shutdown of normalization window, which may be due to the fact that the balance favours antiangiogenic factors and thus results in the substantial pruning of vascular system. Furthermore, appropriate dosing of antiangiogenic drugs is essential for maintaining vascular normalization. Using a judicious dose of antiangiogenic agents would be able to alleviate some abnormal vessels and remodel the rest leading to normalized vessels, avoiding the appearance of resistance to antiangiogenic therapy. Based on the characteristics of vascular normalization, an increasing number of antiangiogenic drugs have been developed and utilized in a manner of new paradigm for treating cancer, which brought in great benefit for cancer patients. Generally, tumor vascular normalization hypothesis has recently opened a door for the rational and appropriate usage of antiangiogenic drugs and combination with chemotherapeutics. Nevertheless, efforts to seize the normalization window have restricted the advancement of vascular normalization therapy in clinical settings due to the lack of circulating biomarkers and the obsessional dosage regimes\textsuperscript{177}.

As discussed previously, the state of vascular normalization is commonly transient. In such a situation, tumor is prone to be more receptive to the delivery of anticancer drugs. However, there seems to be a high extent of variation in the temporal window of vascular normalization for various antiangiogenic drugs\textsuperscript{176}. To this end, the development of delivery strategy and drug design would be able to lead vascular normalization toward a more effective and successful clinical cancer therapy in the future.

NP-based nanomedicine shows great promising to treat cancer. However, safety, efficacy and targeted delivery remain concerns. Recently, inorganic NPs have been shown to disrupt vascular endothelial barrier function and promote metastasis\textsuperscript{177}. A full understanding of the nano–bio interactions will lead to safer nanotherapeutics. Active targeting through transcytosis, especially those with multiple functionalities, has the potential to overcome various biological barriers to tumor sites with improved target specificity, leading to enhancement in efficacy and reduction in the systematic administration-induced off-target side effects. However, they are much more complex, which may face challenges in the manufacturing process and getting approval from regulatory authorities. Future direction will focus on the NPs with a simple design, great biocompatibility and stability, high specificity with the capability to deliver large payloads of therapeutics to targeted sites in tumors.

Author contributions

Jia Li was responsible for the conception and design of the review. Yang Zhao and Jia Li drew the figures and tables. Yang Zhao, Xiangrong Yu and Jia Li drafted and revised the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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