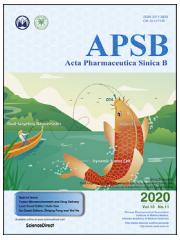
Acta Pharmaceutica Sinica B 2020;10(11):2018–2036

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

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

Yang Zhao[a], **Xiangrong Yu**[b], **Jia Li**[c,*]

[a]*Centre for the Endothelium, Vascular Biology Program, Centenary Institute, The University of Sydney, Sydney NSW 2042, Australia*
[b]*Department of Radiology, Zhuhai People's Hospital, Jinan University, Zhuhai 519000, China*
[c]*Department of Biomedical Sciences, Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney NSW 2109, Australia*

Received 29 June 2020; received in revised form 25 August 2020; accepted 31 August 2020

KEY WORDS

Immune–vascular crosstalk;
Vascular normalization;
Nanoparticles;
Transcytosis;
Immune cells;
Antiangiogenesis;
Immunotherapy;
Tumor microenvironment

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.

*Corresponding author.
E-mail address: jia.li1@mq.edu.au (Jia Li).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2020.09.014

1. Introduction

Tumor vasculature 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[1]. Tumor vasculature 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[2,3]. 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[4]. More importantly, normalization of the tumor vasculature has been elucidated to promote the effectiveness of immunotherapies 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[5,6].

Nanoparticles (NPs) can be tailored to meet a broad range of specific application requirements[7]. 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[8]. 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[9].

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 fulfil 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 anticancer therapy[10]. 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[11,12]. 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[13]. Despite tumor angiogenesis is meant to supply nutrients and blood to the tumor required for energy demand and growth[14], the angiogenic vessels of tumors show chaotic architecture and disturbed barrier function resulting in a hyperpermeable state, and lack of perfusion etc[13]. 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[15,16].

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[4]. 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[13,17]. 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 lumens[18]. 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[19]. Furthermore, vessel walls with uneven diameters include plentiful trans-cellular openings and fenestrations owing in part to compression of the immature walls[20]. 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[21]. 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[22,23]. Nevertheless, two-photon microscopy is regarded as a better option in terms of cellular and extracellular imaging due to its superior spatial resolution[24,25].

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)[26]. 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[27]. 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[28]. 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[29]. At least four signaling pathways participate in the recruitment of mural cells, they include (a) platelet-derived growth factor B (PDGF-B) and PDGF receptor β (PDGFR-β) signaling; (b) sphingosine-1-phosphate-1 (S1P1) and endothelial differentiation sphingolipid G-protein-coupled receptor 1 (EDG1) signaling; (c) Ang1 and Tie2 signaling; and (d) transforming growth factor (TGF)-β signaling[30].

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[31]. 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[32], while other studies show that it exists but is morphologically aberrant[33]. 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[34].

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[35]. 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[36]. 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[37]. 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[38]. 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[39].

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[40]. 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[41]. 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[42]. 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[43].

Tumor hypoxia is a consequence of inadequate oxygen supply induced by extensive abnormalities in the vascular network. Further, the leakiness 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[44]. Chronic hypoxia is commonly due to elevated diffusion distances from capillaries to cells within the disorganized tumor vasculature, resulting in cells far from capillaries to receive less oxygen than needed. It is noteworthy that uncontrolled activation of hypoxia signaling, especially hypoxia-inducible factor-1 (HIF-1), in tumor mass frequently leads to an abnormal, chaotic vascularization that fails to compensate oxygen deficiency[45].

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[3,46]. 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 chemotherapeutics and immunotherapeutic entities, but facilitate attraction of immunosuppressive immune cells (myeloid-derived suppressor cells, neutrophils, Tregs, etc.)[6,47]. 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[15]. 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[48]. 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[49]. 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[50]. To this end, angiogenic blood vessels can be important therapeutic

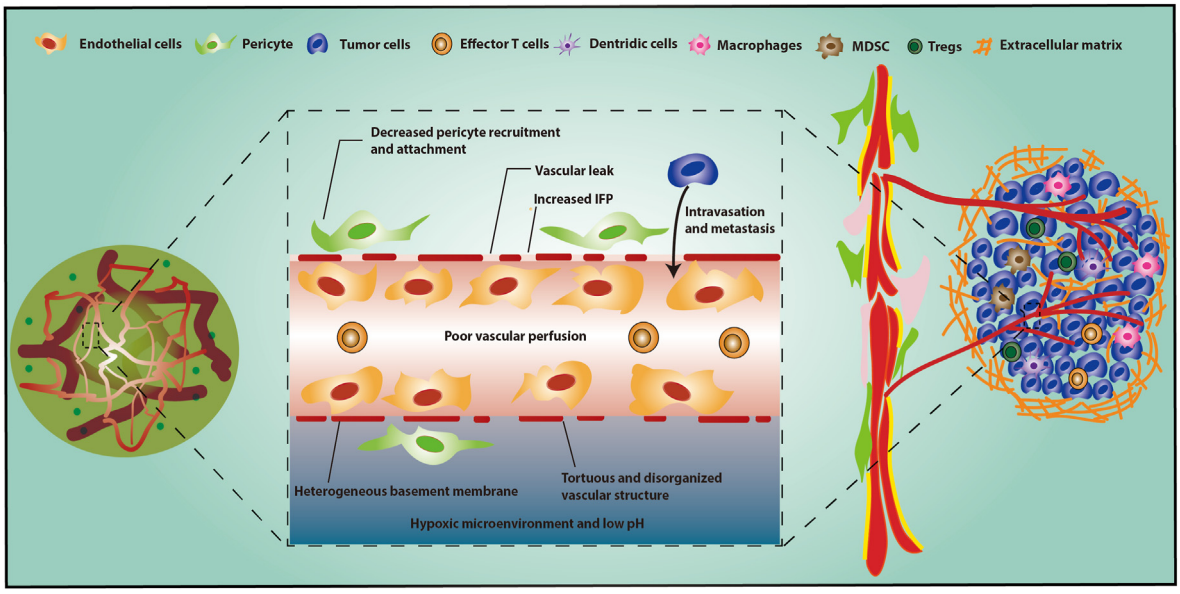


Figure 1 Abnormal tumor vasculature creates immune-suppressive microenvironment. Tumor blood vessels are frequently structurally disorganized and tortuous with poor pericyte coverage and abnormal basement membrane support. Functionally, the blood flow appears to be erratic with heterogeneous distribution 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).

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 reprograming 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[6,51–59].

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[2]. 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[4]. With the correction of abnormal vasculature, the TME can be normalized and ultimately tumor progression is controlled and response to other therapies is improved[13]. 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[61] (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

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
	LTβR	53
	Effector T cell-derived TNFα and LTa	53
Inhibition of endothelial anergy	VCAM1, ICAM1, SELE	54
	Suppression of VEGF-A and b-FGF	55
	Blocking endothelin-1/endothelin-B receptor	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	6

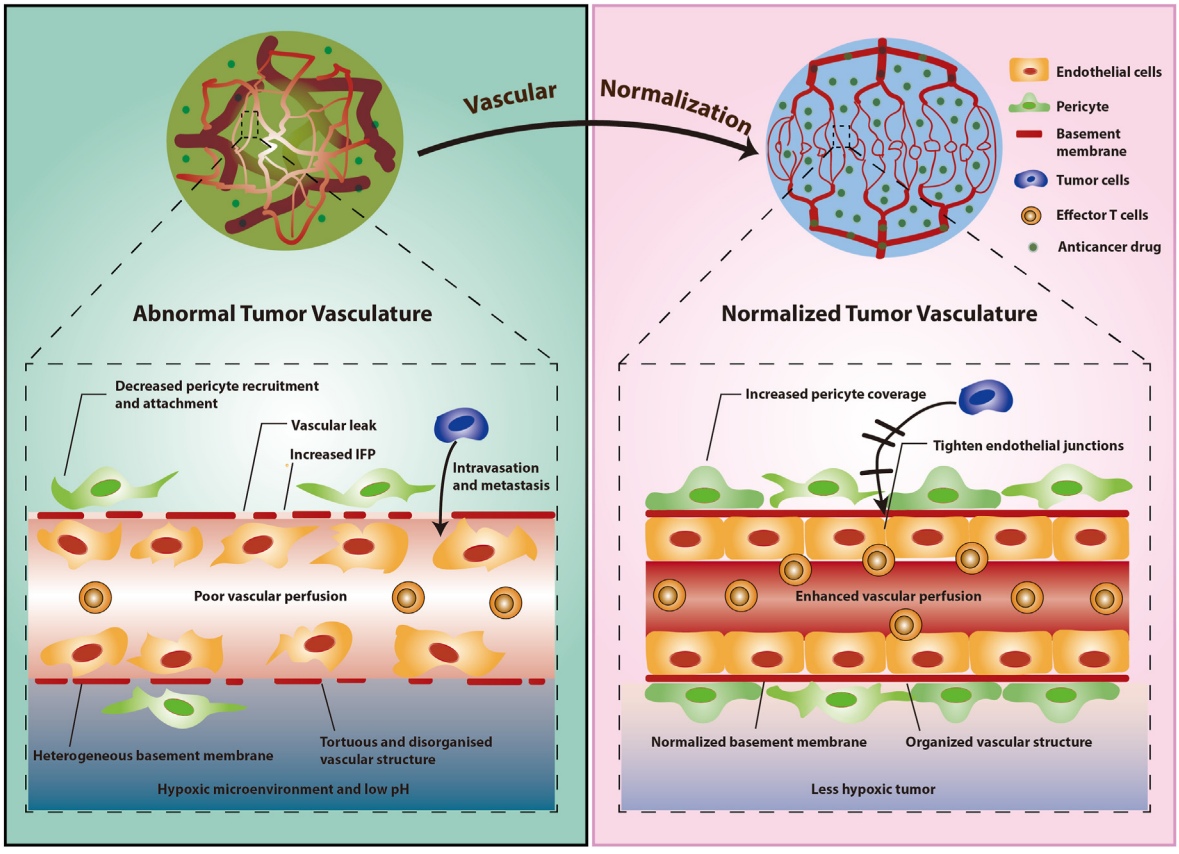


Figure 2 Vascular normalization emerges as a novel anticancer therapeutic strategy. Vascular normalization can reverse the angiogenic blood vessels back towards a mature and stabilized vasculature. The normalized tumor vasculature exhibits enhanced pericyte coverage and a more normal basement membrane support phenotype. The fortified vascular structure also gives rise to the normalized vascular function, which can be reflected by improved vascular perfusion and decreased vascular permeability and hypoxic state. Vascular normalization facilitates the drug delivery and immune cell infiltration, providing the opportunity to develop new combination therapy.

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[50].

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[63]. 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[64]. 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[51]. 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 HEV^{high} tumors than HEV^{low} tumors[52]. 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 LTa, which bind to stromal TNF receptors, thereby bypassing LTβR signaling[53].

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[54]. 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[55]. 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[65,66]. 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[67].

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[5]. 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[56], implying the fundamental role of immune reprograming 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.[6] 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[68]. Further, Yang et al.[57] 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[57], 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[69].

Based on the fact that immune reprograming 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 VEGFR2 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)[70]. Also, the combination of immune checkpoint anti-CTLA-4 blockade (ipilimumab) 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[71]. 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[72].

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[73].

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[58,74]. 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[75]. 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[15,76].

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

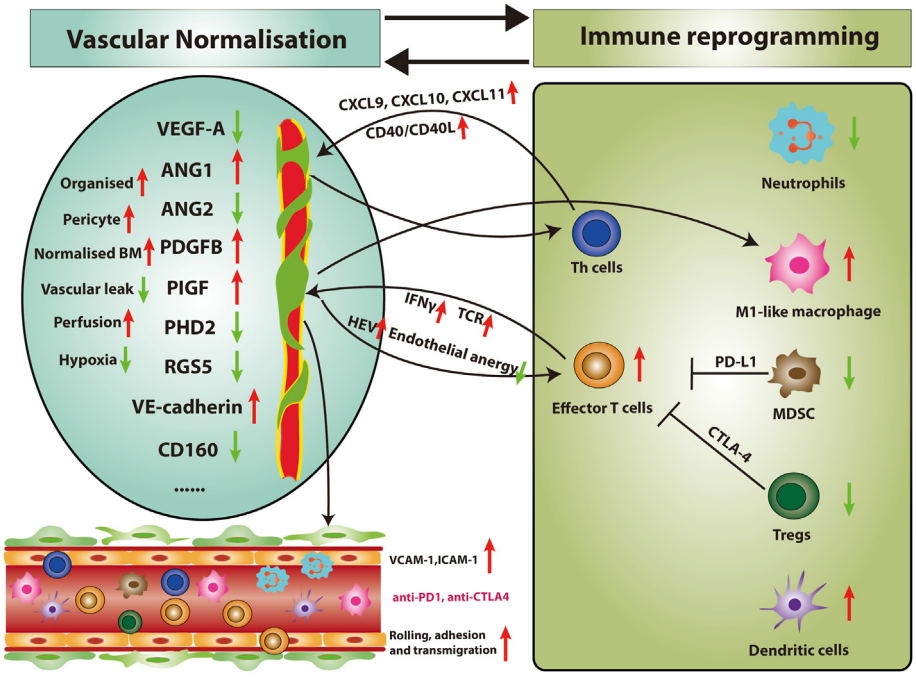


Figure 3 Vascular normalization and immune reprogramming form a positive feedback loop. Tumor vascular normalization mediated by inhibition of some critical angiogenic factors (*e.g.*, VEGF-A and ANG2) or regulation of various pivotal molecules (*e.g.*, PHD2 and RGS5) enhances the infiltration of T cells 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.

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[77–79]. 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[80].

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 (Fig. 3 and Table 1). 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)[81]. Nevertheless, the most fundamental T cell derived factors including TNF, TGF-β, and IFNs, on the opposite, present anti-angiogenic functions[82–84]. Of note, T cells especially Th1 cells secrete IFNγ, whose presence in the tumors is often linked to good prognosis[85]. 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[59]. More interestingly, IFNγ and surface molecule CD40L could upregulate the endothelial adhesion molecules including ICAM1 and SELE, which induce the infiltration of T cells. IFNγ 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[6].

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[86]. 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[87]. 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[88]. 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

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[87,89].

Notably, hypoxic tumor cells, largely due to the abnormal tumor blood vessels with limited diffusion and perfusion, are resistant to chemotherapy[90]. 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[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 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[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 systematically. 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[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.

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 MnO_2 NPs	102
	siVEGF/M2pep conjugated AuNPs	103
Enhance extravasation of nanomedicine into tumor		
Transcytosis-based delivery		
Adsorptive-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 GD16/Dll4 by:	
	PTX/GD16 conjugated aldehyde-PEG-PLA and MPEG-PLA block copolymers	107
Immune cell-based delivery	PTX/cationic liposomes (PTX-CL)-NEs to form PTX-CL/NEs	108
	Liposome-formulated SN-38/ lymphocytes	109
	DOX and PXL/RGD conjugated platelet membrane (PM)-coated nanoparticles (PM-NP)	110

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[13,111]. 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[112]. For example, Huang et al.[3] 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

administration of antiangiogenic agents was required to be confined to a certain therapeutic window. Furthermore, Willett et al.[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[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[94]. 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[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[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[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[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[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[117]. Goel et al.[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 stronger inhibition of tumor growth compared with VEGF inhibition alone[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[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[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[99].

4.1.3. *Targeting other angiogenesis-associated signaling*

Platelet-derived growth factor receptor-B (PDGFB), expressed by endothelial cells, is a well-characterized recruitment signal for pericytes that mainly produce PDGF receptor-β (PDGFRβ)[121]. It has been reported that silencing of PDGF-B signaling decreases pericyte recruitment, which further contributes to vascular permeability[122]. Therefore, restoration of the PDGF-B signaling axis is a pivotal means to fulfil 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 natural occurring tumors[123]. 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 electrospun (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[100]. 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[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[125].

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

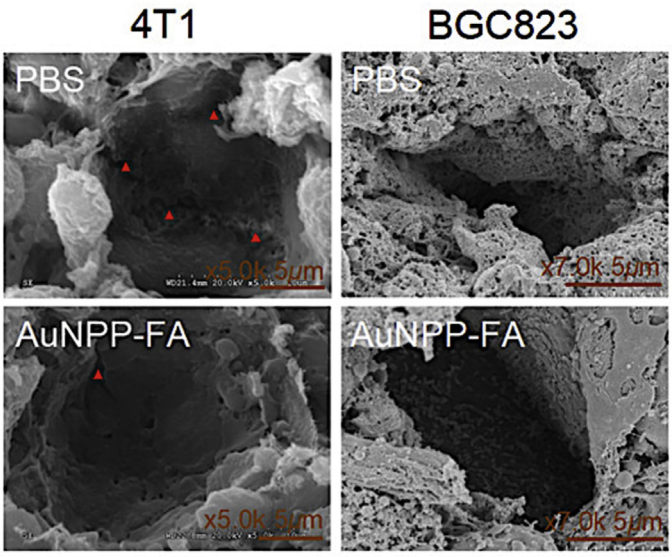


Figure 4 Effects of gold nanoparticles (AuNPs) on tumor vascular normalization. AuNPP-FA normalized tumor vasculature in both 4T1 and BGC823 tumor models. Reprinted with the permission from Ref. 134. Copyright © 2020 American Chemical Society.

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[126]. 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[127]. 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[29]. 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[128]. 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[129]. In addition, Magrini et al.[130] 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 blockage 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[131]. Notably, restoration of endothelial barrier integrity especially inhibiting vascular permeability has been regarded as an effective strategy to achieve vascular normalization. Zhao et al.[132] and Li et al.[133] 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 (Fig. 4[134]). Similar to what Huang et al.[134] and Zhao et al.[132] 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.[135] 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[136]. 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.[137] 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

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[3].

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[138]. 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[139]. 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 electrostatics of NPs. This co-delivery of DOX and IFNγ resulted excellent synergistic antitumor efficiency against B16F10 tumor bearing mice.

Also, Johansson et al.[140] found that low-dose TNFα treatment stabilized the vascular network and enhanced vascular perfusion in the RIP1-Tag5 pancreatic neuroendocrine tumors. More importantly, this inflammatory vascular remodelling induced by low-dose TNFα tremendously boosted the therapy of antitumor vaccination or adoptive T-cell transfer. 3-aminopropyltriethoxysilane (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[141].

A growing body of evidence has uncovered that tumor associated macrophages (TAMs) with pro-angiogenic and immune-suppressive (M2-like) phenotypes are indications of malignant progression[142]. TAM induces tumor vascularization and overexpression of angiogenic VEGF while promoting immunosuppression within the TME. Rolny et al.[23] 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 PIGF. Furthermore, eosinophils are also thought to be necessary components of the immune microenvironment that regulates tumor initiation and progression. It has been shown that activated tumor-associated eosinophils were essential for tumor rejection through secreting chemoattractants 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[143].

Despite that TAMs represent an appealing target for cancer therapy, targeting TAMs, such as inhibition of macrophage recruitment, or enhancement of tumuoricidal activity of macrophages approach has been far from clinic. One of the major obstacles 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[101]. TAMs are highly accumulated in hypoxic regions of tumors. Attenuating tumor hypoxia can be used as a strategy to prime TAMs toward M1-like phenotype, leading to inhibition of tumor growth[102]. Mannose receptor is highly expressed in TAM-M2 macrophages. Conjugation of mannan guide manganese dioxide NPs (MnO2 NPs) to the M2-like TAMs in hypoxic area and reacting with hydrogen peroxide (H_2O_2) to produce O_2, led to the alleviation of hypoxia and regulation of pH in the hypoxic region. Further modification of NPs with hyaluronic acid (HA) reprogram anti-inflammatory, pro-tumoral M2-like TAMs to pro-inflammatory, antitumor M1-like TAMs to further enhance the ability of MnO_2 NPs to lessen tumor hypoxia and modulate chemoresistance. The HA-coated, mannan-conjugated MnO_2 particle (Man-HA-MnO_2) treatment significantly increased tumor oxygenation and down-regulated HIF-1α and VEGF in tumors. Combination treatment of the tumors with Man-HA-MnO_2 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, receptors for folate and mannan 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 tumor 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[103]).

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 nanomedicine hard to reach cancer cells deep in the tumor[144].

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 vessel 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[145,146].

In addition, remodelling of tumor extracellular matrix also promotes tumor-targeting efficiency of NP drug carriers[147]. In

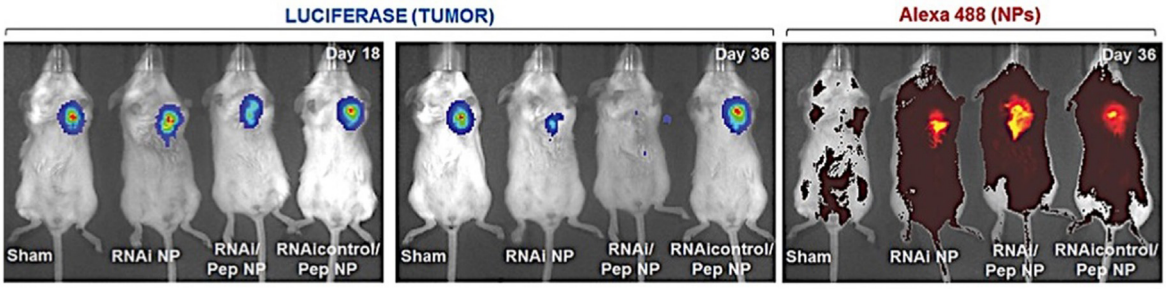


Figure 5 Live imaging of BALB/c nude mice bearing A549-luciferase-C8 human lung adenocarcinoma tumors non-treated (Sham) and treated with both RNAi- and RNAi-M2pep-AuNPs and with RNAicontrol-M2pep-AuNPs (dose of 0.05 mg/kg of siRNA). Reprinted with the permission from Ref. 103. Copyright © 2015 John Wiley & Sons, Inc.

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-β/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[148]. 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[144]. 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[149].

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[150]. 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"[151]. 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[152].

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[153]. 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[154]. In addition, immune cells have been found to enrich in solid tumors, either promote or inhibit tumor development[155]. Therefore, immune cells can act as a "trojan horse" to deliver NP-based therapeutics into the targeted region in tumors[156]. 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[153]. 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[157].

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[104]. 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[158]. Zhou et al.[104] 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 tumors. In cells, γ-glutamyl-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[104].

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 αv family, including αvβ3, αvβ5, and α5β1, are preferentially expressed on tumor vasculature. RGD (Arg−Gly−Asp) is a peptide that specifically binds to integrin αv receptors. Therefore, ligands containing the RGD integrin recognition motif can lead ligands-NPs conjugates to tumor vasculature[159,160]. However, RGD-integrin αv interaction can only accumulate therapeutics in the tumor vasculature without penetration into TME. To overcome this, iRGD, the sequence of which is CRGDKGPDC, 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[161,162]. 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 siPlk1 and miR-200c, showed deep tumor penetration and a significant suppression of the primary tumor growth and marked reduction of metastasis[105]. 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[106]. 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[163]. 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[164]. Moreover, Hu et al.[160] 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[162]. 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, Dll4 is over-expressed to regulate tumor angiogenesis. The highly up-regulated Dll4 on the membrane of tumor vasculature makes Dll4 an attractive target for the NP-based delivery system to achieve targeted therapy. GD16 peptide (H_2N–GRCTNFHNFIYICFPD–$CONH_2$) specifically binds to Dll4 in tumor vasculature with high affinity[165]. 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 Dll4, lead to active transcytosis mediated by GD16-Dll4 interaction and accurate *in vivo* tumor neovasculature targeting property[107].

4.3.2. Immune cell-based delivery

Immune cell infiltration into tumor is a key prognostic marker for tumor progression[156]. Immune cells in blood extravasate and penetrate TME through a highly orchestrated process of diapedesis, which can be either transcellular or paracellular route[166]. 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[109,167,168]. In addition, stem cells have also shown potentials to act as carriers for tumor-targeted delivery of NP-based therapeutics[169]. 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[170,171]. Neutrophils have been used to carry anticancer drugs containing liposomes NPs to treat glioblastoma[108]. 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[108].

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[109]. 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[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[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.*, $\alpha v\beta 3$) 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[110].

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[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.[174] showed the capability of exosomes to efficiently deliver KrasG12D siRNA to target undruggable oncogenic *Kras* in pancreatic tumor cells *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[2,4]. 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[13]. 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[3]. 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[175]. 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[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[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.

References

1. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;**285**:1182–6.
2. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat Med* 2001;**7**:987–9.
3. Huang Y, Yuan J, Righi E, Kamoun WS, Ancukiewicz M, Nezivar J, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. *Proc Natl Acad Sci U S A* 2012;**109**:17561–6.
4. Goel S, Duda DG, Xu L, Munn LL, Boucher Y, Fukumura D, et al. Normalization of the vasculature for treatment of cancer and other diseases. *Physiol Rev* 2011;**91**:1071–121.
5. Huang Y, Kim BYS, Chan CK, Hahn SM, Weissman IL, Jiang W. Improving immune-vascular crosstalk for cancer immunotherapy. *Nat Rev Immunol* 2018;**18**:195–203.
6. Tian L, Goldstein A, Wang H, Ching Lo H, Sun Kim I, Welte T, et al. Mutual regulation of tumour vessel normalization and immunostimulatory reprogramming. *Nature* 2017;**544**:250–4.
7. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnol* 2018;**16**:71.
8. Greish K. Enhanced permeability and retention (EPR) effect for anticancer nanomedicine drug targeting. *Methods Mol Biol* 2010;**624**:25–37.

9. Liu X, Jiang J, Meng H. Transcytosis - an effective targeting strategy that is complementary to "EPR effect" for pancreatic cancer nano drug delivery. *Theranostics* 2019;**9**:8018−25.
10. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med* 2013;**19**:1423−37.
11. Lanitis E, Dangaj D, Irving M, Coukos G. Mechanisms regulating T-cell infiltration and activity in solid tumors. *Ann Oncol* 2017;**28**: xii18−32.
12. Anderson KG, Stromnes IM, Greenberg PD. Obstacles posed by the tumor microenvironment to T cell activity: a case for synergistic therapies. *Canc Cell* 2017;**31**:311−25.
13. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 2005;**307**:58−62.
14. Jain RK. Normalizing tumor microenvironment to treat cancer: bench to bedside to biomarkers. *J Clin Oncol* 2013;**31**:2205−18.
15. Lanitis E, Irving M, Coukos G. Targeting the tumor vasculature to enhance T cell activity. *Curr Opin Immunol* 2015;**33**:55−63.
16. Hendry SA, Farnsworth RH, Solomon B, Achen MG, Stacker SA, Fox SB. The role of the tumor vasculature in the host immune response: implications for therapeutic strategies targeting the tumor microenvironment. *Front Immunol* 2016;**7**:621.
17. De Bock K, De Smet F, Leite De Oliveira R, Anthonis K, Carmeliet P. Endothelial oxygen sensors regulate tumor vessel abnormalization by instructing phalanx endothelial cells. *J Mol Med* 2009;**87**:561−9 (Berl).
18. Lin MI, Yu J, Murata T, Sessa WC. Caveolin-1-deficient mice have increased tumor microvascular permeability, angiogenesis, and growth. *Cancer Res* 2007;**67**:2849−56.
19. Aird WC. Endothelial cell heterogeneity. *Cold Spring Harb Perspect Med* 2012;**2**:a006429.
20. Jain RK. Molecular regulation of vessel maturation. *Nat Med* 2003;**9**: 685−93.
21. Sabo E, Boltenko A, Sova Y, Stein A, Kleinhaus S, Resnick MB. Microscopic analysis and significance of vascular architectural complexity in renal cell carcinoma. *Clin Canc Res* 2001;**7**:533−7.
22. Ewing JR, Brown SL, Lu M, Panda S, Ding G, Knight RA, et al. Model selection in magnetic resonance imaging measurements of vascular permeability: gadomer in a 9L model of rat cerebral tumor. *J Cerebr Blood Flow Metabol* 2006;**26**:310−20.
23. Rolny C, Mazzone M, Tugues S, Laoui D, Johansson I, Coulon C, et al. HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through down-regulation of PlGF. *Canc Cell* 2011;**19**:31−44.
24. Reitan NK, Thuen M, Goa PE, de Lange Davies C. Characterization of tumor microvascular structure and permeability: comparison between magnetic resonance imaging and intravital confocal imaging. *J Biomed Optic* 2010;**15**:036004.
25. Weissleder R, Pittet MJ. Imaging in the era of molecular oncology. *Nature* 2008;**452**:580−9.
26. Stratman AN, Pezoa SA, Farrelly OM, Castranova D, Dye 3rd LE, Butler MG, et al. Interactions between mural cells and endothelial cells stabilize the developing zebrafish dorsal aorta. *Development* 2017;**144**:115−27.
27. Raza A, Franklin MJ, Dudek AZ. Pericytes and vessel maturation during tumor angiogenesis and metastasis. *Am J Hematol* 2010;**85**: 593−8.
28. Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK, McDonald DM. Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *Am J Pathol* 2002;**160**:985−1000.
29. Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat Rev Drug Discov* 2011;**10**:417−27.
30. Jain RK, Booth MF. What brings pericytes to tumor vessels?. *J Clin Invest* 2003;**112**:1134−6.
31. Chen D, Teng JM, North PE, Lapinski PE, King PD. RASA1-dependent cellular export of collagen IV controls blood and lymphatic vascular development. *J Clin Invest* 2019;**129**:3545−61.
32. Paulus W, Roggendorf W, Schuppan D. Immunohistochemical investigation of collagen subtypes in human glioblastomas. *Virchows Arch A Pathol Anat Histopathol* 1988;**413**:325−32.
33. Farnoud MR, Lissak B, Kujas M, Peillon F, Racadot J, Li JY. Specific alterations of the basement membrane and stroma antigens in human pituitary tumours in comparison with the normal anterior pituitary. An immunocytochemical study. *Virchows Arch A Pathol Anat Histopathol* 1992;**421**:449−55.
34. Baluk P, Morikawa S, Haskell A, Mancuso M, McDonald DM. Abnormalities of basement membrane on blood vessels and endothelial sprouts in tumors. *Am J Pathol* 2003;**163**:1801−15.
35. Hida K, Hida Y, Amin DN, Flint AF, Panigrahy D, Morton CC, et al. Tumor-associated endothelial cells with cytogenetic abnormalities. *Cancer Res* 2004;**64**:8249−55.
36. Gillies RJ, Schomack PA, Secomb TW, Raghunand N. Causes and effects of heterogeneous perfusion in tumors. *Neoplasia* 1999;**1**: 197−207.
37. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. *Nat Rev Clin Oncol* 2010;**7**:653−64.
38. Fukumura D, Jain RK. Tumor microenvironment abnormalities: causes, consequences, and strategies to normalize. *J Cell Biochem* 2007;**101**:937−49.
39. Curnis F, Sacchi A, Corti A. Improving chemotherapeutic drug penetration in tumors by vascular targeting and barrier alteration. *J Clin Invest* 2002;**110**:475−82.
40. Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, et al. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. *Proc Natl Acad Sci U S A* 1998;**95**:4607−12.
41. Nagy JA, Dvorak AM, Dvorak HF. Vascular hyperpermeability, angiogenesis, and stroma generation. *Cold Spring Harb Perspect Med* 2012;**2**:a006544.
42. Azzi S, Hebda JK, Gavard J. Vascular permeability and drug delivery in cancers. *Front Oncol* 2013;**3**:211.
43. Kumar P, Shen Q, Pivetti CD, Lee ES, Wu MH, Yuan SY. Molecular mechanisms of endothelial hyperpermeability: implications in inflammation. *Expet Rev Mol Med* 2009;**11**. e19.
44. Siemann DW, Horsman MR. Modulation of the tumor vasculature and oxygenation to improve therapy. *Pharmacol Ther* 2015;**153**: 107−24.
45. Vaupel P. Tumor microenvironmental physiology and its implications for radiation oncology. *Semin Radiat Oncol* 2004;**14**:198−206.
46. Schaaf MB, Garg AD, Agostinis P. Defining the role of the tumor vasculature in antitumor immunity and immunotherapy. *Cell Death Dis* 2018;**9**:115.
47. Chouaib S, Noman MZ, Kosmatopoulos K, Curran MA. Hypoxic stress: obstacles and opportunities for innovative immunotherapy of cancer. *Oncogene* 2017;**36**:439−45.
48. Wei SC, Anang NAS, Sharma R, Andrews MC, Reuben A, Levine JH, et al. Combination anti-CTLA-4 plus anti-PD-1 checkpoint blockade utilizes cellular mechanisms partially distinct from monotherapies. *Proc Natl Acad Sci U S A* 2019;**116**:22699−709.
49. Barrett DM, Grupp SA, June CH. Chimeric antigen receptor- and TCR-modified T cells enter main street and wall street. *J Immunol* 2015;**195**:755−61.
50. Johansson-Percival A, He B, Ganss R. Immunomodulation of tumor vessels: it takes two to tango. *Trends Immunol* 2018;**39**:801−14.
51. Johansson-Percival A, He B, Li ZJ, Kjellen A, Russell K, Li J, et al. *De novo* induction of intratumoral lymphoid structures and vessel normalization enhances immunotherapy in resistant tumors. *Nat Immunol* 2017;**18**:1207−17.
52. Martinet L, Garrido I, Filleron T, Le Guellec S, Bellard E, Fournie JJ, et al. Human solid tumors contain high endothelial venules: association with T- and B-lymphocyte infiltration and favorable prognosis in breast cancer. *Cancer Res* 2011;**71**:5678−87.
53. Peske JD, Thompson ED, Gemta L, Baylis RA, Fu YX, Engelhard VH. Effector lymphocyte-induced lymph node-like

vasculature enables naive T-cell entry into tumours and enhanced anti-tumour immunity. *Nat Commun* 2015;**6**:7114.
54. Buckanovich RJ, Facciabene A, Kim S, Benencia F, Sasaroli D, Balint K, et al. Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. *Nat Med* 2008;**14**:28−36.
55. Tanaka T, Sho M, Takayama T, Wakatsuki K, Matsumoto S, Migita K, et al. Endothelin B receptor expression correlates with tumour angiogenesis and prognosis in oesophageal squamous cell carcinoma. *Br J Canc* 2014;**110**:1027−33.
56. Zheng X, Fang Z, Liu X, Deng S, Zhou P, Wang X, et al. Increased vessel perfusion predicts the efficacy of immune checkpoint blockade. *J Clin Invest* 2018;**128**:2104−15.
57. Yang H, Lee WS, Kong SJ, Kim CG, Kim JH, Chang SK, et al. STING activation reprograms tumor vasculatures and synergizes with VEGFR2 blockade. *J Clin Invest* 2019;**129**:4350−64.
58. Ohm JE, Gabrilovich DI, Sempowski GD, Kisseleva E, Parman KS, Nadaf S, et al. VEGF inhibits T-cell development and may contribute to tumor-induced immune suppression. *Blood* 2003;**101**:4878−86.
59. Strieter RM, Burdick MD, Gomperts BN, Belperio JA, Keane MP. CXC chemokines in angiogenesis. *Cytokine Growth Factor Rev* 2005;**16**:593−609.
60. Duda DG, Jain RK, Willett CG. Antiangiogenics: the potential role of integrating this novel treatment modality with chemoradiation for solid cancers. *J Clin Oncol* 2007;**25**:4033−42.
61. Mattheolabakis G, Mikelis CM. Nanoparticle delivery and tumor vascular normalization: the chicken or the egg?. *Front Oncol* 2019;**9**:1227.
62. Bonaventura P, Shekarian T, Alcazer V, Valladeau-Guilemond J, Valsesia-Wittmann S, Amigorena S, et al. Cold tumors: a therapeutic challenge for immunotherapy. *Front Immunol* 2019;**10**:168.
63. Ager A. High endothelial venules and other blood vessels: critical regulators of lymphoid organ development and function. *Front Immunol* 2017;**8**:45.
64. Hindley JP, Jones E, Smart K, Bridgeman H, Lauder SN, Ondondo B, et al. T-cell trafficking facilitated by high endothelial venules is required for tumor control after regulatory T-cell depletion. *Cancer Res* 2012;**72**:5473−82.
65. Shrimali RK, Yu Z, Theoret MR, Chinnasamy D, Restifo NP, Rosenberg SA. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. *Cancer Res* 2010;**70**:6171−80.
66. Klein D. The tumor vascular endothelium as decision maker in cancer therapy. *Front Oncol* 2018;**8**:367.
67. Georganaki M, van Hooren L, Dimberg A. Vascular targeting to increase the efficiency of immune checkpoint blockade in cancer. *Front Immunol* 2018;**9**:3081.
68. Facciabene A, Peng X, Hagemann IS, Balint K, Barchetti A, Wang LP, et al. Tumour hypoxia promotes tolerance and angiogenesis *via* CCL28 and T(reg) cells. *Nature* 2011;**475**:226−30.
69. Liu Z, Wang Y, Huang Y, Kim BYS, Shan H, Wu D, et al. Tumor vasculatures: a new target for cancer immunotherapy. *Trends Pharmacol Sci* 2019;**40**:613−23.
70. Ciciola P, Cascetta P, Bianco C, Formisano L, Bianco R. Combining immune checkpoint inhibitors with anti-angiogenic agents. *J Clin Med* 2020;**9**:675.
71. Wu X, Li J, Connolly EM, Liao X, Ouyang J, Giobbie-Hurder A, et al. Combined anti-VEGF and anti-CTLA-4 therapy elicits humoral immunity to galectin-1 which is associated with favorable clinical outcomes. *Cancer Immunol Res* 2017;**5**:446−54.
72. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc Natl Acad Sci U S A* 2010;**107**:4275−80.
73. Schmittnaegel M, Rigamonti N, Kadioglu E, Cassara A, Wyser Rmili C, Kiialainen A, et al. Dual angiopoietin-2 and VEGFA inhibition elicits antitumor immunity that is enhanced by PD-1 checkpoint blockade. *Sci Transl Med* 2017;**9**. eaak9670.
74. Ziogas AC, Gavalas NG, Tsiatas M, Tsitsilonis O, Politi E, Terpos E, et al. VEGF directly suppresses activation of T cells from ovarian cancer patients and healthy individuals *via* VEGF receptor Type 2. *Int J Canc* 2012;**130**:857−64.
75. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet AL, et al. VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. *J Exp Med* 2015;**212**:139−48.
76. Motz GT, Santoro SP, Wang LP, Garrabrant T, Lastra RR, Hagemann IS, et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nat Med* 2014;**20**:607−15.
77. Bouzin C, Brouet A, De Vriese J, Dewever J, Feron O. Effects of vascular endothelial growth factor on the lymphocyte-endothelium interactions: identification of caveolin-1 and nitric oxide as control points of endothelial cell anergy. *J Immunol* 2007;**178**:1505−11.
78. Dirkx AE, Oude Egbrink MG, Kuijpers MJ, van der Niet ST, Heijnen VV, Bouma-ter Steege JC, et al. Tumor angiogenesis modulates leukocyte-vessel wall interactions *in vivo* by reducing endothelial adhesion molecule expression. *Cancer Res* 2003;**63**:2322−9.
79. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to modulate antitumor immunity. *Front Immunol* 2018;**9**:978.
80. Ozao-Choy J, Ma G, Kao J, Wang GX, Meseck M, Sung M, et al. The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and modulation of tumor microenvironment for immune-based cancer therapies. *Cancer Res* 2009;**69**:2514−22.
81. Blotnick S, Peoples GE, Freeman MR, Eberlein TJ, Klagsbrun M. T lymphocytes synthesize and export heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor, mitogens for vascular cells and fibroblasts: differential production and release by CD4+ and CD8+ T cells. *Proc Natl Acad Sci U S A* 1994;**91**:2890−4.
82. Fathallah-Shaykh HM, Zhao LJ, Kafrouni AI, Smith GM, Forman J. Gene transfer of IFN-gamma into established brain tumors represses growth by antiangiogenesis. *J Immunol* 2000;**164**:217−22.
83. Friesel R, Komoriya A, Maciag T. Inhibition of endothelial cell proliferation by gamma-interferon. *J Cell Biol* 1987;**104**:689−96.
84. Madri JA, Pratt BM, Tucker AM. Phenotypic modulation of endothelial cells by transforming growth factor-beta depends upon the composition and organization of the extracellular matrix. *J Cell Biol* 1988;**106**:1375−84.
85. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Canc* 2012;**12**:298−306.
86. Stylianopoulos T, Jain RK. Combining two strategies to improve perfusion and drug delivery in solid tumors. *Proc Natl Acad Sci U S A* 2013;**110**:18632−7.
87. Tredan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. *J Natl Cancer Inst* 2007;**99**:1441−54.
88. Dewhirst MW, Secomb TW. Transport of drugs from blood vessels to tumour tissue. *Nat Rev Canc* 2017;**17**:738−50.
89. Heldin CH, Rubin K, Pietras K, Ostman A. High interstitial fluid pressure—an obstacle in cancer therapy. *Nat Rev Canc* 2004;**4**:806−13.
90. Leontieva OV, Natarajan V, Demidenko ZN, Burdelya LG, Gudkov AV, Blagosklonny MV. Hypoxia suppresses conversion from proliferative arrest to cellular senescence. *Proc Natl Acad Sci U S A* 2012;**109**:13314−8.
91. Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. *J Cell Biol* 2012;**196**:395−406.
92. Nia HT, Munn LL, Jain RK. Mapping physical tumor microenvironment and drug delivery. *Clin Canc Res* 2019;**25**:2024−6.
93. Li C, Wang J, Wang Y, Gao H, Wei G, Huang Y, et al. Recent progress in drug delivery. *Acta Pharm Sin B* 2019;**9**:1145−62.
94. Li F, Huang J, Ji D, Meng Q, Wang C, Chen S, et al. Azithromycin effectively inhibits tumor angiogenesis by suppressing vascular endothelial growth factor receptor 2-mediated signaling pathways in lung cancer. *Oncol Lett* 2017;**14**:89−96.

95. Ding X, Su Y, Wang C, Zhang F, Chen K, Wang Y, et al. Synergistic suppression of tumor angiogenesis by the co-delivering of vascular endothelial growth factor targeted siRNA and candesartan mediated by functionalized carbon nanovectors. *ACS Appl Mater Interfaces* 2017;**9**:23353−69.
96. Huang S, Shao K, Liu Y, Kuang Y, Li J, An S, et al. Tumor-targeting and microenvironment-responsive smart nanoparticles for combination therapy of antiangiogenesis and apoptosis. *ACS Nano* 2013;**7**:2860−71.
97. Martinez JO, Evangelopoulos M, Karun V, Shegog E, Wang JA, Boada C, et al. The effect of multistage nanovector targeting of VEGFR2 positive tumor endothelia on cell adhesion and local payload accumulation. *Biomaterials* 2014;**35**:9824−32.
98. Liu ZL, You CL, Wang B, Lin JH, Hu XF, Shan XY, et al. Construction of Ang2-siRNA chitosan magnetic nanoparticles and the effect on Ang2 gene expression in human malignant melanoma cells. *Oncol Lett* 2016;**11**:3992−8.
99. Zhang L, Qi Y, Min H, Ni C, Wang F, Wang B, et al. Cooperatively responsive peptide nanotherapeutic that regulates angiopoietin receptor tie2 activity in tumor microenvironment to prevent breast tumor relapse after chemotherapy. *ACS Nano* 2019;**13**:5091−102.
100. Tsao CJ, Pandolfi L, Wang X, Minardi S, Lupo C, Evangelopoulos M, et al. Electrospun patch functionalized with nanoparticles allows for spatiotemporal release of VEGF and PDGF-BB promoting *in vivo* neovascularization. *ACS Appl Mater Interfaces* 2018;**10**:44344−53.
101. Penn CA, Yang K, Zong H, Lim JY, Cole A, Yang D, et al. Therapeutic impact of nanoparticle therapy targeting tumor-associated macrophages. *Mol Canc Therapeut* 2018;**17**:96−106.
102. Song M, Liu T, Shi C, Zhang X, Chen X. Bioconjugated manganese dioxide nanoparticles enhance chemotherapy response by priming tumor-associated macrophages toward M1-like phenotype and attenuating tumor hypoxia. *ACS Nano* 2016;**10**:633−47.
103. Conde J, Bao C, Tan Y, Cui D, Edelman ER, Azevedo HS, et al. Dual targeted immunotherapy *via in vivo* delivery of biohybrid RNAi-peptide nanoparticles to tumour-associated macrophages and cancer cells. *Adv Funct Mater* 2015;**25**:4183−94.
104. Zhou Q, Shao S, Wang J, Xu C, Xiang J, Piao Y, et al. Enzyme-activatable polymer-drug conjugate augments tumour penetration and treatment efficacy. *Nat Nanotechnol* 2019;**14**:799−809.
105. Wang Y, Xie Y, Kilchrist KV, Li J, Duvall CL, Oupicky D. Endosomolytic and tumor-penetrating mesoporous silica nanoparticles for siRNA/miRNA combination cancer therapy. *ACS Appl Mater Interfaces* 2020;**12**:4308−22.
106. Li X, Wu M, Pan L, Shi J. Tumor vascular-targeted co-delivery of anti-angiogenesis and chemotherapeutic agents by mesoporous silica nanoparticle-based drug delivery system for synergetic therapy of tumor. *Int J Nanomed* 2016;**11**:93−105.
107. Liu YR, Guan YY, Luan X, Lu Q, Wang C, Liu HJ, et al. Delta-like ligand 4-targeted nanomedicine for antiangiogenic cancer therapy. *Biomaterials* 2015;**42**:161−71.
108. Xue J, Zhao Z, Zhang L, Xue L, Shen S, Wen Y, et al. Neutrophil-mediated anticancer drug delivery for suppression of postoperative malignant glioma recurrence. *Nat Nanotechnol* 2017;**12**:692−700.
109. Huang B, Abraham WD, Zheng Y, Bustamante Lopez SC, Luo SS, Irvine DJ. Active targeting of chemotherapy to disseminated tumors using nanoparticle-carrying T cells. *Sci Transl Med* 2015;**7**:291−4.
110. Wu L, Xie W, Zan HM, Liu Z, Wang G, Wang Y, et al. Platelet membrane-coated nanoparticles for targeted drug delivery and local chemo-photothermal therapy of orthotopic hepatocellular carcinoma. *J Mater Chem B* 2020;**8**:4648−59.
111. Chung AS, Lee J, Ferrara N. Targeting the tumour vasculature: insights from physiological angiogenesis. *Nat Rev Canc* 2010;**10**:505−14.
112. Heist RS, Duda DG, Sahani DV, Ancukiewicz M, Fidias P, Sequist LV, et al. Improved tumor vascularization after anti-VEGF therapy with carboplatin and nab-paclitaxel associates with survival in lung cancer. *Proc Natl Acad Sci U S A* 2015;**112**:1547−52.
113. Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* 2004;**10**:145−7.
114. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J Clin Oncol* 2008;**26**:2013−9.
115. Jiang T, Olson ES, Nguyen QT, Roy M, Jennings PA, Tsien RY. Tumor imaging by means of proteolytic activation of cell-penetrating peptides. *Proc Natl Acad Sci U S A* 2004;**101**:17867−72.
116. Cascone T, Heymach JV. Targeting the angiopoietin/Tie2 pathway: cutting tumor vessels with a double-edged sword?. *J Clin Oncol* 2012;**30**:441−4.
117. Zhang J, Fukuhara S, Sako K, Takenouchi T, Kitani H, Kume T, et al. Angiopoietin-1/Tie2 signal augments basal Notch signal controlling vascular quiescence by inducing delta-like 4 expression through AKT-mediated activation of beta-catenin. *J Biol Chem* 2011;**286**:8055−66.
118. Goel S, Gupta N, Walcott BP, Snuderl M, Kesler CT, Kirkpatrick ND, et al. Effects of vascular-endothelial protein tyrosine phosphatase inhibition on breast cancer vasculature and metastatic progression. *J Natl Cancer Inst* 2013;**105**:1188−201.
119. Peterson TE, Kirkpatrick ND, Huang Y, Farrar CT, Marijt KA, Kloepper J, et al. Dual inhibition of Ang-2 and VEGF receptors normalizes tumor vasculature and prolongs survival in glioblastoma by altering macrophages. *Proc Natl Acad Sci U S A* 2016;**113**:4470−5.
120. Khan OF, Zaia EW, Jhunjhunwala S, Xue W, Cai W, Yun DS, et al. Dendrimer-inspired nanomaterials for the *in vivo* delivery of siRNA to lung vasculature. *Nano Lett* 2015;**15**:3008−16.
121. Armulik A, Genove G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell* 2011;**21**:193−215.
122. Gaengel K, Genove G, Armulik A, Betsholtz C. Endothelial-mural cell signaling in vascular development and angiogenesis. *Arterioscler Thromb Vasc Biol* 2009;**29**:630−8.
123. Hedlund EM, Hosaka K, Zhong Z, Cao R, Cao Y. Malignant cell-derived PlGF promotes normalization and remodeling of the tumor vasculature. *Proc Natl Acad Sci U S A* 2009;**106**:17505−10.
124. Roca C, Adams RH. Regulation of vascular morphogenesis by Notch signaling. *Genes Dev* 2007;**21**:2511−24.
125. Phng LK, Gerhardt H. Angiogenesis: a team effort coordinated by notch. *Dev Cell* 2009;**16**:196−208.
126. Takeda K, Ichiki T, Narabayashi E, Inanaga K, Miyazaki R, Hashimoto T, et al. Inhibition of prolyl hydroxylase domain-containing protein suppressed lipopolysaccharide-induced TNF-alpha expression. *Arterioscler Thromb Vasc Biol* 2009;**29**:2132−7.
127. Mazzone M, Dettori D, de Oliveira RL, Loges S, Schmidt T, Jonckx B, et al. Heterozygous deficiency of PHD2 restores tumor oxygenation and inhibits metastasis *via* endothelial normalization. *Cell* 2009;**136**:839−51.
128. Hamzah J, Jugold M, Kiessling F, Rigby P, Manzur M, Marti HH, et al. Vascular normalization in Rgs5-deficient tumours promotes immune destruction. *Nature* 2008;**453**:410−4.
129. Sawada J, Urakami T, Li F, Urakami A, Zhu W, Fukuda M, et al. Small GTPase R-Ras regulates integrity and functionality of tumor blood vessels. *Canc Cell* 2012;**22**:235−49.
130. Magrini E, Villa A, Angiolini F, Doni A, Mazzarol G, Rudini N, et al. Endothelial deficiency of L1 reduces tumor angiogenesis and promotes vessel normalization. *J Clin Invest* 2014;**124**:4335−50.
131. Chabot S, Jabrane-Ferrat N, Bigot K, Tabiasco J, Provost A, Golzio M, et al. A novel antiangiogenic and vascular normalization therapy targeted against human CD160 receptor. *J Exp Med* 2011;**208**:973−86.
132. Zhao Y, Ting KK, Li J, Cogger VC, Chen J, Johansson-Percival A, et al. Targeting vascular endothelial-cadherin in tumor-associated

blood vessels promotes T-cell-mediated immunotherapy. *Cancer Res* 2017;**77**:4434–47.
133. Li J, Zhao Y, Choi J, Ting KK, Coleman P, Chen J, et al. Targeting miR-27a/VE-cadherin interactions rescues cerebral cavernous malformations in mice. *PLoS Biol* 2020;**18**. e3000734.
134. Huang N, Liu Y, Fang Y, Zheng S, Wu J, Wang M, et al. Gold nanoparticles induce tumor vessel normalization and impair metastasis by inhibiting endothelial Smad2/3 signaling. *ACS Nano* 2020: 7940–58.
135. Li X, Kostareli E, Suffner J, Garbi N, Hammerling GJ. Efficient Treg depletion induces T-cell infiltration and rejection of large tumors. *Eur J Immunol* 2010;**40**:3325–35.
136. Sautes-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat Rev Canc* 2019;**19**:307–25.
137. Bauer R, Udonta F, Wroblewski M, Ben-Batalla I, Santos IM, Taverna F, et al. Blockade of myeloid-derived suppressor cell expansion with all-trans retinoic acid increases the efficacy of antiangiogenic therapy. *Cancer Res* 2018;**78**:3220–32.
138. Kammertoens T, Friese C, Arina A, Idel C, Briesemeister D, Rothe M, et al. Tumour ischaemia by interferon-γ resembles physiological blood vessel regression. *Nature* 2017;**545**:98–102.
139. Yin Y, Hu Q, Xu C, Qiao Q, Qin X, Song Q, et al. Co-delivery of doxorubicin and interferon-gamma by thermosensitive nanoparticles for cancer immunochemotherapy. *Mol Pharm* 2018;**15**:4161–72.
140. Johansson A, Hamzah J, Payne CJ, Ganss R. Tumor-targeted TNFalpha stabilizes tumor vessels and enhances active immunotherapy. *Proc Natl Acad Sci U S A* 2012;**109**:7841–6.
141. Teo P, Wang X, Chen B, Zhang H, Yang X, Huang Y, et al. Complex of TNF-alpha and modified Fe3O4 nanoparticles suppresses tumor growth by magnetic induction hyperthermia. *Cancer Biother Radiopharm* 2017;**32**:379–86.
142. Siveen KS, Kuttan G. Role of macrophages in tumour progression. *Immunol Lett* 2009;**123**:97–102.
143. Carretero R, Sektioglu IM, Garbi N, Salgado OC, Beckhove P, Hammerling GJ. Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells. *Nat Immunol* 2015;**16**:609–17.
144. Zuo ZQ, Chen KG, Yu XY, Zhao G, Shen S, Cao ZT, et al. Promoting tumor penetration of nanoparticles for cancer stem cell therapy by TGF-beta signaling pathway inhibition. *Biomaterials* 2016;**82**: 48–59.
145. Chauhan VP, Stylianopoulos T, Martin JD, Popovic Z, Chen O, Kamoun WS, et al. Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. *Nat Nanotechnol* 2012;**7**:383–8.
146. Jiang W, Huang Y, An Y, Kim BY. Remodeling tumor vasculature to enhance delivery of intermediate-sized nanoparticles. *ACS Nano* 2015;**9**:8689–96.
147. Lee S, Han H, Koo H, Na JH, Yoon HY, Lee KE, et al. Extracellular matrix remodeling *in vivo* for enhancing tumor-targeting efficiency of nanoparticle drug carriers using the pulsed high intensity focused ultrasound. *J Control Release* 2017;**263**:68–78.
148. Panagi M, Voutouri C, Mpekris F, Papageorgis P, Martin MR, Martin JD, et al. TGF-beta inhibition combined with cytotoxic nanomedicine normalizes triple negative breast cancer microenvironment towards anti-tumor immunity. *Theranostics* 2020;**10**: 1910–22.
149. Cun X, Ruan S, Chen J, Zhang L, Li J, He Q, et al. A dual strategy to improve the penetration and treatment of breast cancer by combining shrinking nanoparticles with collagen depletion by losartan. *Acta Biomater* 2016;**31**:186–96.
150. Chen Y, Liu X, Yuan H, Yang Z, von Roemeling CA, Qie Y, et al. Therapeutic remodeling of the tumor microenvironment enhances nanoparticle delivery. *Adv Sci* 2019;**6**:1802070 (Weinh).
151. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res* 1986;**46**:6387–92.
152. Chauhan VP, Jain RK. Strategies for advancing cancer nanomedicine. *Nat Mater* 2013;**12**:958–62.
153. Sindhwani S, Syed AM, Ngai J, Kingston BR, Maiorino L, Rothschild J, et al. The entry of nanoparticles into solid tumours. *Nat Mater* 2020;**19**:566–75.
154. Zhou Y, Chen X, Cao J, Gao H. Overcoming the biological barriers in the tumor microenvironment for improving drug delivery and efficacy. *J Mater Chem B* 2020;**8**:6765–81.
155. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med* 2018;**24**:541–50.
156. Pasto A, Giordano F, Evangelopoulos M, Amadori A, Tasciotti E. Cell membrane protein functionalization of nanoparticles as a new tumor-targeting strategy. *Clin Transl Med* 2019;**8**:8.
157. Pandit S, Dutta D, Nie S. Active transcytosis and new opportunities for cancer nanomedicine. *Nat Mater* 2020;**19**:478–80.
158. Liu Y, Tan J, Zhang Y, Zhuang J, Ge M, Shi B, et al. Visualizing glioma margins by real-time tracking of gamma-glutamyltranspeptidase activity. *Biomaterials* 2018;**173**:1–10.
159. Murphy EA, Majeti BK, Barnes LA, Makale M, Weis SM, Lutu-Fuga K, et al. Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis. *Proc Natl Acad Sci U S A* 2008;**105**: 9343–8.
160. Hu C, Yang X, Liu R, Ruan S, Zhou Y, Xiao W, et al. Coadministration of iRGD with multistage responsive nanoparticles enhanced tumor targeting and penetration abilities for breast cancer therapy. *ACS Appl Mater Interfaces* 2018;**10**:22571–9.
161. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, et al. Tissue-penetrating delivery of compounds and nanoparticles into tumors. *Canc Cell* 2009;**16**:510–20.
162. Liu X, Lin P, Perrett I, Lin J, Liao YP, Chang CH, et al. Tumor-penetrating peptide enhances transcytosis of silicasome-based chemotherapy for pancreatic cancer. *J Clin Invest* 2017;**127**: 2007–18.
163. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Greenwald DR, et al. Coadministration of a tumor-penetrating peptide enhances the efficacy of cancer drugs. *Science* 2010;**328**: 1031–5.
164. Yang X, Hu C, Tong F, Liu R, Zhou Y, Qin L, et al. Tumor microenvironment-responsive dual drug dimer-loaded PEGylated bilirubin nanoparticles for improved drug delivery and enhanced immune-chemotherapy of breast cancer. *Adv Funct Mater* 2019;**29**: 1901896.
165. Zoller F, Markert A, Barthe P, Zhao W, Weichert W, Askoxylakis V, et al. Combination of phage display and molecular grafting generates highly specific tumor-targeting miniproteins. *Angew Chem Int Ed Engl* 2012;**51**:13136–9.
166. Muller WA. Getting leukocytes to the site of inflammation. *Vet Pathol* 2013;**50**:7–22.
167. Choi MR, Stanton-Maxey KJ, Stanley JK, Levin CS, Bardhan R, Akin D, et al. A cellular Trojan Horse for delivery of therapeutic nanoparticles into tumors. *Nano Lett* 2007;**7**:3759–65.
168. Stephan MT, Moon JJ, Um SH, Bershteyn A, Irvine DJ. Therapeutic cell engineering with surface-conjugated synthetic nanoparticles. *Nat Med* 2010;**16**:1035–41.
169. Huang X, Zhang F, Wang H, Niu G, Choi KY, Swierczewska M, et al. Mesenchymal stem cell-based cell engineering with multifunctional mesoporous silica nanoparticles for tumor delivery. *Biomaterials* 2013;**34**:1772–80.
170. Shaul ME, Fridlender ZG. Tumour-associated neutrophils in patients with cancer. *Nat Rev Clin Oncol* 2019;**16**:601–20.

171. Nemeth T, Sperandio M, Mocsai A. Neutrophils as emerging therapeutic targets. *Nat Rev Drug Discov* 2020;**19**:253−75.
172. Hu Q, Sun W, Qian C, Wang C, Bomba HN, Gu Z. Anticancer platelet-mimicking nanovehicles. *Adv Mater* 2015;**27**:7043−50.
173. Hu Q, Sun W, Qian C, Bomba HN, Xin H, Gu Z. Relay drug delivery for amplifying targeting signal and enhancing anticancer efficacy. *Adv Mater* 2017;**29**:1605803.
174. Kamerkar S, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA, et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* 2017;**546**:498−503.
175. Du S, Xiong H, Xu C, Lu Y, Yao J. Attempts to strengthen and simplify the tumor vascular normalization strategy using tumor vessel normalization promoting nanomedicines. *Biomater Sci* 2019;**7**:1147−60.
176. Hernandez-Agudo E, Mondejar T, Soto-Montenegro ML, Megias D, Mouron S, Sanchez J, et al. Monitoring vascular normalization induced by antiangiogenic treatment with (18)F-fluoromisonidazole-PET. *Mol Oncol* 2016;**10**:704−18.
177. Peng F, Setyawati MI, Tee JK, Ding X, Wang J, Nga ME, et al. Nanoparticles promote *in vivo* breast cancer cell intravasation and extravasation by inducing endothelial leakiness. *Nat Nanotechnol* 2019;**14**:279−86.