Modulation of Tumor Vasculature Network: Key Strategies

Mohammad Taleb, Niloufar Mohammadkhani, Farbod Bahreini, Muhammad Ovais, and Guangjun Nie*

Tumor cells, owing to their relentless division, require increased nutrient supply. This is made possible by angiogenesis, that is, the formation of new blood vessels increases the blood flow toward tumor cells, thereby promoting the supply of oxygen and vital nutrients required for the proliferation of tumor cells. Decreasing the blood flow to the tumor cells can effectively halt tumor progression and possibly cure cancer. However, practically, the mechanism is not as straightforward as it seems. In this review, the role of angiogenesis is focused on in the development of tumors and cancer metastasis and the potential of tumor vascular normalization for the reduction of angiogenesis, which results in the suppression of tumor cells. An insight into various strategies, including pharmacological inhibition of angiogenesis, genetic models, and nanoparticle-based novel treatment strategies for vascular normalization, is provided. In addition, the effect of vessel normalization and tumor microenvironment modulation on the delivery of nanoparticle-based drugs to the target tumor cells is discussed. The critical role of monitoring vascular normalization is also highlighted, without which selecting the most efficient strategy for tumor vascular normalization is possible. Therefore, efficiently combating cancer and its devastating effects remains an unsolved puzzle.

1. Introduction

Neovascularization in tumors is the most vital process whereby new vascular networks arise from existing ones that support tumor growth and dissemination. The diffusion distance from capillaries to cells is about 10–30 μm in normal tissues that exceed to 100–200 μm in cancer tissues and causes depletion of oxygen and nutrients in the cells. Created hypoxia microenvironment promotes the expression of hypoxia-inducible factor-1 (HIF-1) that consequently induces the transcription of several angiogenic factors, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and platelet-derived growth factor (PDGF) to sustain rapid tumor growth. [31] Tumor vessels are structurally disorganized and have abnormal features, including increased permeability and leakiness that cause inadequate perfusion of tumor tissues. [2,3] Blood vessels of normal tissues are formed in a controlled manner, and their inner walls are lined by endothelial cells (ECs), which are transformed into the tumor ECs (TECs) in the tumor vessels. Loss of polarity that is contributed with defects in centrosome function is the hallmark of TECs, resulting in accumulation and detachment of cells from the vessel walls. Pericytes help keep blood vessels healthy by surrounding and warping the endothelium and filling in the gaps between ECs. Pericytes are functionally associated with vessel maturation and their communication with ECs regulates angiogenesis capillary diameter and permeability. Pericytes are held in place by vascular basement membrane (BM) and regulate membrane organization via catalyzing deposition of proteins. Furthermore, the detachment of pericytes during tumor growth makes vessels less contractile and dysfunctional, due to which they are unable to control the vascular permeability and regulate flow and therefore enhance tumor metastatic potential. It is also indicated that vascular structural instability increases the hematocrit and thus the viscosity of tumor blood. Therefore, the reduced intravascular pressure gradient in leaky vessels and their impaired blood flow rate can obstruct the distribution of antitumor therapeutics. The same features of the tumor microenvironment (TME) impaired the emigration and infiltration of immune cells into the tumor site.

Approaches incorporating imaging modalities and preclinical studies have explored the influence of antiangiogenesis
therapeutics on the structure of tumor vasculature, their permeability, perfusion, and the magnitude and heterogeneity of intratumoral distribution of drugs. Their method, known as vascular normalization, confirmed the influence of vessel decompression on improving intravascular transportation of therapeutics and enhancing the level of immune cell infiltration. Key advances in cancer therapy need a novel therapeutic agent with a novel mode of action to improve its therapeutic output and synergy with conventional therapies. Therapeutic approaches using nano-drug delivery systems (NDDSs) are guaranteed by nanotherapeutics’ specific targeting ability, which leads to enhanced drug delivery plus substantially reduced side effects. Nanomedicines offer new possibilities to overcome vascular abnormalities and strengthen the cycle of improved therapeutics delivery to the tumor sites. Having the benefits of combinational approaches, nanotherapeutics facilitate the co-delivery of vessel normalization agents, chemotherapeutics, and immunotherapeutic agents. Recent studies also show strategies combining vascular normalization and extracellular matrix (ECM) remodeling that can significantly improve the delivery of nanomedicines to solid tumors. Combinational vascular normalization and immunotherapy have been a topic of intense research interest. Nanotherapeutics can also be designed to perform more precisely based on the physiological differences between cancer and healthy tissues.

This review comprehensively detailed the tumor vascular normalization and provided insight into strategies including pharmacological inhibition of angiogenesis, genes-based therapeutic approaches, and nanoparticle (NP)-based novel treatment strategies for vascular normalization.

2. The Structure and Pathology of the Tumor Vasculature Network

Tumor vessels differ from normal tissue in many ways, such as size, shape, tortuosity, and branching pattern. Perfusion in tumor tissue is affected by the specific branching pattern and abnormal diameters. All vessel structures exhibit abnormalities in details. Specific vessel structures are not maintained. In large vessels, for example, it is only the BM and endothelium. At the edges of the tumor, tumor vessels are heterogeneous more than at its center, and the chaotic structure of the center is more pronounced.

2.1. Tumor Endothelial Cell Features

EC proliferation in mature vessels is lower than in tumor tissues, and their turnover time depends on time, while in tumor tissues it is determined by growth factors. VEGF is essential for the growth of host vessels that support tumor growth. As new tumors form, EC comes into tumor cells that produce VEGF, which is necessary for proliferation and alterations in their permeability. ECs proliferate 50–200 times more frequently in tumors compared to normal tissues. Proliferation in some areas of the tumor vasculature leads to angiogenesis, while in others it results in apoptosis in parallel with necrosis and vessel regression. The structure of tumor-associated endothelium is defective. Features such as gaps and discontinuities are common that enhance permeability and hemorrhage. There are not many complex structures establishing contact between cells. Cell–cell junction abnormalities and other changes can increase the leakiness of ECs. In contrast, media can be used for imaging tumors and for delivering macromolecular therapeutics based on the correlation between the grade of the tumor and its malignant potential. Additionally, it may facilitate the dissemination of tumor cells and cause metastasis. Several other endothelial features can be observed in the endothelium of tumor vessels, including transendothelial holes, intercellular gaps, vesiculo-vacuolar organelles (VVOs), transendothelial holes, and endothelial fenestrae. The expression of cell adhesion molecules is also different in TECs from normal ones. A defect in VE-cadherin expression occurs in tumor vessels, resulting in destabilization and abnormal remodeling. Many abnormal gene expression patterns occur in TECs.

2.2. Tumor Basement Membrane

In tumor vessels, the BM that surrounds EC and pericytes might contain extra layers totally dissociated from the cells. Structure proteins are digested by enzymes to make molecules that are active. Growth factors are also bound by BM, which is involved in angiogenesis.

2.3. Tumor Pericyte

New vessels are stabilized and surrounded by pericytes, which are perivascular cells (PVCs). EC is in direct contact with the processes that extend around and along microvascular tubes. In several types of murine tumor, immunohistochemistry staining of sections reveals 73%–92% of endothelial sprouts. The recruitment of pericyte is higher in breast and colon cancers than in gliomas or renal cell carcinomas. A majority of tumor pericytes do not connect with EC well, have atypical shape, paradoxically extend cytoplasmic processes farther away from vessel walls, and have extra layers of loosely fitting BM. PDGF signaling pathways appear to be altered in their abnormalities. A number of in vivo models have shown an increase in pericyte recruitment when tumor cells express platelet-derived growth factor B (PDGFB). In addition, genetically reducing PDGFB-R expression by embryonic pericyte reduced recruitment. In mice implanted with Lewis lung carcinomas, EC coverage was significantly reduced.

2.4. Vascular Co-Option

Angiogenesis in cancer cells differs from normal angiogenesis, in that, cancer cells migrate to other locations, establish metastasis, and create new vessels. As a result, vessels retract owing to apoptosis of the EC, which may be mediated by the Ang-2 molecule. Finally, tumor angiogenesis is promoted by VEGF and Ang-2 working in concert at the periphery of a growing tumor mass. When tumor cells metastasize to or are implanted within vascularized tissues, they often have immediate access to blood vessels. Co-opting adjacent vessels, they grow as cuffs around them immediately. As a result of the coopting of the vessels, a robust host defense mechanism is activated by autocrine
induction of Ang-2, and regression of the co-opted vessels results in extensive tumor death that is followed by regression of the co-opted vessels. Neoangiogenesis is a strategy used by tumors that have overcome this regression. Tumors that have been regressed upregulate their expression of VEGF, perhaps as their oxygen levels drop as a result of losing vascular support. This is similar to the way destabilizing signals provided by Ang-2 potentiate the angiogenic response when combined with VEGF in normal vascular remodeling. In many solid tumors, newly formed vessels continue to overexpress Ang-2, preventing them from forming a vascular system that is well-differentiated and stable.[21] It may be determined whether new tumor vessels continue to grow or contract during transformation based on the balance between VEGF and Ang-2. Figure 1 illustrates a schematic comparison of the information transfer effect on flow and oxygenation in healthy and tumor networks.[22]

3. Approaches to Vessels Normalization

Antiangiogenic cancer therapy is defined as inhibition of the vessel formation that inhibits tumor growth through vascular regression. This therapeutic approach is known as vascular normalization.[23–25] Vascular normalization approaches have been developed based on the various studies on pro-angiogenic and antiangiogenic molecules and molecular and genetic agents in ECs and novel genetic models.[23] Tumor vascularization is initiated directly by cancer cells or induced by stromal cells. There is a balance between pro-angiogenic factors and endogenous angiogenic in normal tissues that their disruption resulted in the continuous development of vessels.[26] In turn, the expression of pro-angiogenic molecules is increased by the progression of malignant cancers.[24] This would lead to a rapid and abnormal vascularization in the tumor, hypoxia condition facilitated metastasis.[2,27] VEGF is a ligand produced by stroma and cancer cells that can bind tightly to the corresponding receptors, vascular endothelial growth-factor receptor (VEGFR)-1 and VEGFR-2. Interaction with these receptors then activates multiple downstream pathways and ultimately results in EC proliferation and migration. Ferrara’s group in 1993 reported the use of anti-VEGF monoclonal antibody in nude mice bearing xenografts as a significant inhibitor of tumor growth, which leads to a decrease in vascular density, reduction in metastasis, and delayed growth in subcutaneous xenografts of colorectal cancer. Further, tumor hypoxia is also decreased due to the anti-vascular effects of anti-VEGF therapy and leads to the less metastatic ability of tumors. Rather than inhibiting the neovascularization by utilizing antiangiogenic therapy, the main idea on vascular normalization is returning the abnormal function and structure toward a normal state, consequently normalized TME. In another words, vessel normalization is reverting the function and structure of tumor vessels, correcting the abnormality and also controlling the therapeutic responsiveness of TME. In recent years, a plenty of clinical and preclinical studies have validated this hypothesis.[2,23,28–31] In vascular normalization therapeutic approaches, even not perfect modification in the structure and function of vessels improves blood flow in tumors and reduces hypoxia and acidosis.[31,32] Therefore, the normalization of vessels hinders invasation and extravasation of cancer cells and reduces metastasis.[23] In the normalization of tumor vessels, there are many key angiogenic molecules and genes that are directly implicated. Various genes play a different function in vascular stability, oxygen sensing, and pericyte function confirmed in vessel normalizations. However, blocking the VEGF signaling is a validated mechanism in this regard. VEGF is a growth-promoting factor that affects the proliferation and survival of ECs and enhances the permeability of vessels. Pharmacological VEGF signaling pathway inhibitors can restore the balance between antiangiogenic and pro-angiogenic signaling and lead them to the balanced mode. In the following section, we introduced these pharmacological compounds that are divided based on their mechanism of action.

3.1. Anti-VEGF Therapeutics

Pharmacological inhibitors engaged different antiangiogenesis mechanisms to block the pathway of VEGF signaling. Inhibiting the binding of VEGF to its receptors is mainly known as therapeutic strategy. VEGF has different receptors such as VEGFR-1, VEGFR-2, neuropilin-1 (NRP-1), and NRP-2. Using antibodies as pharmacological inhibitors of the VEGF pathway can directly block the interaction of VEGF with receptors.[25] Anti-human antibody A4.6.1. and its developed model, bevacizumab, use similar mechanisms in blockage of VEGF signaling pathway by targeting and neutralizing VEGF-A and preventing its specific binding to the receptor.[23] The application of neutralizing A4.6.1 antibody was first reported by Fan Yuan et al. in 1996.[33] They implanted three human tumors (melanoma, glioblastoma multiforme, and colorectal) into severe combined immunodeficient mice and analyzed the function and structure of the tumor vessels in real time using the intravital microscopic technique. Reduction of vessel tortuosity and permeability and significant decrease of tumor vessel diameter were observed after administration of A4.6.1. that verified the therapeutic benefits of the neutralization of tumor cell-derived VEGF in tumor microvasculature. However, the normalization effect due to the
intravenous injection of A.4.6.1 is not stable, which reversed to abnormal phenotype on the fifth-day post-administration. By continuing the therapy and neutralization of VEGF, normalization features were replaced by vascular progression. This evidence represented the transient nature of the normalization induced by pharmacological inhibitors. Following the aforementioned study, more evidence were reported to support the use of A.4.6.1 as a normalization factor in human tumor xenograft, and also its benefits in the combination with radiation therapy show a synergic effect in the tumor therapy compared with treatment alone. Bevacizumab has been developed based on the A.4.6.1 antibody to neutralize all isofoms of human VEGF-A and is approved by the FDA as antiangiogenic therapy. Promising results from the literature on combinational therapy of bevacizumab and traditional chemotherapy proved their synergistic benefits. The effect of bevacizumab on tumor vascular function was examined by Dickson and colleagues using human neuroblastoma xenografts. Similar to the A.4.6.1 therapy, the reduction in vessels’ diameter, length, density, and tortuosity caused a normalized and mature phenotype of tumor vessels within 24 h after treatment, resulting in improved vascular maturation index (VMI). Clinical and preclinical studies also revealed that following treatments using bevacizumab in combination with chemotherapy drugs in the penetration of systemically administrated chemotherapeutics are improved, which delayed tumor growth and enhanced the overall survival of patients with advanced lung cancer. This combinational therapy showed the maximal benefits; that is, the maximal retraction of tumors happened when bevacizumab therapy started 3 days before using chemotherapy agents.

Amount of delivered chemotherapy enhanced if administered during the vascular normalization window. Reduction of vessel permeability in rats bearing human breast cancer xenografts was observed after therapy with bevacizumab. Studies on mice bearing breast cancer, melanoma, and ovarian cancer of both humans and murine also resulted in similar findings. In both human and murine tumors, maturation of the vascular system (increased VMII) and improvement of function (reduce hypoxia) was demonstrated after 2–4 days of treatment. Again, tumor growth control depended on specific treatment time (utilization of bevacizumab 48 h before radiation therapy). In addition to these features, improvement in tumor hypoxia was also observed over a similar time frame in the treatment of glioblastoma (GBM) via bevacizumab, and good results in tumor-delayed growth was observed when combined with radiotherapy.

To demonstrate the benefits of combined therapy of anti-VEGF with radiotherapy and immunotherapy, an anti-mouse VEGF antibody was used. It synergizes the effect of anti-VEGF therapy with radiation in the sensitization of cancer cells and vessel normalization. Also, the infiltration of T-cells was increased by vessel normalizations. In melanoma cases of murine, treatment with anti-VEGF antibody and transfer the pre-activated antitumor immune cells (adoptive cell transfer ACT). Monotherapy in comparison with combined anti-VEGF therapy administrated one dose before the ACT was ineffective. Normalization study by aflibercept was examined in a murine model of pancreatic cancer (beta-cell derived). Vascular regression and neovascular sprouts were reduced by inhibiting the VEGF signaling within 7 days. It is even evidence of the maturation of vessels with reduction of ECs tighten and fenestration association between ECs and PVCs.

3.2. Anti-VEGFR Therapeutics

Several receptors of ECs bind to VEGF (VEGFR-1, VEGFR-2, NRP-1, and NRP-2). Among these receptors, VEGFR-2 mediates most properties of VEGF in angiogenesis. In tumor vascularity, it is expressed at a higher level on ECs. However, VEGFR-1 binds to VEGF with a higher affinity than VEGFR-2, but its roles in angiogenesis are not as clear as VEGFR-2. As VEGFR-1 is expressed in bone marrow–derived progenitor or stem cells, therefore, it might be involved in adult vasculogenesis directly, or indirectly in tumor angiogenesis. NRP receptors function as co-receptors of VEGFR-2, which regulate their activity and may also have direct pro-angiogenic effects.

VEGF receptors can also be targeted by pharmacological agents that induce vessel normalization of tumors. DC101, VEGF-2 monoclonal antibody of rat anti-mouse, blocking antibody of dimerization of VEGF-2 and VEGF-3, and VEGF receptor tyrosine kinase inhibitors (TKIs). TKIs have a different inhibitory activity despite the VEGFRs; such as PDGFRs and c-kit and act in competition with the binding site of ATP of the catalytic domain of tyrosine kinases. In tumor angiogenesis, the blocking of the VEGF–VEGFR-2 signaling pathway with DC101 is an interesting factor in identifying its effect on the vascular system. This agent was utilized in many studies to normalize tumor vessels.

The effect of DC101 was reported by Rakesh et al. in 2004 in mice bearing breast, colorectal, lung, and GBM tumors. Vessels diameter and microvessel density were reduced after 3 days of DC101 administration, and PVC coverage was increased. During this treatment period, the BM was also modified, and collagen IV coverage in vessels was noticed to be normal. Intersitial fluid pressure (IFP) of vessels decreased and led to less permeability whereas pressure gradient of transvascular hydrostatic increased, which functionally have improved in vessels (Figure 2). Continuing the anti-VEGF therapy makes the regression in vascularization by cutting the immature vessels that have adequate BM and lack PVC coverage. These results have been reported in other tumors by treating with DC101, such as androgen-dependent male breast cancer, colon cancer, hepatocellular carcinoma, and bladder cancer. Synergistic effects of radiation therapy and vessel normalization occur with DC101 in GBM and other tumors. By a window of improved oxygenation after 2–8 days since starting the treatment, the structure and function of vessels in GBMs were normalized with DC101. Radiotherapy during the period of window time would have a better effect. Studies on DC101 demonstrated many mechanisms of blocking VEGFR2 in GBM. One of the main pathways which are regulated by DC101 is tumor Ang-1 gene expression. It is expressed by PVCs, and binding and activation of Tie-2/TEK receptors on ECs lead to a reduction in vascular permeability and promotion in vascular integrity and maturation. Overexpression of Ang-2 or downregulation of Tie-2 by pharmacological blocking or genetic modeling after DC101 therapy compromises vascular normalization in GBM. The BM in GBM tumor vessels is thick and irregular.
that can be reorganized by another mechanism. By blocking VEGFR2, an attached and thinner BM monolayer is restored that approves the VEGF’s role in the abnormal BM. The thickness of the BM is decreased using DC101 therapy by enhancing collagen IV degradation, and the function of vessels can be improved. All these studies demonstrated that the phenotype of vessels in tumors is normalized by blocking VEGFR2 and depends on the TME.

3.3. Agents Inhibiting Receptor Tyrosine Kinase

Many small molecules are approved for clinical use that can inhibit tyrosine kinase of VEGFRs, such as sunitinib, pazopanib, and sorafenib. In many regards, they are different from antibodies. Tyrosine kinase inhibitors inhibit the tyrosine kinase domain of receptors without any contribution of VEGF family members to their receptors. However, it demonstrated positive results in preclinical studies to normalize vessels in structure and function by utilizing TKIs. However, in clinical trials, the procedure was not as successful as preclinical data showed. Another vital point of TKIs is their toxicity profile compared to anti-VEGF antibodies. These inconsistent effects of TKIs in anti-VEGF antibodies may have some reasons such as off-target effect or short half-life of circulation, for example, PDGFR blockade. The effects of TKI and antibody therapy on vessel structure generally are the same as each other, such as vessel diameter, microvascular density, and permeability. However, the mechanisms of the molecular effect of TKIs are more complex and are not clear. One of the TKIs of VEGFR1, VEGFR2, VEGFR3, PDGFRb, and c-kit is axitinib, which reduces microvessel density and the number of endothelial germination and increases the vicinity of ECs and PVCs in some cancer models. Sunitinib (Sutent) is another inhibitor of VEGFRs, colony-stimulating factor-1, ret, c-kit, and fli-3 receptors that have a similar effect of an enhanced number of PVC-covered vessels by utilizing in the treatment of ectopic human glioma xenografts. Rakesh and colleagues evaluated AZD2171 (cediranib), a TKI in VEGFR1, VEGFR2, VEGFR3, PDGFRs, and c-kit, in a preclinical study of GBM models. The effect of cediranib in GBM xenografts in mice is the same as the study of DC101, that is, decreased vessel diameter and weakening BM, and the association between PVCs and ECs tightens after 2 days of therapy. Vessel normalization has been seen by different changes in enhancing vessel coverage by PVCs in treating with an inhibitor of VEGFR2, FGFR, PDGFRs, and FGF (TSU68); and a pan-VEGFR TKI (KRN951) in syngeneic colorectal cancer in rat bearing models within 4 days after starting the treatment.

TKIs of both PDGFRs and VEGFRs are more effective in PVC coverage. Inhibiting the VEGF/VEGFR2 pathway could block the recruitment of PVCs in PDGFR-β in vessel walls. Hence, when
VEGFR2 activity is suppressed by TKI, the PVC coverage will be increased in vessel.[58] An increase in PDGF-β-mediated PVC recruitment has a reverse effect on EC proliferation and tumor growth, which is the opposite of PDGF inhibition. It is postulated that this phenomenon can be due to pericyte-mediated inhibition of EC excessive proliferation.[59] Nevertheless, this finding regarding the effect of PDGF on the EC proliferation varies between tumor type and TME.[55] Therapeutic benefits of vessels function in different aspects after TKI therapy has been demonstrated. Vascular permeability in the axitinib study was decreased to 50 nM microspheres.[54] Even by a 86% reduction in the tumor vascularidity, the delivery of larger molecules was improved through the remaining vessels. Drug delivery of large molecules after treatment with axitinib increased, and the permeability of vessels was reduced. Likewise, sunitinib therapy in gliomas enhanced chemotherapy (temozolomide) delivery and reduced IFP in tumors.[60,61] In these studies, sunitinib induced vessel normalization by an increase in chemotherapy penetration, which is associated with a decrease in the thickness of collagen IV in abnormal vasculature basements. In the study of sunitinib, the effect on drug delivery was reported for low doses only, which approved the importance of vessel normalization in an antiangiogenesis study. The extravasational drug load can lead to vessel regression, so it impedes the delivery of cytotoxic drugs into the tumors.[60]. It can result from a failure of a high dose of TKIs combined with chemotherapy during a clinical study. Results of Semaxanib in melanoma xenografts showed an improvement in vessel normalization by better blood flow in tumors and oxygenation and reduction of tumor IFP by TSU68 therapy.[23] After 2 days of commencing the vessel normalization, the permeability of vessels was reduced and extended for 8 days.

3.4. Phosphatidylinositol Glycan Anchor Biosynthesis Class F (PIGF)-Directed Agents

Placental growth factor PIGF is a member of the VEGF family involved in tumor angiogenesis and has a regulatory effect on vessel normalization. PIGF can promote several regulations in tumors, ischemic diseases, and inflammatory diseases by stimulating ECs growth, recruitment of macrophages, mobilization of bone marrow cells, and macrophage polarization modifications when it binds to VEGFR1, NRP-1, and NRP-2.[21] PIGF is not normally expressed in tissue molecules but is overexpressed in various tumors.[63] There are conflicting results in pharmacological blocking of PIGF.[19,63,64] While Van de Veire and colleagues have illustrated that the inhibition of PIGF can block the vessel abnormalization in tumor models, Bais and coworkers manifested that such inhibition has no effect on reducing the growth rate of primary tumors.[64,65] Blocking PIGF by monoclonal anti murine antibody has controlled tumor growth in melanoma, lymphoma, pancreatic carcinoma, and colon carcinoma in murine models.[63] Many factors such as reduction in tumor angiogenesis, apoptosis of ECs, and vascular pruning affect tumor growth. The effect of ECs losing is more prevalent than PVCs losing, which results in the maturation of vessel structure and reduces the hypoxia in tumors. Using these antibodies demonstrated effective results of cytotoxic chemotherapy. Other effects of antibodies include reduction in the growth of carcinogens, induction of murine hepatocellular carcinoma transgenically, and normalization of uniform vessels.[66] These results are not confirmed generally, as another group has reported limited effect for the different antibodies of PIGF on tumor growth in combination therapy with anti-VEGF or monotherapy. But in both studies, the reduction of metastasis was reported by blocking PIGF.[19,63]

Nonetheless, treatment of human solid tumors using anti-VEGF monotherapy despite promising preclinical studies has not been clinically successful.[26,27] For example, preclinical studies confirmed that the administration of bevacizumab (monoclonal antibody against human VEGF) as anti-VEGF therapy improves patients’ outcomes significantly. However, the response rate in patients with metastatic colorectal cancer that received bevacizumab monotherapy was only 3.3%. Similarly, in patients with metastatic breast cancer, overall survival of 6.7% was observed for the same therapeutic approach.[67] Also, in other cases, such as ovarian cancer or recurrent glioblastoma, anti-VEGF monotherapy has been proven effective but not as strong as that makes tumor shrinkage. According to the mentioned reports, anti-VEGF therapy alone is not effective for inducing regression in tumor vessels. However, the combination of anti-VEGF therapy and chemotherapy in phase III of clinical trials showed significant improvement in survival cases as compared to chemotherapy alone. Several cases have further discussed this synergistic effect of anti-VEGF antibody therapy with chemotherapy in some malignancies.[68,69] It can be described by the primary intention of anti-VEGF therapy that is designed to starve tumors of nutrients and promote vascular regression. In contrast, the efficacy of chemotherapy depends on tumor blood flow. This evidence seems counterintuitive but proved by clinical data from administering compounds with blood vessel pruning effects.

4. Other Therapeutic Effects of VEGF Pathway Blocking

4.1. Improvement of Immune Responses

The immnosuppressive effect of VEGF has been primarily described. Dendritic cells are antigen-presenting cells and play a crucial role as antitumor activators of the immune system. As reported in both in vitro and in vivo studies, these cells’ maturation and common function are impeded by VEGF.[70-72] In addition, infiltration of regulatory T cells (Tregs) and Gr1 myeloid suppressor cells are increased in tumors on account of VEGF overexpression, resulting in a further immunosuppressive TME: conversely, blockade of VEGFA–VEGFR pathway in metastatic colorectal cancer patients and colon cancer–bearing mice indicated a remarkable decrease in tumor-associated Tregs proliferation.[74-76] Likewise, sunitinib monotherapy of tumor-bearing mice resulted in the depletion of myeloid-derived suppressor cells (MDSCs) in the tumor, spleen, and circulation. A twofold increase in the number of CD8 T cells with significantly enhanced activation status was reported as well.[76]

As reported in some experiments, the entry of leukocytes into tumors is prevented since leukocyte adhesion molecules expressed on ECs are downregulated by VEGF.[23,77] Some other
studies also revealed that VEGF aggravates the expression of adhesion molecules on angiogenic vessels, furthering adhesion and rolling of natural killer (NK) cells.[23] Available data approves synergistic impact of combined anti-VEGF therapy and immuno-therapy; additionally, cytotoxic T cells infiltrate more efficiently into tumors following vessel normalization effect of anti-VEGF therapeutics. The impaired characteristics of the tumor niche, due to its abnormal vasculature, negatively impact the infiltration of immune cells into the tumor, and thus the immune responses of these cells. Vessel normalization affects the function and structure of tumor vessels, corrects the abnormality, and also controls the therapeutic responsiveness of TME. The indigenous immune activity is promoted due to the normalization effect of anti-VEGF monotherapy on the tumor niche. In vivo study of Dirkx and colleagues on tumor-bearing mice demonstrated that through administration of antiangiogenic monotherapy, endothelium–leukocyte interplay would be increased consistent with the significant tumor infiltration of host leukocytes such as cytotoxic T lymphocytes.[78] Also, most of the patients on bevacizumab treatment showed higher levels of soluble E-cadherin and soluble E-selectin than the baseline.[79] It is assumed that the triggering of VCAM-1 and E-selectin (leukocyte-adhesion molecules) accounts for the induction of rolling and adhesion of leukocytes. Likewise, anti-VEGFR2 monotherapy in the other study was effective in increasing tumor-infiltrating CD4 and CD8 T-cells.[77] In conformity with these data, antitumor immune responses by promoting immune cell infiltration into tumors might be improved by anti-VEGF therapy–mediated vascular normalization; hence, immunological effects of anti-VEGF-based therapy approaches are indeed worthy of a better investigation in clinical cases.

4.2. Enhancement of Drug Delivery

Rakesh K. Jain in 2001 introduced the vessel normalization hypothesis suggesting that despite depleting vessels, submaximal doses of antiangiogenics considerably maintain the standard function and structure of tumor vessels and promote drug delivery.[29] Although in a series of preclinical studies, significant drug delivery enhancement has been reported during the normalization window, the impact of antiangiogenic agents on the intratumoral distribution of drugs in human tumors is among the most challenging area of study, and a small number of cancer patients have been assessed to date. In the study conducted by Muggia et al., the pharmacokinetics of PEGylated liposomal doxorubicin (PLD) was examined in patients with recurrent epithelial ovarian cancer.[80] PLD concurrently administrated with bevacizumab or alone. Simultaneous administration of PLD with bevacizumab resulted in an increase of PLD’s $t_{1/2}$ (half-life) in keeping with a remarkable decrease in serum PLD levels. This suggests that improvement of vasculature functionality plus a decrease in IFP may play a pivotal role in maintaining intratumoral PLD regardless of reduced permeability of vessels. In a more recent clinical study, neoadjuvant bevacizumab therapy strategy in HER2-negative breast cancer patients reduced the IFP and promoted PVC coverage, resulting in a more normalized and better-perfused vasculature phenotype and enhanced drug delivery.[81] Moreover, Park et al. reported that simultaneous activation of Tie2 and inhibition of Ang2 (using an antibody named ABTAA) in three different preclinical tumor models not only improved blood perfusion but also led to a decrease in both tumor growth and metastatic spread but elevated chemotherapeutics delivery into tumors.[82] Tumor-associated macrophages (TAM) are typically polarized toward a more M2-like phenotype, that increases angiogenesis and immunosuppression.[83] Data indicates that TAM repolarization from M2-like phenotype toward M1-like phenotype, which is classically activated and of more antiangiogenic features, mediates vascular normalization. Rolny et al. were the pioneers who focused on the histidine-rich glycoprotein (HRG) impact on macrophage polarization.[64] HRG is a plasma protein which through the suppression of the PIGF signaling protects against tumor growth and metastasis, via modulation of macrophage polarization and induction of vascular normalization.[83,84] In line with the previous studies, Lammers’ team examined the impact of macrophage-mediated vessel normalization on the tumor accumulation and penetration of prototypic polymeric drug carriers. Using multimodal and multiscale optical imaging, they indicated that the accumulation of fluorophore-labeled polymers in tumors and their penetration out of tumor blood vessels deep into the interstitium is promoted through the normalization of tumor vasculature[85] (Figure 3).

Figure 3. The effects of histidine-rich glycoprotein (HRG) expression on the polarization of M2-like tumor-associated macrophages (TAM), which is alternatively activated and pro-angiogenic phenotype toward M1-like TAM, which is a classically activated and antiangiogenic phenotype. This polarization leads to the vascular normalization phenotype in tumors through the downregulation of PIGF. Reproduced with permission.[85] Copyright 2011, Elsevier.
5. Nanoengineering Normalization

Therapeutics generally suffer from poor pharmacokinetics, which are forced to the repeated administration. In comparison to the direct administration of a specific compound (traditional drug delivery systems), nanoformulation is considered to considerably improve the impact of therapeutics.\cite{ref_86} High toxicity, poor specificity, poor aqueous solubility, and unfavorable pharmacokinetics are other obstacles in conventional therapeutic systems.\cite{ref_87} However, NPs and NP-based drug delivery systems are promising alternatives to resolve the mentioned issues.\cite{ref_88} NPs with elaborate designs are a versatile platform for drug delivery. The engineering of multifunctional nanodrugs demonstrates new possibilities to manipulate the TME. In addition, they are seen to be highly effective in improving the efficacy of chemotherapeutics by normalizing tumor vessels and modifying TME. Nanoengineered normalizations approaches are mostly designed by recruiting whether NPs, liposomes, micelle-based therapies, or gene-based therapies. This section focuses on the effect of each of these nanoengineered approaches on tumor vessel normalization and TME modulation. Nonetheless, it should be noted that shifting from traditional drug delivery systems to NP-based delivery systems could be problematic as our knowledge for engineering NPs capable of delivering frequently used therapeutics is limited. Furthermore, biophysical and biochemical limitations in developing nanocarriers should be considered. Here, we would also discuss some of these limitations if applicable.

5.1. NPs

Aiming to achieve vessel normalization, nano delivery strategies have been implemented to target tumor vasculature. Jain et al. pioneered the study for nano delivery approaches concerning normalization of tumor angiogenesis when they unveiled that tumor vasculature normalization and subsequently reduction of the intratumoral IFP resulted from VEGFR2 targeting promotes NP delivery in a size-dependent manner.\cite{ref_89} However, further studies revealed intratumoral accumulation of larger NPs following VEGFR2 inhibition; yet a more homogeneous distribution of smaller NPs was demonstrated inside the tumor.\cite{ref_90}

It should be mentioned that according to the unfavorable impact of collagen deposition on increased IFP and consequently reduced NP delivery, recent efforts of vessel normalization have led to targeting both the TME and the ECM through the...
coadministration of anti-VEGF and anti-TGF-β, which has shown enhanced intratumoral nanomedicine delivery.²⁹¹ EC functions such as angiogenesis are mediated by the growth factor Nogo-B.²⁹² Surface charge switchable polymeric NP was developed to knock down Nogo-B receptor, resulting in breast tumor vascular normalization in vivo and metastasis prevention through reversion of epithelial–mesenchymal transition (EMT) (Figure 4).²⁹³ Despite many advantages known for the NP-based drug delivery methods (e.g., lower degradation and less toxic side effects of drugs), Wilhelm and colleagues believe that a better selective therapy using NPs is not achieved yet in the course of cancer treatment.²⁸⁶,²⁹⁴ When contemplating the application of such engineered methods to deliver therapeutic agents, it should be considered that the long-term effect of nanomaterials on human metabolism needs to be measured precisely to determine if any adverse effect is correlated to the NPs.

5.2. Liposomes

Since 1995, when the FDA approved Doxil, a PEGylated delivering doxorubicin, numerous positive and negative results are obtained from the administration of liposomal drugs in the treatment of cancer.²⁹⁵ Despite the multitude of advantages of recruiting liposomes in delivery systems including the possibility of carrying both positively charged and negatively charged therapeutics, these carriers have low stability, short half-life, and are sensitive to oxidation and hydrolysis.²⁹⁶ These obstacles have limited the application of liposomes as nanocarriers for treating cancer. In contrast, some of these negative features such as short half-life can be considered useful when designing vaccines against cancer. Studies have shown that liposomes are great candidates for the adjuvant-delivery systems in designing vaccines.²⁹⁷ Several studies have focused on the recruitment of liposomes as a useful method for the delivery of therapeutic agents. A valuable and functional example in this regard is the application of gold NPs (AuNPs) in the tumor vessel normalization. AuNPs are composed of an inorganic gold core encircled by an organic monolayer. The outer layer provides easy and custom surface modification for optimum and specific delivery to the target. The noticeable optical characteristic of AuNPs supports photothermal therapeutic applications due to efficient heat generation coming from excellent near-infrared (NIR) light absorbance.²⁹⁸ In various tumor types, AuNPs have been used for vessel normalization. Administration of human recombinant endostatin encapsulated with AuNP in non-small cell lung cancer (NSCLC) represented transient vessel normalization characteristics.²⁹⁹ Notably, in a novel strategy, aptamer-mediated co-delivery of erlotinib (anti-endothelial growth factor receptor (EGFR)) and survivin (antiapoptotic protein) short hairpin RNA in combination with chloroquine substantially impeded tumor growth in EGFR-mutated NSCLC.³⁰⁰ Additionally, AuNPs demonstrated significant potential in the blockade of metastasis in melanoma due to their vascular normalization effects.³⁰¹ Normalized tumor vasculature in a cediranib-treated breast cancer model elevated tumor retention of enzyme-responsive size-changeable AuNPs, confirming that combinatorial approaches could be an influential strategy for breast tumor imaging and treatment.³⁰²

5.3. Micelle-Based Therapies

One of the most effective approaches in the delivery of water-insoluble therapeutic agents is loading the agent inside a polymeric micelle. Engineering such micelle-loaded particles would enable the delivery of water-insoluble agents. However, it should be considered that the drug-loading capacity of the desired micelle is significantly affected by the structural and chemical compatibility of the micelle core and the therapeutic agent.³⁰³ In any case, if designed carefully, such micelles have manifested desirable outcomes in many studies. In a study of cyclooxygenase (COX)-2 inhibitor, celecoxib, which is considered as the TME-normalizing mediator, the uptake of paclitaxel-loaded micelles in xenografts of human lung adenocarcinomas was improved.³⁰⁴ Irinotecan-treated glioblastoma tumors mostly show normalized vessels featured by enhanced PVC coverage and reduced vascular diameter. Data manifested that the pharmacokinetic profile of chemotherapeutics, including irinotecan, doxorubicin, and vincristine as well as their potency in tumor inhibition, significantly improved following intravenous administration of their lipid-based nanoparticulate formulations in orthotopic glioblastoma.³⁰⁵ Nonetheless, such therapy approaches should be developed further as at the current stage, micelles have manifested poor drug-loading efficiency, insufficient cellular interaction of micelles and cancerous cells, which lead to insufficient cellular uptake.³⁰⁶ Thus, micelles should be improved in design and structure to overcome these barriers.

5.4. Gene-Based Therapies

Gene-based therapeutic approaches such as RNA interference (RNAi) technology have been developed by using foreign genomic material to correct or alleviate inherited and acquired genetic errors in the host cells.³⁰⁷ In tumor treatment, RNAi emerged as a beneficial therapeutic tool to downregulate genes that directly or indirectly are responsible for the abnormal proliferation of cancerous cells. Nanomaterials delivering these therapeutic nucleic acids have been used widely to normalization of vascularatures in the tumor. Recent evidence reveals that microRNAs, including the miR-200 family, contribute to the regulation of different stages of angiogenesis. Inhibition of both tumor growth and metastasis followed tumor vasculature normalization through NP-mediated delivery of miR-200 in ovarian, lung, renal, and breast adenocarcinomas.³⁰⁸ Furthermore, encapsulated irinotecan and miR-200, respectively, with peptide-modified liposomes and solid lipid NPs (SLN) demonstrated that effective delivery of these pH-sensitive targeting NPs may provide a beneficial therapy approach for promising colorectal cancer treatment.³⁰⁹ However, when interpreting the obtained data with microRNAs, it should be noticed that microRNAs can affect several genes in different organs simultaneously. Hence, the desired microRNA for targeted therapy should be well selected.

In addition to being efficacious therapeutic agents, nucleic acids can serve as a building block to assemble sophisticated and biocompatible nano-assemblies. Son et al. introduced hybrid RNA-based AuNP assemblies (RNAi–AuNP) to integrate the beneficial biological function of RNAi and photothermal therapeutic function of AuNPs (Figure 5).³¹⁰ In this design, a different
Figure 5. Schematic illustration of siRNA–AuNPs nanocluster formation for enhanced delivery of therapeutic anti-VEGF siRNA and combination with strong photothermal therapy. a) Sense and antisense strands of anti-VEGF siRNA separately conjugated to the AuNPs via thiol-ligand chemistry, b) RNA-conjugated AuNPs serve as a building block of nanostructures, c) various self-assembly formations of different building block geometries via complementary siRNA base-pairing, d) cationic PEI polymer encapsulated nano assemblies to enhance their stability and cellular uptake. Higher coupled surface resonance of Au-nanoclusters in comparison to individual NPs provides strong potential for use as photothermal therapy agents. Moreover, Du and colleagues utilized an NP-based drug to observe its effect on the normalization of tumor blood vessels in mice. In this study, they recruited a lipid derivative conjugate (LGC) that was constructed of antiangiogenic agents with gemcitabine (a chemotherapy medication) and low-molecular-weight heparin (LMWH). Employing fluorescein isothiocyanate–conjugated dextran as a probe to analyze tumor vascular leakage, they observed that the extravascular fluorescence in LGC, gemcitabine, and LMWH-treated tumors was significantly reduced when compared to the control group. Furthermore, they used the HIF-1α immunohistochemistry and laser speckle contrast analysis system to assess the oxygen level of TME and tumor vascular perfusion, respectively. Results indicated that HIF-1α decreased significantly in LGC NP-treated mice. Also, LGC was seen to remarkably improve the perfusion extent on day 6 in the normalization window. It can be concluded that the LGC NPs are effective for normalizing the tumor vascular. The LGC NPs were also found to improve the efficacy of chemotherapeutics in treating liver cancer in mice. However, genomics and proteomics investigations are needed to confirm the effect of such method in clinical studies. Reproduced with permission. Copyright 2017, Ivyspring International Publisher.
number of sense and antisense RNA strands of anti-VEGF siRNA were separately conjugated to the surface thiol groups of AuNPs. Self-assembly of siRNA conjugated AuNPs through complementary base-pairing interactions in RNA stands from condensed and compact nanostructures with a high loading of therapeutic siRNA. Coupled surface plasmon and strong absorption of NIR wavelengths in larger AuNPs assemblies rather than individual NPs provide efficient heat conversion that verifies the potential of using as a photothermal therapy agent. Furthermore, the stability and effectiveness of intracellular delivery of nano-clusters have been enhanced via coating by cationic branched polyethyleneimine (PEI) polymers. Measuring the VEGF protein expression level in the cancer cells and tumor-bearing mice confirmed the efficacy of gene silencing using the hybrid nano-assembly and imposed sufficient combinational therapeutic potential for cancer therapy (Table 1).

### Table 1. Summary of drugs that affect tumor vascularization.

| Drug              | In combination with | Nanodrug delivery approach | Target[s]/mechanism of action                                    | Reference[s] |
|-------------------|---------------------|----------------------------|------------------------------------------------------------------|--------------|
| Bevacizumab       | Irinotecan and temozolomide | –                          | VEGF-A                                                           | [23,67,79]   |
| DC101             | Abraxane            | –                          | VEGF–VEGFR2 signaling, tumor Ang-1                               | [23,46,51,52,128] |
| Sunitinib         | –                   | –                          | VEGFR tyrosine kinase (TK), colony stimulating factor 1, ret-cskit, Flt3 | [60,61,76]   |
| Pazopanib         | –                   | –                          | VEGFR TK, colony stimulating factor 1, ret-cskit, Flt3           | [60,61,76]   |
| Sorafenib         | –                   | –                          | VEGFR TK, colony stimulating factor 1, ret-cskit, Flt3           | [60,61,76]   |
| Axitinib          | –                   | –                          | VEGFR1, VEGFR2, VEGFR3, PDGFRb, c-kit TK                        | [41,54]      |
| TSU 68            | KRN951              | –                          | VEGFR2, FGFR, Platelet-derived growth factor receptor beta (PDGRbS), FGFR Pan-VEGFR TK | [23]         |
| Doxorubicin       | Bevacizumab         | PEGylated liposome         | Reduced IFP                                                      | [81]         |
| Neoadjuvant bevacizumab | –             | –                          | Reduced IFP and enhanced PVC coverage                           | [81]         |
| ABTAA             | –                   | –                          | Tie2, Ang2                                                       | [82]         |
| Histidine-rich glycoprotein | –         | –                          | PI/GF signaling                                                  | [62]         |
| NP-NgBR           | –                   | NP                         | Nago-B receptor                                                 | [93]         |
| Human recombinant endostatin | –       | AuNP                       | VEGF, FGFR                                                     | [98]         |
| AZD2171 (cediranib) | –                    | Enzyme-responsive size-changeable AuNPs | VEGFR1, VEGFR2, VEGFR3, PDGFRb, c-kit TK TK | [101,147]   |
| Celecoxib         | Paclitaxel          | Micelles                   | Cox-2                                                            | [103]        |
| Irinotecan        | –                   | Lipid-based NP             | Enhanced PVC coverage and reduced vascular diameter            | [104]        |
| Irinotecan        | miR-200             | Solid lipid NP and peptide-modified liposomes | VEGFR1             | [108]        |
| RNAi              | –                   | AuNP                       | VEGF                                                            | [109]        |
| Gemcitabine       | Low-molecular-weight hairpin lipid derivative conjugate | –                          | HIF-1α                                                          | [146]        |
| Erlotinib         | Human-serum-albumin-bound paclitaxel | –                          | VEGF, EGF                                                       | [112]        |
| Cisplatin         | Chloroquine         | –                          | Notch-1 signaling                                               | [135]        |
| Rapamycin         | Cisplatin           | Poly (lactin-co-glycolic acid) NPs | mTOR signaling                                                  | [56,138-140] |

VEGF: vascular endothelial growth factor; PDGF: platelet-derived growth factor; IFP: interstitial fluid pressure; FGF: fibroblast growth factor; HIF: hypoxia-inducible factor; NP: nanoparticle; EGF: epidermal growth factor.

### 6. Effect of TME Modulation and Tumor Vascular Normalization on Nanomedicine Drug Delivery

The TME is distinguished by several features, including hypoxia, tortuous neovascularure, low extracellular pH, and increased ECM network concentrations. These barriers are contributed
to drug delivery failure and chemoresistance.\[110,111\] Moreover, as blood vessels of tumors are poorly organized and leaky, the IFP inside tumors is increased and blood supply to the tumor is reduced. This feature reduces drug delivery to the tumor cells.\[89\] Nevertheless, considering the enhanced permeability and retention (EPR) effect, which states that molecules with particular diameter and size (e.g., NPs and macromolecule drugs) accumulate more in tumor tissues compared to the normal tissues, the permeability of nanodrugs to the tumor cells is increased. In contrast, several studies have shown that TME modulation and blood vessel normalization increase nanomedicine drug delivery to the tumor cells, manifesting controversies when the EPR effect is considered\[88\] (Figure 6). This section reviews the characteristics of NP-based drugs in TME, modulated TME, and normalized vessels of tumor tissues. Furthermore, drugs that increase drug delivery by modulating the TME and/or blood vessel normalization will be discussed.

6.1. Barriers of NP-Based Drugs Transportation in TME

It is well studied that three barriers hold the efficacy of NP-based drugs transportation to the TME: abnormal tumor vasculature and elevated IFP, dense ECM, and stromal cells.\[103\]

6.1.1. Abnormal Tumor Vasculature and Elevated IFP

The abnormal and atypical tumor vasculature is characterized by dilation, tortuosity, insufficient blood supply (associated with IFP), and heterogeneous distribution.\[112\] The leaky nature of tumor vessels and tortuosity promote the compromised blood flow in tumors since the high tortuosity of tumor blood vessels causes a promoted geometric resistance, which then reduces the blood flow. Inadequate blood flow causes poor delivery of the drugs that are systematically administrated.\[103\] IFP is defined as a class of stress that is exerted by fluids. Studies have shown that the IFP is consistently increased in tumors. Fluid flow is associated with fluid drainage by the lymphatic vessels, all over the tumor interstitium and the flow alongside the tumor vessels.\[113\] The IFP is seen to abolish the fluid pressure gradient that motivates a rapid convective penetration into tumors (a flow-driven process), which would ultimately hinder drug delivery to tumors.\[114\] This issue restricts drug penetration in the transvascular (along vessel walls into tumors) and interstitial (across tumor tissue) to decelerate the diffusion rate.\[106\] This seems more problematic in solid tumors when therapeutics with large sizes (e.g., drug-loaded NP) are advised.\[112\]

Figure 6. A schematic representation of the effect of tumor vessel normalization on the efficiency of intratumoral IFP and drug delivery. Hypoxia condition and IFP within tumor are reduced in normalized tumor vessels, which in turn elevate the drug delivery efficiency. Nanocarrier size is a limiting factor for optimal targeting and delivery, the efficiency of which is inversely proportional to the nanocarrier size, under vessel normalization conditions. Reproduced under the terms of the Creative Commons CC-BY license.\[108\] Copyright 2019, The Authors. Published by Frontiers Media S.A.

6.1.2. Dense ECM

It is well-established that the TME is owning a complex structure being composed of a wide range of ECM.\[115\] The ECM network mainly contains a complex compound of near 300 components, including functional enzymes like lysyl oxidase, matrix metalloproteinase, and macromolecules such as fibronectin, hyaluronic acid, and collagen.\[116,117\] ECM is seen to be frequently disorganized in tumors. This can mainly occur in desmoplastic tumors such as breast and pancreatic cancers.\[118\] It is observed that high collagen levels of ECM (i.e., dense ECM) restrict the transportation of NPs > 100 nm in dense ECM.\[119\] In addition to the role of dense ECM in limiting the vascular transportation of NP-based drugs, it isolates tumor cells inside nest-like structures at a definite distance from the collapsed vessel, leading to the prevention of homogeneous distribution of NP-based drugs.\[120,121\] In contrast, in tumors with high permeability, NP-based drugs can penetrate the tumor tissues much easier than desmoplastic tumors.\[122\]

6.1.3. Stromal Cells

Stromal cells include pericytes, TAM, and tumor-associated fibroblast (TAF). Pericytes are mainly located in the prevascular space that affects the delivery of NP-based drugs. Accumulating evidence has shown that either immature, leaky vessels with slight coverage or overmature vessels with excessive coverage of pericytes are unfavorable for NP-based therapies. Excessive pericyte coverage is regularly detected in desmoplastic tumors that restrict NP-based therapeutics transportation by decreasing the endothelial gap. This limitation mainly occurs for larger NPs.\[122\] TAF is a vital cancer-associated inflammatory cell that participates in the immune evasion of tumor cells, which occurs due to the TME characteristics and angiogenesis that ultimately leads to tumor growth.\[123\] Inflammatory cells are demonstrated to be linked with off-target uptake of NP-based drugs due to their phagocytic properties. It is manifested that the affinity of NPs to TAM is four times greater than the affinity of NPs to cancer cells, showing that TAM can significantly reduce NPs uptake by tumor cells.\[124\] TAF is vastly reported to act in the progression of cancer. TAF is seen to be rich in desmoplastic tumors and plays a pivotal role in the production of EMC for the isolation of tumor cells in a
nest. TAF is known to hold the central part of tumor stroma and supplies the binding-site barrier for interstitial transportation of NP-based drugs. TAF also is related to dense EMC, which compresses tumor vessels, and affects the vascular transportation on NP-based drugs. Miao and colleagues have shown that TAF uptakes anisamide ligand–modified NPs seven times higher than other cells due to differentials in the expression level of the sigma receptor, which acts in cellular differentiation between TAF and other cells.[125,126]

6.2. Blood Vessel Normalization and TME Modulation Increases Transportation of NP-Based Drugs

As discussed, tumor blood vessels and TME limit the delivery of NP-based drugs to their targets, specifically when a large-sized NP is being delivered. Several studies have addressed this issue and investigated the impact of normalized tumor blood vessels and modulated TME on the delivery of NP-based therapeutics. For instance, using bacterial collagenase to treat sarcoma and melanoma xenografts with high collagen content can increase the diffusion of antibodies such as immunoglobulin G (with a hydrodynamic residue of about 4.5 nm).[127] In an in vivo study on mice, Chen and coworkers have shown that erlotinib can significantly affect the expression of VEGF and EGF in colorectal cancer, murine breast cancer, and squamous cell carcinoma.[112] The same study demonstrated that after the treatment with erlotinib, the tumor vasculatures are remarkably normalized while the hypoxia in the TME is reduced. Recruiting immunofluorescent imaging, they illustrated that tumor tissue uptake of a 100 nm NP-based drug (human serum albumin–bound paclitaxel) increases after treatment with erlotinib. This occurs due to the role of erlotinib in the enhancement of tumor perfusion.

Another drug that is reported to enhance the delivery of NPs to the tumor tissue is DC101, of which mechanism in TME and role on tumor blood vessels normalization are described in Section 3.2. Chauhan and colleagues have shown that in orthotopic tumors, 5 mg kg\(^{-1}\) of DC101 can temporarily reduce vessel diameter.[89] This dose of DC101 was seen to improve the transvascular flux (defined as NP penetration rate) of 12 nm particles by 2.7-fold. However, no improvement was detected for larger NPs. Additionally, a 3.1-fold improvement in transvascular flux was determined when 10 mg kg\(^{-1}\) of DC101 was used. Similar to above, a dose of 5 mg kg\(^{-1}\), the doubled doses of this drug did not improve the delivery of larger NPs. To demonstrate the finding of their study, Chauhan and colleagues further compared the result of two combination therapies using 5 mg kg\(^{-1}\) of DC101 and 2 mg kg\(^{-1}\) of Doxil (which has a diameter of about 100 nm) and Abraxane (which has a diameter of about 10 nm). They observed that while vascular normalization with DC101 improved the efficacy of Abraxane, it did not show any impact on the effectiveness of Doxil, which validated their findings. Later, Huang and coworkers manifested that treatment with DC101 improves the delivery of anticancer agents (with 12 nm in size) in a dose-dependent and time-dependent manner by normalizing the tumor vasculature.[128]

Celecoxib, a food and drug administration (FDA)-approved drug, is also reported to modulate the TME and normalize tumor blood vessels.[103] Celecoxib is an inhibitor of COX-2, which is recognized to be upregulated in multiple cancer-related pathways that regulate apoptosis, cell proliferation, angiogenesis, and multi-drug resistance.[129] Modulation of TME and tumor blood vessel normalization after treatment with celecoxib-enhanced paclitaxel-loaded micelles uptake in xenografts of human lung adenocarcinomas.[103] Celecoxib is a member of nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs not only are responsible for COX inhibition but also trigger the function of inflammation resolution mediators. This would lead to the enhancement of vascular permeability.[130] Increasing evidence suggests that NSAIDs apply their tumor-suppressive activity on premalignant lesions in human tumors. NSAIDs also are observed to play a significant role in angiogenesis inhibition. However, our understanding of the role of NSAIDs on the ECM is still insufficient.[71,131]

Figure 7 depicts anti-inflammatory strategy to improve drug delivery to tumors.[130] Bevacizumab is another FDA-approved drug that is used for cancer treatment. Bevacizumab is a recombinant monoclonal antibody that targets VEGF in vascular ECs and obstructs tumor angiogenesis.[112] Bevacizumab is seen to be effective for normalizing tumor vessels when applied in metastatic colorectal cancer treatment and was detected to enhance the delivery of NP-based drugs in tumor treatment.[103,131] The role of imatinib mesylate, which also participates in the normalization of vessel tumors, in the delivery of NPs is investigated by Zhang and coworkers.[114] Imatinib mesylate was reported to significantly decrease the accumulation of NPs with a size of \(\approx 110\) nm. However, NPs with about 23 nm of size were more accumulated when treatment with imatinib mesylate was applied. Chloroquine is another medication that is evaluated in cancer treatment. Studies have illustrated that chloroquine effectively induces cell cycle arrest and, consequently, apoptosis in several cancers.[102] Chloroquine is also seen to function as a suppressor in cancer cell invasion. Maes et al. have described that chloroquine normalizes tumor vessels by increasing Notch1 signaling in ECs.[135] They observed that chloroquine-mediated tumor vessel normalization enhances drug delivery of NP-based drugs and improves the efficacy of chemotherapy in melanoma.

Rapamycin (also known as sirolimus) is a suppressor of T and B cells and is being used to prevent organ transplantation rejection and also acts as a tumor suppressor and angiogenesis suppressor.[136,137] Rapamycin is recognized as a mammalian target of rapamycin (mTOR) signaling pathway inhibitor that leads to reduced levels of VEGF.[138] Cisplatin is a chemotherapy medication is applied to treat several cancers, including breast cancer and cervical cancer. However, the clinical efficacy of cisplatin is frequently limited due to the primary and/or secondary resistance.[139] It is understood that rapamycin can increase the sensitivity of cells to cisplatin by downregulating antiapoptotic proteins.[140] In a well-designed study, Guo and colleagues developed an encapsulated drug containing rapamycin and cisplatin to evaluate the synergetic effect of these drugs on cancer therapy.[35] Using solvent displacement technique, they developed poly(lactic-co-glycolic acid) (PLGA) NPs capable of encapsulating rapamycin and cisplatin cores. The synergetic effect of these drugs in PLGA enhanced the delivery of NPs to the tumor by modulating and normalizing the TME. In this study, Guo reported that cisplatin cores and rapamycin in PLGA can significantly decrease the quantity of TAFs and expression level of collagen in xenograft
tumors, which explains the antiangiogenic effect of this NP on the normalization of the tumor blood vessel. This clarifies the role of this injectable medication in the improvement of NP-based drug penetration to the tumor.

A similar approach to vessel normalization, ECM normalization aims to remodel the microenvironment to replicate that of healthy tissue rather than destroy each component of the ECM.7,32 Cell reprogramming agents tend to be most effective in achieving this goal.32 During the pathological progression of cancer, ECM undergoes changes that are made evident when it changes composition/properties, and strategies are developed which can enhance tumor penetration and diffusion of drugs and nano-formulations throughout solid tumors.

Overproduction/deposition of collagen contributes significantly to fibrosis as a therapeutic target. Thus, targeting the collagenous component of the ECM is of great interest. Inhibiting

Figure 7. Anti-inflammatory treatment is proposed as an alternative to antiangiogenic treatment to induce vascular normalization. Both approaches can improve perfusion and drug delivery as they lead to reduction in tumor vessel wall permeability. Reproduced with permission.[130] Copyright 2017, Elsevier.
collagen synthesis, destroying collagen in the stroma, impairing collagen cross-linking, and blocking collagen interactions are some of the possible approaches. There are two forms of collagen: intact triple-helical collagen and denatured collagen. The production of intact collagens increases in fibrotic conditions such as cancer.\textsuperscript{[15,16]} Inhibiting TGF signaling and the subsequent alteration of how it regulates collagen synthesis has been the most common method to reduce collagen synthesis.

7. Monitoring of Tumor Vascular Normalization

Given that drug administration strictly within the normalized time window is of significant importance, conducting precise monitoring of the time window plays a vital role. Currently, histological staining, including microvessel density, tumor perfusion, vascular morphology, and permeability, is a gold standard for detecting tumor vasculature normalization. Nevertheless, performing the aforementioned detecting strategies in the clinic is almost complicated as they could not be repeated in the same individual, hence, the trend of the normalization time window is difficult to be dynamically monitored. Accordingly, establishing a relatively convenient and repeatable vessel normalization monitoring strategy would be of high value. Of note, novel insights of monitoring vascular normalization have been provided from available data. Although currently several monitoring technologies are implemented in studies, to date, no agreement on validating a single method has yet arrived.

Hypoxia condition facilitated metastasis

The vascular density and perfusion as well as bifurcation of vessels and the oxygen content of a tumor can be visualized by the CT technique. Indirect indications of hemodynamic and morphological indices plus extravasation in various tumor districts are provided by the contrast medium in the contrast-enhanced CT method. Although clinical studies validate the capacity of CT for detecting vascular morphology, tumor perfusion, and response to antiangiogenic therapy, several factors, including scanning solutions, analysis software, and personal bias, affect CT perfusion imaging, indicating the essential need for verification of the sensitivity and reliability of perfusion CT. At present, CT cannot confirm vessel normalization, and more research is required to support this.\textsuperscript{[141]}

Magnetic resonance imaging (MRI) is a versatile, noninvasive, and repeatable detecting method utilized for dynamic measurement of both function and morphology of the target (e.g., vascular changes) in a short time.\textsuperscript{[142]} Clinical studies of tumor vascular normalization have used dynamic contrast-enhanced MRI (DCE–MRI), functional imaging to evaluate antiangiogenic effects\textsuperscript{[143]} and detect hypoxia changes. Notwithstanding that several technological and methodological indicators, including treatment schedule, contrast agent, and tumors type, can simply influence the quality of a DCE–MRI vessel normalization study. Thus, further evidence and a consolidated platform need to be designed. Positron emission tomography (PET) is a molecular imaging method used to supply data about biomolecule metabolism and angiogenesis based upon proteins’ activity.\textsuperscript{[144]} Single-photon emission computed tomography (SPECT) is a nuclear medicine tomographic imaging technology capable of providing 3D information. Both of these techniques are subsets of emission computed tomography (ECT) technology. However, the stability, target affinity, and specificity need to be improved. Furthermore, the slow speed of accumulation of tracers as well as a substantial amount of radioactive metabolites is among the other disadvantages for this detecting method.\textsuperscript{[141]}

Blood flow and morphological features of vessels are quantitatively assessed through a mathematical model provided by another functional technology, dynamic contrast-enhanced ultrasoundography (DCE–US), which uses Doppler ultrasound and perfusion software.\textsuperscript{[145]} DCE–US can be used to evaluate tumor vessel normalization due to its low cost and risk plus providing reproducible data, whereas intestine gas affects data from DCE–US, and also abdominal and pelvic tumors’ blood flow is incapable to be accurately monitored.\textsuperscript{[141]} Intriguingly, with the burgeoning interest of plasma or serum proteomics concerning the characterization of reliable biomarkers for the prediction of tumor progression and detecting vascular normalization, including PIGF, VEGF, soluble VEGFR-1/R2, Ang2, soluble Tie2, SDF1α, and collagen IV, blood has become a promising sample according to its easy availability and sensitivity over the antiangiogenic treatment period. This monitoring method has a confirmed potency to predict cancer prognosis and recurrence, and also metabolic alterations of the body can be displayed. Nonetheless, various tumor types can influence serum proteins; hence, a unified standard has to be formed for this technique.\textsuperscript{[141]}

8. Conclusion

With ever-increasing cases of cancer patients diagnosed each year, the economic burden on individuals who have cancer, the society, the health system, and the country increase substantially. Understanding cancer biology is of core importance for the proper diagnosis and treatment of cancer. Angiogenesis makes it possible for tumor cells to get more blood, which helps them grow. Since angiogenesis significantly changes the morphology of blood vessels, the study of such changes can diagnose tumor formation in the body. A detailed understanding of how angiogenesis takes place provides valuable insight into how these changes can be reversed to suppress blood supply toward tumor cells. Angiogenesis can be reversed using antiangiogenic therapies, which involve inhibiting blood vessel formation. Such antiangiogenic therapies involve blockage of disorganized and immature vessels rendering tumors susceptible to inhibition. The use of anti-VEGF monoclonal antibodies showed promising results in suppressing tumors in mice; however, similar results were not replicated during experimentation on tumor suppression in humans. This problem can be resolved by a combination of anti-VEGF therapy with chemotherapy. Normalization of tissue vasculature is among the novel techniques for curing tumors and holds much promise. Several approaches can achieve this, including direct pharmacological inhibition, indirect inhibition, and genetic modeling. Direct pharmacological inhibition usually focuses on VEGF pathway inhibition. In contrast, the indirect approaches targets alteration of pro-angiogenic factors produced by malignant cells advent of nanotechnology has also introduced new frontiers in the treatment of cancer. Owing to the leaky nature of vessel walls and abnormal vasculature, efficient delivery
of NPs targeting tumor cells has gained much interest and is the center of contemplation in cancer research. NPs, due to their small size, have greater efficacy of drug delivery than the rest of the contemporary drugs.

The efficacy of these therapeutic techniques requires the administration of drugs within the normalized time window. Studying the morphological features of vessels using staining techniques is a valuable monitoring technique for vasculature normalization and optimizing the time window. Similarly, advanced techniques like CT-Scan, PET-Scan, DCE-US, and MRI can also be utilized to accurately identify normalized time window. Identification of normalized time window is not an easy task due to several complications, but still, it is of prime importance to improve the efficacy of cancer therapeutics. Indeed, the concept of vascular normalization has paved new pathways for research into finding an efficient and potent cure for cancer. However, optimizing protocols and efficient monitoring of anti-angiogenesis requires much more effort and research.

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Conflict of Interest

The authors declare no conflict of interest.

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angiogenesis, cancer, chemotherapy, genetic models, nanoparticles, vasculature

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Mohammad Taleb got his Ph.D. from National Center for Nanoscience and Technology, China. He obtained his BS degree from Shahid Beheshti University of Iran in 2015. His main interests are designing bioinspired materials to overcome the barriers in tumor therapy and nanomedicine as well as targeting and regulating tumor microenvironment mediated by intelligent functional nanomaterials.

Niloufar Mohammadkhani obtained her M.Sc. in Clinical Biochemistry from Shahid Beheshti University of Medical Sciences in 2020. Her main research interest is understanding the underlying effects of tumor microenvironment players on tumor cell proliferation and metastasis.

Farbod Bahreini obtained his B.Sc. in Cell and Molecular Biology from the University of Guilan in 2017. He has obtained his M.Sc. in Biochemistry at Tarbiat Modares University, Tehran, Iran. His research interest is understanding the molecular mechanism of cancer progression and metastasis as well as developing noninvasive biomarkers for early detection of cancer.

Muhammad Ovais obtained his BS degree in Biotechnology from the University of Peshawar in 2015 and his MS degree in 2017 from Quaid-i-Azam University. The latter year he joined Professor Chen’s group as a Ph.D. candidate at CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China. His research interest lies in the development of advanced nano/bio-materials for cancer theranostic, immuno-engineering, and nano catalytic applications.

Guangjun Nie is a Principal Investigator at the National Center for Nanoscience and Technology of China and an Associate Editor of Nano Letters. Prof. Nie’s research activity has generated a collection of interdisciplinary works in the fields of nanobiology, nanomedicine, and blood physiology, comprising over 200 papers published in Nature Biotechnology, Nature Biomedical Engineering, Nature Communications, Science Translational Medicines, Nature Reviewers Materials, Blood, Nano Letters, ACS Nano, among others.