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Nano-targeting vascular remodeling in cancer: Recent developments and future directions

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ABSTRACT

Tumor growth and progression are strictly dependent on the adequate blood supply of oxygen and nutrients. The formation of new blood vessels and vascular networks is essential to ensure this demand. Blood vessels also facilitate the invasion of cancer cells into nearby tissues and their subsequent metastasis. Tumor cells represent the main driver of the neovascularization process through the direct or indirect, by neighboring non-cancer cells, release of pro-angiogenic molecules. The mediators (e.g., growth factors and extracellular matrix components), signaling pathways, cellular components, and processes (e.g., endothelial cell proliferation and migration) activated in tumor angiogenesis are similar to those involved in normal vascular development, except they lack efficient control mechanisms. Consequently, newly formed tumor vessels are typically fragile and hyper-permeable with a reduced and erratic blood flow. Targeting the tumor vasculature has been the focus of intense research over the last 20 years. However, despite the initial interest and expectations, the systemic use of anti-angiogenic drugs has not always led to therapeutic breakthroughs and, in some cases, has been associated with the development of tumor adaptive resistance resulting in a more aggressive phenotype. Therefore, new therapeutic approaches have focused on combining anti-angiogenic agents with chemotherapy or immunotherapy and/or optimizing (normalizing) the structure and function of tumor blood vessels to ensure a more efficient drug delivery. In this context, nanomedicine offers the significant advantage of targeting and releasing anti-angiogenic drugs at specific sites, minimizing toxicity in healthy tissues. Several nanoparticles possess intrinsic modulatory effects on angiogenesis, while others have been developed to facilitate drug delivery in association with chemotherapy, thermotherapy, radiotherapy or in response to specific stimuli within the tumor environment (e.g., enzymes, ions, redox potential) or exogenous stimuli (e.g., temperature, electricity, magnetic fields, and ultrasound). Other nanoparticles can modify, under specific conditions, their physical properties (e.g., dimensions, structure, and interactions) to increase penetration in tumor cells. This review provides a comprehensive appraisal of the critical modulators of tumor vascular biology, the most promising nano-strategies that specifically target such modulators, and the directions for future research and clinical applications.

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Table 1
Examples of nano-strategies targeting vascular remodeling in cancer.

Nanoparticle	Ligand or additional treatment	Target	Purpose	Type of cancer	Reference
Superparamagnetic iron oxide nanoparticles	Cytosine deaminase, 5-flucytosine, endostatin, Lenti6.3-CD-EGFP, Lenti6.3-ES-Monomer-DsRed, N-alkyl-polyethylenimine 2 kDa, TNF-α gene	Endothelial progenitor cells, TNF-α	Treatment, imaging	Liver, lung	[45–47,165]
Gold nanoparticles	Folic acid, copolymer poly(ethylene glycol)-*b*-poly(diethylaminoethyl acrylate), recombinant human endostatin, nuclear localization signal peptides, dexamethasone, withaferin A, ultrasound, RGD peptide, captopril, VEGF-siRNA, radiation therapy, radiofrequency ablation, thermal therapy, cryosurgery therapy, cold plasma	Pericytes, migration and invasion, epithelial to mesenchymal transition, VEGF, TNF-α	Treatment	Breast, stomach, colon, melanoma, ovary, liver, glioma, head and neck, renal, leiomyoma	[59,87,92,93, 96,97,114,115, 132,133,138, 139,166–169]
Ferritin-based nanoparticles	Endothelial protein C receptor-targeting peptides, protease activated receptor-1-activating peptides, cisplatin	Pericytes	Treatment	Lung, breast	[61]
Doxorubicin nanomedicine	Tranilast, programmed cell death-1 and cytotoxic T-lymphocyte-associated antigen-4 antibodies	Pericytes	Treatment	Breast	[62]
Nanoparticles loaded with a lipid-anchored near-infrared fluorophore	α-melittin	Endothelial cells	Treatment	Liver metastasis	[66]
Chondroitin sulfate-sorbitan ester nanoparticles	miR-20a	Endothelial cells	Treatment	Liver metastasis	[68]
Chitosan nanoparticles	Pigment epithelium derived factor receptor PLXDC1 siRNA, anti-RhoA siRNA, VEGF-siRNA, bevacizumab, VEGF and Bcl-2 dual-targeted VEGF-siRNA, VEGF and interleukin-4-siRNA, contrast-enhanced computerized X-ray tomography, ang-2-siRNA	Endothelial cells, migration and invasion, VEGF, FGF, angiopoietins	Diagnosis, treatment	Liver, ovary, breast, epidermoid, prostate, esophagus, melanoma	[67,69,91, 153–155,196, 225]
F3 peptide nanoparticles	Cisplatin	Endothelial cells	Treatment	Ovary	[70]
Mesoporous silica nanoparticles	Combretastatin A4, doxorubicin, all-trans retinoic acid, interleukin-2	Migration and invasion, VEGF, TGF-β	Treatment	Cervix, lung, melanoma	[95,143,192]
Cerium oxide nanoparticles	–	Proliferation	Treatment	Ovary	[101]
Cationic liquid nanoparticle conjugated with Arg-Gly-Asp peptide	siVEGFR2	Proliferation	Treatment	Lung metastasis	[103]
ECO (1-aminoethyl)iminobis(N-oleicylsteinyl-1-aminoethyl) propionamide) nanoparticle	β3 integrin-specific silencing RNA cyclic RGD peptide	Epithelial to mesenchymal transition	Treatment	Breast	[107]
Nanoscale coordination polymers with an ethylenediamine component	miR-655–3p	Epithelial to mesenchymal transition	Treatment	Liver metastasis	[110]
Liposome-based nanoparticles	Simvastatin, paclitaxel, VEGF-siRNA, etoposide, doxorubicin, hyperthermia	Epithelial to mesenchymal transition, VEGF, TNF-α	Treatment	Lung, breast, glioma	[113,150,151, 171]
Calcium carbonate nanoparticles	VEGF-C-siRNA	VEGF	Treatment	Colon	[141]
Bioinorganic nanoparticles composed of polyelectrolyte-albumin complex and manganese oxide	Ionizing radiation	VEGF	Treatment	Breast	[146]
Lipid-calcium-phosphate nanoparticles	VEGF-A-siRNA, photodynamic therapy	VEGF	Treatment	Head and neck	[147]
Vapreotide-modified core-shell nanoparticles	VEGF-siRNA, paclitaxel	VEGF	Treatment	Breast	[148]
Organic nanoparticles composed of a tripeptide lipid and sucrose laurate	VEGF-siRNA, paclitaxel	VEGF	Treatment	Lung	[149]
Carbon (60)(OH)(20) nanoparticles	–	TNF-α	Treatment	Lung	[174]
Bio-responsive nanoparticles constructed by β-cyclodextrin-grafted heparin and pH-sensitive pseudorotaxane	Doxorubicin, TGF-β receptor inhibitor SB431542	TGF-β	Treatment	Breast	[181]
Heparanase-driven sequential release of β-cyclodextrin grafted heparin nanoparticles	Doxorubicin, TGF-β receptor inhibitor SB431542, ferrocene	TGF-β	Treatment	Breast	[183]
pH-responsive clustered nanoparticles	TGF-β inhibitor LY2157299, siRNA targeting PD-L1	TGF-β	Treatment	Pancreas	[184]
Polyethylenimine-modified carboxyl-styrene/acrylamide nano-spheres	Unmethylated cytosine-phosphate-guanine oligodeoxynucleotides, TGF-β inhibitor LY2157299	TGF-β	Treatment	Liver	[185]
CGKRK-modified nanoparticles and biomimetic nanoparticles	Fraxinellone, siRNA-loaded lipid-coated calcium phosphate	TGF-β	Treatment	Pancreas	[189]
PEG nanoparticles			Treatment		

(*continued on next page*)

Table 1 (*continued*)

Nanoparticle	Ligand or additional treatment	Target	Purpose	Type of cancer	Reference
	Anti-VEGF antibodies, doxorubicin, polo-like kinase 1 oncogene silencing, TGF-β inhibitor, SN38, LY364947, paclitaxel, PDGFR-β-high affinity cyclic peptide	VEGF, TGF-β, FGF, PDGF, MMPs		Breast, lung, multiple myeloma, colon	[157,190,201, 214,233,235]
Nano emulsions	Fraxinellone, tumor-specific peptide vaccine	TGF-β	Treatment	Melanoma	[191]
Polymer-based nanoparticles	siRNA-FGF-BP	FGF	Treatment	Colon	[200]
Nanoparticles of different size (23–110 nm)	Imatinib mesylate	PDGF	Treatment	Lung	[215]
Gelatin-based nanoparticles	Paclitaxel, 5-aminolevulinic acid, doxorubicin	MMPs	Treatment	Melanoma, sarcoma	[236,237]
HEKMS nanoscale micelles	Doxorubicin	MMPs	Treatment	Breast	[238]
Size-controllable-MMP-2-responsive nanoparticles	CD47 peptide, doxorubicin	MMPs	Treatment	Fibrosarcoma	[239]
Gallium nanoparticles	Gamma radiation	MMPs	Treatment	Liver	[240]
As_2O_3/Fe_3O_4 nanoparticles	Magnetic fluid hyperthermia	MMPs	Treatment	Cervix	[245]

Legend: VEGF, vascular endothelial growth factor; PLXDC1, plexin domain containing 1; VEGFR2, vascular endothelial growth factor receptor 2; TNF-α, tumor necrosis factor alfa; TGF-β, transforming growth factor beta; PD-L1, programmed death-ligand 1; FGF, fibroblast growth factor; FGF-BP, FGF-binding protein; PDGF, platelet derived growth factor; PDGFR-β, platelet-derived growth factor receptor beta; Ang-2, angiopoietin-2; PEG, polyethylene glycol; MMPs, matrix metalloproteinases.

1. Introduction

Cancer cells are particularly dependent on oxygen and nutrients for their growth. This supply is normally provided by the vasculature and the tumor microenvironment (TME) [1]. The vasculature is also essential for cancer cells to metastasize through their entry into the circulatory system, arrest and adhesion to the endothelium, exit from the circulation and, finally, colonization of remote sites [2]. The generation of new blood vessels (tumor angiogenesis) is thus a key process for solid tumor growth and dissemination. This proposition is further supported by the observation that rapidly growing tumors are highly vascularized compared to those that are slowly progressing [3]. Tumor angiogenesis requires the presence of responsive capillaries, small yet highly structured blood vessels consisting of a layer of endothelial cells (ECs) surrounded and supported by pericytes and, occasionally, smooth muscle cells [3,4]. However, tumor angiogenesis is not the only neovascularization mechanism involved in supporting tumor growth and meeting the increased requirement of oxygen and nutrients [5]. Indeed, new blood vessel formation can also occur through vasculogenesis, vascular mimicry, or trans-differentiation of cancer stem cells (CSCs) [4].

EC activation, proliferation and migration, regulated by specific growth factors, represent critical steps in tumor angiogenesis [4,6]. In tumor neovascularization, ECs originate from the trans-differentiation of CSCs [7,8], whereas in tumor vasculogenesis they are recruited from endothelial progenitor cells (EPCs) [9–11]. The pericytes, a cell type that wraps around the blood vessels providing structural support to ECs, are also crucial in tumor angiogenesis by contributing to the development and the maintenance of the vasculature and regulating blood flow and vessel permeability [12,13]. However, tumor vascularization is a complex process that involves several other cells and mediators, such as the vascular endothelial growth factor (VEGF), the platelet-derived growth factor (PDGF), the fibroblast growth factor (FGF), angiopoietins, and chemokines [3]. It is important to emphasize that new tumor vessels are morphologically and functionally different from normal vessels. Indeed, the tumor vasculature typically consists of an intricate network of highly leaky vessels due to the loss of EC junctions and pericytes coverage, favouring a reduced and erratic blood flow [3,14]. The mismatch between reduced blood flow and greater oxygen demand by tumor cells increases the risk of intra-tumoral hypoxia [15]. Despite this dysregulated vasculature environment, cancer cells can still proliferate and develop drug resistance [16]. One reason for this disorganized vascular network is the persistent activation of pro-angiogenic signaling pathways with a resulting imbalance between pro- and anti-angiogenic factors [4].

Over the last 10–15 years, anti-angiogenesis therapies based on the systemic use of anti-angiogenic drugs, while effective in some patient groups, have often failed to meet the expectations typical of a breakthrough treatment. In some cases, their use has induced tumor adaptive resistance mechanisms resulting in a more aggressive phenotype [17,18]. An important factor in this process is the anti-angiogenesis-induced tumor hypoxia, which can stimulate the recruitment of other pro-angiogenic cells with further revascularization and remodeling [19,20]. In this context, the hypoxia-associated transcription factors hypoxia-inducible factor-1 alpha (HIF-1α) and factor-2 alpha (HIF-2α) are critically involved in blood vessel formation [21–23]. Their activity is tightly regulated by oxygen tension through oxygen sensors such as HIF prolyl-hydroxylases and factor inhibiting HIF-1 enzymes [24–26]. Hypoxia and HIF-α subunits attract EPCs from the bone marrow and promote their differentiation into ECs by regulating VEGF activity and stimulating the production of pro-angiogenic molecules, e.g., VEGF receptor 1 and 2 (VEGFR1 and VEGFR2), FGF, PDGF, angiopoietins, and transforming growth factor-beta (TGF-β) [22,27,28]. These molecules can also modulate the activity of extracellular matrix enzymes, matrix metalloproteinases (MMPs), to promote pre-existing vessel sprouting/splitting and allow ECs to migrate across the extracellular matrix (ECM) in response to chemoattractants [28].

The above-mentioned limitations have stimulated the search for new therapeutic approaches, such as combining anti-angiogenic agents with chemotherapy or immunotherapy [29]. Other anti-angiogenesis strategies aim at normalizing the structure and the function of tumor blood vessels to ensure a more efficient anti-cancer drug delivery and accumulation [14,30,31]. In this context, nanomedicine, the use of materials with nanoscale dimensions, typically between 1 and 100 nm, is emerging as the new frontier in cancer therapy [32]. Nanomaterials allow the sustained delivery of drugs to the target sites, with associated advantages including the increased solubility and stability of the drug, the enhanced biodistribution and bioavailability, and the reduced dose required, with consequent reduced toxicity in non-cancer tissues. Additionally, nanocarriers can simultaneously carry multiple and diverse therapeutic agents and integrate them with molecular imaging agents with theragnostic properties [33–36].

This article presents a comprehensive overview and critical appraisal of the key “druggable” modulators of tumor vascular biology, the recent advances in the use of nanomaterials and relevant therapeutic approaches to target these modulators, and the challenges and opportunities for future research and clinical applications.

2. Tumor vascular biology: Components, targets, and nano-strategies

2.1. Cell types

2.1.1. Nano-strategies targeting endothelial progenitor cells

EPCs, first described by Asahara et al. in 1997 [37], are a type of circulating cells that can reach neovascularized sites, differentiate into ECs, and actively participate in angiogenesis in models of ischemia, wound healing, and tumor growth [37–39]. In 2007, Nolan et al. reported that in the early stages of tumor development there is a high recruitment of bone marrow (BM)-derived VEGFR2-positive EPCs, in response to tumor-derived VEGF, which are subsequently incorporated in the lumen of nascent vessels. By contrast, in the more advanced stages, the relative contribution of EPCs decreases due to the increasing presence of non-BM-derived vessels from the periphery [40]. These observations suggest that, in the presence of high concentrations of VEGF during the early stages of carcinogenesis, a consistent amount of EPCs is recruited and transformed into ECs during tumor growth. ECs subsequently secrete additional regulatory factors, which leads to a new balance between the number of EPCs and ECs in the growing tumor. This balance, however, seems to be different according to specific types of tumors [41]. Importantly, EPCs are able to promote the switch from micro- to macro-metastasis by ensuring the development of neo-vessels and the paracrine secretion of several growth factors and molecular signals [42].

EPCs represent an attractive target for medications transported by nanoparticles. However, this strategy has primarily been investigated in cardiovascular research and only a relatively small number of studies have focused on cancer (Table 1) [43,44]. Chen et al. combined superparamagnetic iron oxide (SPIO) nanoparticle labeled EPCs with the "suicide gene" cytosine deaminase (CD), which converts 5-flucytosine (5-FC) into 5-fluorouracil (5-FU), to treat grafted liver carcinomas, and tracked them with 7.0 T magnetic resonance imaging (MRI) [45]. Subsequently, they used CD and the anti-angiogenic agent endostatin (ES)-transfected EPCs, and cultured and transfected mouse BM-derived EPCs with Lenti6.3-CD-EGFP and Lenti6.3-ES-Monomer-DsRed labeled with SPIO nanoparticles against hepatocellular carcinoma. In particular, DiD (lipophilic fluorescent dye)-labeled EPCs were injected into normal mice and mice with liver carcinoma (H22 cells injected into the abdominal cavity of C57BL/6 J male mice) through the caudal vein. The tumor volumes in the EPC + SPIO + CD/5-FC + ES group were ~82% smaller when compared to the EPC + SPIO + LV (lentivirus, empty vector control) group. Furthermore, the median survival time was significantly longer in the EPC + SPIO + CD/5-FC + ES group, 89 vs. 35 days [46]. In another study, labeled EPCs with the non-viral gene carrier N-alkyl-polyethylenimine 2 kDa (PEI2k)-stabilized SPIO were successfully used to facilitate the MRI visualization of EPCs in a mouse xenograft model of lung carcinoma [47].

Therefore, this technology can be used not only for therapeutic purposes but also to track the involvement of EPCs in tumor neovascularization. However, given the relatively limited number of studies investigating this nano-strategy, additional research is warranted to confirm the benefits and the safety of nano-targeting EPCs in liver cancer and other types of cancer.

2.1.2. Nano-strategies targeting pericytes

These important components of the TME, initially described as "adventitial cells" by Rouget in the 19th century [48], were renamed "pericytes" by Zimmermann in 1923 [49]. The role of pericytes in tumor initiation and progression, as well as in drug delivery and local accumulation, is the focus of intense research. Pericytes are elongated, slender, branch-shaped cells with cytoplasmatic projections extending longitudinally and circumferentially around the vessels, between the endothelial wall and the basement membrane [50]. They play a critical role in vessel development, homeostatic maintenance, and regulation of vascular permeability, immunological processes, and exchange between the bloodstream and the peripheral tissues [50,51]. A stable pericyte coverage depends on several critical molecular pathways and mediators, e.g., TGF-β, angiopoietin-1/Tie-2, and PDGF. Pericytes are essential both in physiological and in tumor angiogenesis. An intense direct or paracrine crosstalk between ECs and pericytes is necessary to facilitate blood vessel formation, maturation, and stabilization in physiological angiogenesis. In this context, pericytes secrete growth factors and proteases that stimulate and control EC proliferation and migration [51]. Similar events occur during cancer angiogenesis. However, in this case, pericyte detachment, a process that is also associated with cellular alterations and abnormal cytoplasmic projections, is a critical step to facilitate ECs proliferation and migration while at the same time creating tumor vessels that are excessively disorganized, tortuous, and leaky [52]. The increased number of surrounding pericytes has been associated with higher degrees of tumor progression and poor prognosis, particularly in melanoma and renal cell carcinoma [53].

Pericytes are also involved in tumor dissemination and metastasis. In 2006, Xian et al. reported that the detachment of pericytes from the vessels of primary tumors, and alterations in their communication with ECs, can increase metastatic ability [54]. This finding was subsequently confirmed in patients with colorectal and breast cancer [55,56]. Although the molecular events involved in this phenomenon remain elusive, it has been hypothesized that pericytes act as a physical barrier that limits the transfer of tumor cells into the bloodstream and their eventual seeding in distant tissues. Welti et al. reported, in an experimental model of lung cancer, an enhanced seeding of metastasis in lung regions with a relatively low pericyte coverage, suggesting that pericytes represent an important barrier to cancer cell dissemination [57]. These features have been investigated in studies with nanoparticle anti-cancer treatments (Table 1).

Looprasertkul et al. treated human placental pericytes with 20 nm gold nanoparticles (AuNPs), a type of nanoparticle that has previously been shown to exert anti-angiogenic effects in human umbilical vein ECs (HUVECs), at a concentration of 30 ppm [58]. AuNPs significantly inhibited proliferation, reduced PDGFR-β mRNA expression, and decreased pericyte migration [58]. Ultrastructural analysis of pericytes revealed the accumulation of gold nanoparticles in late endosomes, autolysosomes, and mitochondria. The latter were often swollen or damaged and capillary tube formation was reduced [58]. In three-dimensional capillary tubes, derived from cocultured AuNP-treated pericytes and HUVECs, pericytes were often round-shaped and lacked their typical process extensions, resulting in incomplete tube formation [58]. Another study investigated the anti-cancer effects of the conjugation of copolymer poly(ethylene glycol)-*b*-poly(diethylaminoethyl acrylate) (PEG-PDEAEA) with folic acid-modified gold nanoparticles (AuNPP-FA), in absence of concomitant chemotherapeutics. The treatment inhibited tumor proliferation and metastasis in breast adenocarcinoma (4T1 cells injected into Balb/c mice; reduction in tumor volume and number of lung metastases, ~38% and ~65% respectively) and gastric carcinoma (BGC823 cells injected into Balb/c mice; reduction in tumor volume, ~33%) [59]. The use of folic acid was justified by the frequent overexpression of folate receptors in tumors [60]. AuNPP-FA also normalized the structure and the function of the tumor vasculature by increasing pericyte coverage and strengthening the tight junctions by upregulating the levels of VE-cadherin, an essential cell adhesion molecule, in ECs. No apparent pathological effects were detected in non-cancer tissues in vivo [59]. Ferritin-based protein C nanoparticles (PCNs) were tested in the Lewis lung carcinoma allograft and MMTV-PyMT spontaneous breast cancer models [61]. The PCNs consisted of endothelial protein C receptor (EPCR)-targeting peptides (PC-Gla) and protease-activated receptor-1 (PAR-1)-activating peptides (TRAP). A TRAP-small ferritin (sFn)-PC-Gla (TFG) or a matrix metalloproteinase-2 (MMP-2) cleavage site were inserted between sFn and the PC-Gla domain (TFMG). The nanoparticles significantly reduced the degree of local hypoxia and increased pericyte coverage. This, in

turn, was associated with ~70% inhibition of tumor growth, ~85% reduction in metastasis, and prolonged survival in the studied cancers [61]. The coadministration of cisplatin or doxorubicin with PCNs exerted a synergistic effect on tumor growth suppression, ~50% reduction in tumor volume compared to both chemotherapeutics, by improving drug delivery through an increase in blood perfusion [61]. Another study investigated the combination of the TGF-β inhibitor tranilast, an approved antifibrotic and antihistamine drug, and Doxil, the first FDA-approved nano-drug, a PEGylated liposomal formulation of doxorubicin nanomedicine (size ~85 nm in diameter) designed to avoid premature elimination of the nanocarrier by the reticuloendothelial system. The treatment increased intra-tumoural vessel diameter and pericyte coverage in triple-negative breast cancer mouse models, 4T1 and E0771 cells inoculated into Balb/c and C57BL/6 mice. These effects were associated with a significant normalization of the interstitial space, primarily due to the tranilast-mediated reduction in collagen and hyaluronic acid content and the consequent increase in tissue perfusion and oxygenation. Notably, the increased perfusion facilitated the redirection of local tumor-associated macrophages from an "immunosuppressive" to an "immunosupportive" phenotype. An ~80% reduction in tumor volume with an immunotherapy cocktail of programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibodies was also observed [62].

The results of these studies support the potential benefits of nanomedicine approaches designed, among other effects, to restore pericyte coverage and, consequently, vascular permeability and TME composition, particularly in breast, gastric and lung cancer. The available evidence suggests that specific nanoparticles can directly target pericyte structure and function without additional chemotherapeutic strategies. In other studies, nanoparticle treatment improved the effect of chemotherapeutics, e.g., cisplatin and doxorubicin, on tumor growth. However, this nano-strategy warrants further research in other types of cancer and with different combinations of chemotherapeutics and agents specifically targeting the TME and the interstitial space.

2.1.3. *Nano-strategies targeting endothelial cells*

Targeting tumor endothelial cells (TECs) represents another important strategy in cancer therapy since these cell types regulate several crucial aspects of tumor biology [63]. Alike tumor blood vessels, which differ morphologically and phenotypically from normal blood vessels, TECs also differ in various aspects from normal ECs. Phenotypically, TECs display irregular shape and size and, consequently, form a discontinuous monolayer with loosely associated pericytes and a basement membrane with irregular thickness and type IV collagen content. As a result of these alterations the structural and functional integrity of the barrier is compromised [64,65]. Compared to normal ECs, TECs also exhibit differences in proliferation, migration ability, responses to growth factors, and gene expression profile [65]. Further differences are also present within populations of TECs, e.g., between highly metastatic and minimally metastatic TECs, where the former are more proliferative, motile and sensitive to VEGF than the latter [65]. This heterogeneity can potentially allow the development of specific nanodrug delivery systems to selectively target specific TECs, avoiding toxicity in normal endothelial layers in non-cancer tissues (Table 1).

In a recent study, Yu et al. targeted liver sinusoidal ECs (LSECs), the first hepatic cell line that interacts with circulating metastatic cells and a type of antigen-presenting cells that are responsible for hepatic immunologic tolerance, with nanoparticles containing α-melittin, a natural anti-cancer agent in honeybee venom [66]. The intravenous administration of these nanoparticles caused the rapid activation of LSECs, with a consequent change in the local content of several cytokines and chemokines. The most affected were interleukin-1α, chemokine ligand 9, C-X-C motif chemokine ligand 10, chemokine ligand 13, chemokine ligand 3, C-X-C motif chemokine ligand 1, chemokine ligand 4, and chemokine ligand 5. This, in turn, facilitated the switch of the local immunologic environment to an activated state, with increased content

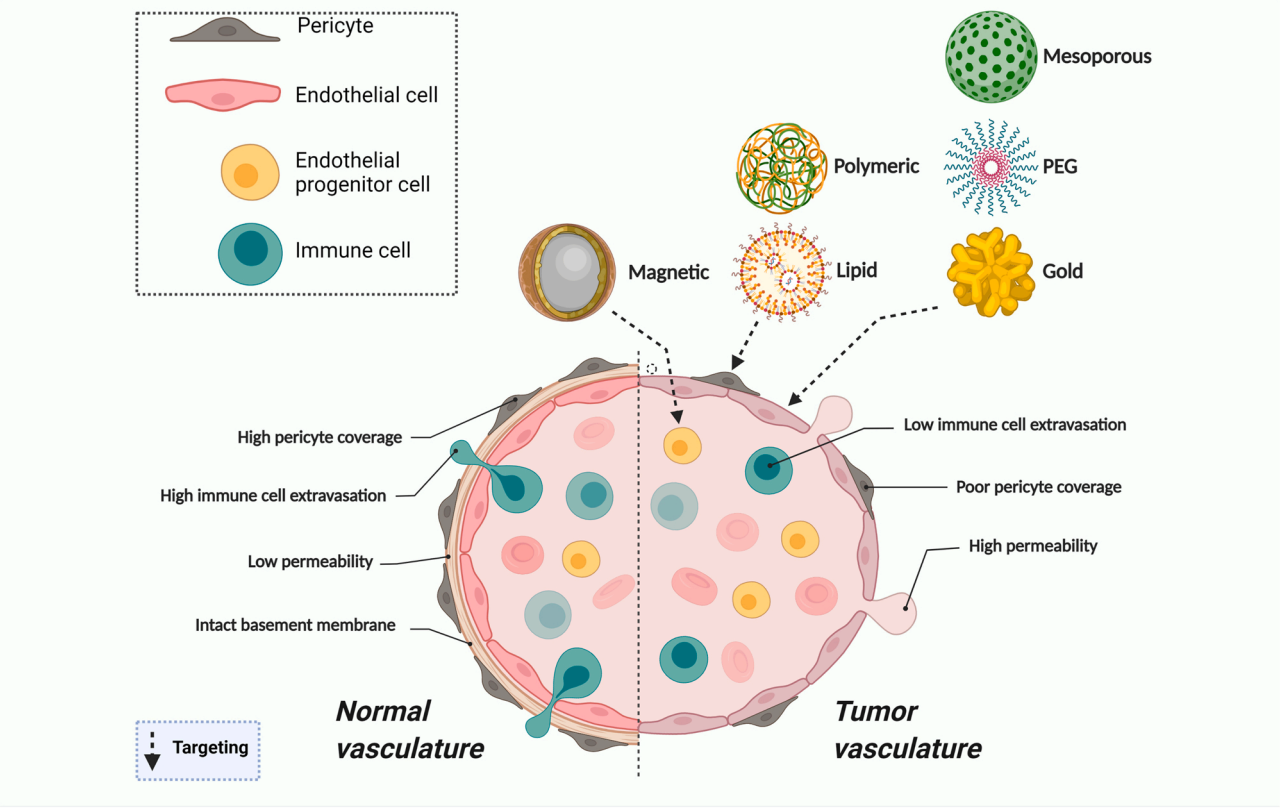

Fig. 1. Description of the principal nanoparticles used to target the main cellular components of the vasculature. PEG, polyethylene glycol.

of natural killer cells, natural killer T cells, macrophages, neutrophils, B cells, and $CD4^+$ and $CD8^+$ cells. A reduced formation of metastatic lesions in three spontaneous liver metastatic tumor models (melanoma, B16F10, triple negative breast cancer, 4T-1, and colon carcinoma, CT26, cells injected into the spleen of C57BL/6 and Balb/c mice), with a survival rate between 37.5% and 70% at 100 days, was also observed. Notably, the α-melittin-NPs-induced changes in cytokines and chemokines were not associated with the development of hepatotoxicity after single or multiple doses. The ability of nanoparticles to enter liver ECs has been further investigated by Xu et al. [67]. In this study, siVEGF-loaded CS-SS9R/BSA-cRGD nanoparticles successfully entered HUVEC EA.hy926 cells and suppressed proliferation and angiogenesis by downregulating VEGF mRNA expression in a liver tumor-bearing mice model consisting of Bel-7402 cells injected into Balb/c nude mice. The treatment also reduced tumor volume by ~78% compared to saline or siVEGF [67]. In addition, chondroitin sulfate-sorbitan ester nanoparticles conjugated with the cancer suppressor micro-RNA miR-20a (size 143 nm) were developed along with a delivery system targeting LSECs [68]. This strategy was prompted by the results of a whole genome miRNA expression analysis of these cells, in the context of liver metastasis in colorectal cancer, which showed a repressed expression of miR-20a. This was associated with a concomitant upregulation of its protein targets, particularly E2F1, contributing to the metabolic reprogramming of cancer cells, and ARHGAP1, contributing to cancer cell proliferation, migration, and invasion. The NPs-mediated restoration of normal mir-20a levels in a murine model of colon cancer with liver metastasis, consisting of the intrasplenic inoculation of C26 cells in Balb/c mice, led to the downregulation of E2F1 and ARHGAP1. This was associated with a ~70% reduction in tumor-infiltrated LSECs [68]. In other studies, the pigment epithelium-derived factor receptor PLXDC1 siRNA-incorporated chitosan nanoparticle (CH-NP/siRNA), coated with hyaluronic acid, has been shown to successfully target the CD44 receptors in TECs. The nanoparticle suppressed microvessel density and reduced tumor growth by ~91% compared to control siRNA in an experimental model of ovarian cancer consisting of female Balb/c mice receiving an intraperitoneal injection of A2780 cells [69].

These and other pivotal studies have stimulated further research on developing more effective nanoparticle-based delivering systems in cancer treatment. Winer et al. developed an anti-vascular tumor therapeutic using the F3 peptide to target cisplatin-loaded nanoparticles (F3-Cis-NPs) to tumor vessels [70]. Although F3-Cis-Np bound with high specificity to both human ovarian tumor cells and TECs in vitro, it only exhibited cytotoxic activity against the latter [70]. Importantly, in various murine ovarian tumor and human tumor xenograft models, F3-Cis-NPs caused a ~3.5-fold regression in tumor volume compared to cisplatin or F3-NP treatment and vascular necrosis. No significant liver, renal, and bone marrow toxicity was observed. More recently, Ara et al. developed a high affinity and specific DNA aptamer as a new ligand for use in liposomal nanoparticles to deliver anti-angiogenic drugs to the tumor vasculature [71]. Other authors have developed dual antibody-coated dendrimers or flavone-based polymers to enhance the control of circulating tumor cells and their hetero-adhesion to ECs for metastasis prevention [72,73]. The issue is particularly intriguing and further delivering systems targeting TECs are expected to be developed in the near future.

Pending the results of these studies, the available evidence suggests that nano-strategies, with or without concomitant chemotherapeutics, e.g., cisplatin, targeting TECs might be particularly effective in colon cancer induced liver metastasis and ovarian cancer. A schematic representation of the principal nanoparticles used to target the main cellular components of the vasculature is described in Fig. 1.

2.2. Mechanisms

Several events drive the metastatic potential of cancer cells, including proliferation and migration of ECs and the transition of endothelial and epithelial cells into a tumorigenic mesenchymal phenotype [74–77]. Standard cancer treatments targeting these mechanisms, e.g., chemotherapy and radiotherapy, are often ineffective because of cancer resistance, lack of selectivity against the target cells, and severe toxicity [78]. For these reasons, nanoparticles are emerging as an alternative option to overcome these hurdles by enhancing target specificity [78–81].

2.2.1. Nano-strategies targeting migration and invasion

Migration and invasion of ECs are an essential component of angiogenesis in the TME. New vessels take shape as ECs respond to cues which either guide or restrain their movement [82]. Cell migration involves repeated extension and contraction cycles generated by finger-like and sheet-like extensions known as filopodia and lamellipodia, respectively, as well as contractile stress fibers [83,84]. These cell protrusions attach to a substrate in the matrix and generate a physical force to heave the cell body forward [85]. The assembly of filopodia and lamellipodia is largely facilitated by the energy-dependent polymerization of cytoskeletal globular actin subunits (G-actin) into helical filaments (F-actin), which retain intrinsic ATPase activity [82]. The invasion process also involves the degradation of ECM components by proteases to facilitate new vessel formation in nearby territories [86]. As such, mechanisms that mediate migration and invasion are potential targets for nanoparticles arresting tumor-associated angiogenesis (Table 1).

Gold nanoparticles have been shown to suppress $VEGF_{165}$-induced HUVEC migration and vessel formation [74]. Morphological assessment using atom force microscopy showed that the treated ECs lacked the proper development of filopodia and lamellipodia, indicating that gold nanoparticles mediated their effect, at least in part, via an impairment of cytoskeletal reorganization. Moreover, the phosphorylation of the signaling molecule, Akt, which is involved in VEGFR2/PI3K-mediated cell migration, was inhibited in a dose-dependent fashion [74]. In addition to targeting signaling molecules that regulate migration, nanoparticles can also restrain migration by enhancing the structural stability of the endothelial barrier. For example, the administration of gold nanoparticles into mice models of melanoma using B16F10 cells increased the expression of the ZO-1 junctional adapter protein, enabling cell-cell interaction in the endothelium. This prevented the transfer of tumor cells into the circulation and reduced the risk of lung metastasis by ~70%. The decrease in migration and invasion was also attributed to the diminished levels of MMP-2 expression, which prevented the degradation of the ECM [87].

Another approach to suppress EC migration consists in targeting the mechanical effects of shear stress, which triggers HUVECs to undergo polarized actin remodeling and subsequent migration and invasion in the direction of the blood flow [82,88,89]. These processes are dependent on the initial release of intracellular calcium, followed by the activation of downstream Rho GTPases and the subsequent assembly of stress fibers [89]. This culminates in cell body contraction and simultaneous elongation of the protrusions in the direction of the shear stress, facilitating ECs migration [89]. Not surprisingly, the overexpression of RhoA, a key component of the Rho GTPases, is associated with a poor prognosis in cancer patients [90]. It was previously reported that intravenously injected nanoparticles are capable of inhibiting RhoA activity, alleviating MDA-MB-231-induced aggressive breast cancer in mice [91]. Specifically, chitosan-coated nanoparticles (ChNPs) enclosing anti-RhoA small interfering RNA (siRNA), administered in two doses (low, 150 μg/Kg, or high, 1500 μg/Kg) every three days for 30 days, significantly inhibited tumor growth, > 90% and 100% inhibition with low- and high-dose, respectively. The anti-tumor effects of the nanoparticles rapidly disappeared after treatment discontinuation. The observed necrotic areas within tumors were due to a reduced number of ECs and inhibition of angiogenesis, corroborating the importance of RhoA in migration and formation of new vessels. No treatment-associated toxic effects were observed in terms of changes in

body weight, histology (liver, spleen, heart, lungs, and kidneys), and biochemical markers of liver, renal, and bone function [91].

Besides siRNAs, nanoparticles conjugated to specific drugs are also effective in suppressing angiogenesis. For example, conjugation of gold nanoparticles with the approved anti-angiogenic drug, recombinant human endostatin (rhES), significantly prevented neovascularization in colorectal cancer xenografts consisting of SW620 cells inoculated into Balb/c mice [92,93]. rhES inhibits the pro-angiogenic molecule, anterior gradient 2 (AGR2), which activates downstream proteins, including MMP-2 and c-Myc, that drive migration and invasion [94]. In parallel, HUVECs exhibited diminished migratory capacity and decreased tube formation in vitro. Notably, the gold nanoparticles conjugated to rhES produced a more potent effect on angiogenesis than gold nanoparticles or rhES alone [93]. A synergistic effect was also observed in other studies, in which β-tubulin damage was more significant when the anti-angiogenic drug combretastatin A4 and the chemotherapeutic agent doxorubicin were both conjugated with mesoporous silica nanoparticles, when compared with treatment with the two agents in isolation [95]. These results paralleled those in vivo, as the tumor growth of HeLa tumor bearing Kunming mice was almost completely inhibited by combined combretastatin A4 and doxorubicin conjugated with mesoporous silica nanoparticles, when compared to the two drugs alone. Although still scarce, these promising findings highlight the presence of several migratory and invasive mechanisms, particularly signaling enzymes and pro-angiogenic proteins, that may be efficiently targeted by nanoparticles.

To broaden the therapeutic potential of nanoparticles, it may also be worth considering their anti-migratory role in tumor cells. For example, it has been reported that the pro-migratory effect of pro-angiogenic chemokines is virtually lost if cancer cells are pre-treated with gold nanoparticles [96]. Specifically, fewer lung tumor nodules were formed after intravenous injection of MDA-MB-231 breast cancer cells pre-treated with gold nanoparticles. This was attributed to the down-regulation of genes involved in energy generation [96]. As a result, there was an inadequate assembly of cytoskeletal components required for cell migration. As cytoskeletal rearrangement is a highly energy-dependent process, the reduced expression of genes related to mitochondrial oxidative phosphorylation and glycolysis led to diminished levels of ATP, ~40%, and subsequent prevention of F-actin cytoskeletal assembly [96]. Another mechanism by which gold nanoparticles can abrogate migration involves the increase in cell and nuclear stiffness. Ovarian serous adenocarcinoma cells (HEYA8) treated with conjugated gold nanoparticles containing nuclear localization signal peptides exhibited decreased cell motility [97]. The nanoparticles aggregated and localized in the nuclear membranes, without internalization into the nucleus. This phenomenon was associated with increased cell and nuclear stiffness, secondary to the overexpression of lamin A/C and the interaction with nuclear membrane-associated proteins that form the integrity-providing nuclear lamina [97].

Collectively, the available findings suggest that several nanoparticles can suppress migration and invasion in colorectal cancer, triple negative breast cancer, and ovarian cancer. Their effect may be potentiated by conjugation with specific siRNAs and chemotherapies, e.g., recombinant human endostatin, combretastatin A4, and doxorubicin. However, given the relatively small number of published xenograft studies, additional research is warranted to confirm the anti-tumor effect and the safety of this nano-strategy in vivo.

2.2.2. *Nano-strategies targeting proliferation*

In addition to migration, the proliferation of ECs, another critical process underlying angiogenesis, can also be targeted to manipulate cancer-associated vascular remodeling (Table 1) [98]. In this context, silver nanoparticles (AgNPs) are inherently cytotoxic and genotoxic against human microvascular endothelial cells (HMECs) and circulating EPCs, thereby arresting their proliferation and angiogenic capacity [80,99]. This effect has been attributed to the depletion of HIF-1α [80]. Proliferation, cell viability and capillary-like tube formation by HUVECs have also been shown to be disrupted by hydroxyapatite nanoparticles, primarily through the inhibition of phosphatidylinositol-3-kinase (PI3K)/Akt/endothelial nitric oxide (NO) synthase (eNOS) [100]. The proliferation of ECs, in the context of ovarian cancer, has also been targeted by cerium oxide nanoparticles, also known as nanoceria (NCe) [101]. In nude mice models of ovarian cancer using the human ovarian cancer cell line A2780, the intraperitoneal administration of NCe significantly attenuated EC proliferation and increased apoptosis, resulting in diminished microvessel density and angiogenesis within the TME. This effect was mediated by the ability of NCe to serve as long-lasting scavengers of free radicals, thereby bypassing the favorable hypoxic environment required for efficient tumor growth [101]. Notably, NCe did not alter VEGF concentrations, further suggesting that their effect is primarily directed to the ECs, and not mediated. NCe also suppressed tumor growth in vivo, with a ~33% reduction in tumor weight compared to vehicle, without inducing toxic effects, therefore offering a potentially safe and effective anti-angiogenic strategy [101].

Cationic liquid nanoparticles (LNPs) have also been used to inhibit neovascularization in cancer tissues by specifically targeting vessels in metastatic and inflammatory sites [102]. The conjugation of the LNP YSK05 with the Arg-Gly-Asp (RGD) peptide, a cyclic peptide that targets αVβ3 integrin, particularly expressed in TECs, has been shown to further enhance the selectivity against TECs. In mice bearing the breast cancer cell line 4T1, an experimental model of lung metastasis, the systemic injection of siVEGFR2 encapsulated in LNPs conjugated with RGD peptide (LNP-RGD) at 1, 3, 5, 7, and 9 days post-implantation resulted in a significant VEGFR2 mRNA knockdown only in the cancer tissues, with consequent anti-proliferative effects against TECs [103]. Once again, the effect was dependent on the type of ligand associated with the LNP. Specifically, LNPs conjugated to polyethylene glycol (PEG) did not effectively accumulate within the tumor sites compared to non-PEG-LNPs. Despite these promising findings, treatment with LNP-RGD did not significantly prolong survival in the lung metastasis mice model when compared to control treatment [103].

Activation of the PI3K/Akt/mTOR pathway, observed in most cancers, is critical for inducing the angiogenic switch, characterized by the shift of the balance between pro- and anti-angiogenic factors towards a pro-angiogenic phenotype, with consequent enhancement of tumor growth and progression [104]. In yet another example of utilizing nanoparticles as drug carriers, PLGA (poly-lactic-co-glycolic acid) based nanoparticles were loaded with the PI3K inhibitor LY294002 (2-(4-morpholinyl)− 8-phenyl-chromone) (LY-NPs). Following the efficient internalization in HUVECs, the LY-NPs caused the complete inhibition of PI3K and downstream Akt, with consequent suppression of tube formation and TECs proliferation. The injection of LY-NPs in zebrafish xenograft tumor models also inhibited B16/F10- and MDA-MB-231-cell induced angiogenesis [104]. Inhibition of the PI3K/Akt/mTOR pathway has also been reported with silica nanoparticles [105]. This resulted in impaired cell proliferation and autophagy. The latter was thought to be associated with mTOR inhibition and manifested with the presence of autophagosomes and damaged mitochondria [105,106]. Additional effects, particularly eNOS inhibition and inducible NOS (iNOS) activation, also led to endothelial dysfunction and contributed to autophagy [105]. It should be noted, however, that the systemic, non-cancer-specific, disruption of these processes in ECs can also lead to impaired vasodilation and a pro-inflammatory response. This, in turn, can accelerate vascular damage and the development of atherosclerosis and hypertension [105].

Therefore, barring nanoceria, the available evidence remains inconclusive regarding the in vivo efficacy of nano-strategies targeting ECs proliferation. Furthermore, the potential clinical application of silica nanoparticles in cancer patients requires further studies to better ascertain their potential cardiovascular toxicity.

2.2.3. Nano-strategies targeting epithelial to mesenchymal transition

As previously discussed, newly formed tumor blood vessels are typically immature and characterized by significant structural and functional abnormalities [87]. This contributes to the development of hypoxia and activation of HIF-1α within the TME, ultimately inducing tumor epithelial cells (TEpCs) to undergo epithelial to mesenchymal transition (EMT). Specifically, TEpCs acquire a mesenchymal phenotype without adequate cell-cell and cell-ECM adhesion. As a result, these cells have an increased propensity to migrate and metastasize. EMT can also lead to vasculogenic mimicry, a process in which TEpCs, together with components of the degraded ECM, can form their own vessel-like tube structures [76,87]. Thus, targeting EMT might favor the normalization of the tumor vasculature, suppressing hypoxia, counteracting the expression of mesenchymal associated proteins, and inhibiting the degradation of the ECM. Recent studies have investigated the effects of nanoparticles against EMT (Table 1).

The injection of gold nanoparticles into murine models of melanoma (B16F10 cell line) reduced the risk of lung metastases by approximately 70% and normalized endothelial morphology, with a consequent reduction in local hypoxia. Concomitantly, vimentin, a marker of EMT, was significantly reduced compared to controls, while E-cadherin, a critical cell-to-cell adhesion protein, was significantly upregulated [87]. The AuNPs-mediated suppression of EMT was also associated with a reduced expression of c-Myc, a nuclear phosphoprotein that is often upregulated in cancer and functions as a transcription factor that induces the expression of several proto-oncogenes and reduces epithelial cell adhesion proteins in tumor cells [87]. The results of this study suggest that gold nanoparticles are promising candidates for the successful targeting of EMT.

EMT can also be induced by the pleiotropic effects of TGF-β in triple-negative breast cancer. TGF-β stimulates the expression of β3 integrin which is required for EMT and subsequent metastasis [107]. Nanoparticles have been developed to abrogate EMT by halting the expression of β3 integrin in triple-negative breast cancer. The lipid-based nanoparticle ECO (1-aminoethyl)iminobis(N-oleicylsteinyl-1-amino ethyl)propanamide) was used as a carrier for β3 integrin-specific silencing RNA (siβ3). The ECO/siβ3 complex significantly reduced the expression of β3 integrin and, consequently, EMT, invasion, and metastasis of MDA-MB-231 breast cancer cells [107]. Conjugation of ECO/siβ3 with cyclic RGD peptide (cRGD) further enhanced the cellular uptake of the nanoparticle and reduced β3 integrin expression in post-EMT and metastatic NME cells [107]. Decreased β3 transcripts were also evident upon injection of cRGD/ECO/siβ3 NPs into mice engrafted with MDA-MB-231 tumors. This was associated with a ~66% reduction in tumor weight and suppressed vascularization compared to controls. Notably, the mesenchymal marker fibronectin was also significantly reduced, indicating that the reversal of EMT played a vital role in the reduced tumor aggressiveness [107].

Among the factors that allow cancer cells to evade the immune response, is the expression of the immune checkpoint molecule, programmed cell death ligand 1 (PD-L1) [108]. This “don’t see me” signal bypasses the cytotoxic effect of CD8 + T cells, enabling the cancer cell to continue replicating [109]. However, certain cancer therapies, such as radiotherapy for gastric cancer treatment, are capable of stimulating not only cytotoxic T cells and antigen-presenting immune cells (APCs) but also EMT of tumor cells and expression of PD-L1 [108]. Therefore, combining radiotherapy with immune checkpoint inhibitors might increase therapeutic efficacy and minimize drug resistance. Indeed, it has been reported that, in tandem with radiotherapy, the use of PEG-based nanoparticles delivering microRNA-200c (miR-200c) can reverse EMT and prevent PD-L1 expression in human gastric adenocarcinoma cells. Specifically, miR-200c binds to the 3’ untranslatable region of the PD-L1 transcript, preventing its translation and suppressing the expression of the anti-inflammatory cytokine, TGF-β [108]. Another important miR for the suppression of EMT, miR-655–3p [110], is significantly downregulated in several cancers, including hepatocellular carcinoma, and in individuals with metastasis, with consequent poor prognosis [111,112]. The therapeutic benefit of miR-655–3p was investigated using a NP-mediated delivery approach. Specifically, nanoscale coordination polymers (NCPs) enclosing miR-655–3p were shown to effectively transfect human colorectal cancer HCT116 cells, with consequent suppression of EMT [110]. Notably, the enclosed miRs could only exert their effect if the NCP included an ethylenediamine component (PtEN), corroborating the importance of conjugated substances to optimize the efficiency of nanoparticles. The suppression of EMT was mainly due to the maintained cell-cell adhesion secondary to a reduced β-catenin nuclear translocation [110]. The systemic administration of PtEN/miR-655–3p in a xenogeneic liver metastasis model of HCT116 significantly reduced hepatic colonization and virtually suppressed tumor burden, assessed with bioluminescence, when compared to treatment with 5% dextrose or PtEN/NT. No toxicity or accumulation in the lung, liver, or kidney were observed [110]. The results of these studies also highlight the significant potential of nanoparticles as highly effective transporters of miRs, in view of their capacity to protect the latter from heat and RNAses.

In addition to increasing cancer cell invasiveness and metastatic potential, EMT is also instrumental in conferring drug resistance [113]. The latter partly arises from alterations in lipid metabolism in cancer cells. Specifically, cholesterol-rich membrane lipid rafts function as scaffolds through which ECM and cell adhesion occurs (via integrin-containing focal adhesion complexes). TGF-β-induced EMT requires the presence of lipid rafts, and depletion of cholesterol reverses epithelial tumor cell plasticity and the associated drug resistance [113]. As such, cholesterol metabolism represents a potential target to reverse the EMT phenotype in tumor cells. Recently, liposome-based NPs (LNPs) enclosing the cholesterol-lowering drug simvastatin (SV) have been shown to downregulate lipid rafts and the subsequent formation of focal adhesions. Because focal adhesions serve as signal transmitters which dictate the activity of the cell in response to the ECM, there was also inhibition of the signaling cascade involving integrin/focal adhesion kinase (FAK)/extracellular-signal-regulated-kinase (ERK). This caused reversal of EMT in paclitaxel-resistant variants of non-small-cell lung cancer cells (A549T). These cells are generally resistant to the anti-cancer drug paclitaxel (PTX), however the injection of LNP-SV-PTX complexes not only reversed the tumorigenic phenotype in mouse A549T xenograft models, with a tumor inhibition rate of ~90% compared to PTX, ~45%, and PTX/SV, ~70%, but also increased cell sensitivity to the co-delivered PTX. In order to enhance intra-tumoral specificity, LNPs were modified with hairpin-like cell-penetrating peptide KC26 and co-loaded with SV and PTX. KC26 is a ligand for the TME-associated protease, legumain, and cleavage of the peptide exposes its cell-penetrating sequence, allowing the efficient uptake of LNPs by cancer cells. The nanoparticle complexes also reversed the phenotype of tumor-associated macrophages (TAMs) to antitumor M1 phenotypes by disrupting macrophage cholesterol metabolism. Because TAMs secrete the EMT-inducing TGF-β, this also contributed to EMT reversal [113]. This study highlights the multi-faceted applications of nanoparticles, including loading with synergistic anti-cancer therapies while at the same time improving the sensitivity of tumor cells to the treatment. In another study, gold nanoparticles were used to sensitize EMT experienced tumor cells to drugs. Given their ability to reverse EMT, glucocorticoids (GCs) have been used to enhance drug sensitivity of transitioned tumor cells. Conjugation of gold nanoparticles with the synthetic GC dexamethasone was shown to target GC receptors on tumor cells [114]. This led to an enhanced response of cancer cells to the co-loaded anti-cancer drug withaferin A (WF) [114]. GC-receptor dependent selective cytotoxicity was observed in the targeted tumor cells (CT26 mouse colon adenocarcinoma and B16F10 murine melanoma) but not in non-cancer cells [114]. Intraperitoneal injection of the AuNP-GC-WF complexes in mice with induced B16F10 melanoma was associated with a ~75% reduction in tumor weight compared to treatment with WF or GC, and EMT reversal through downregulation of

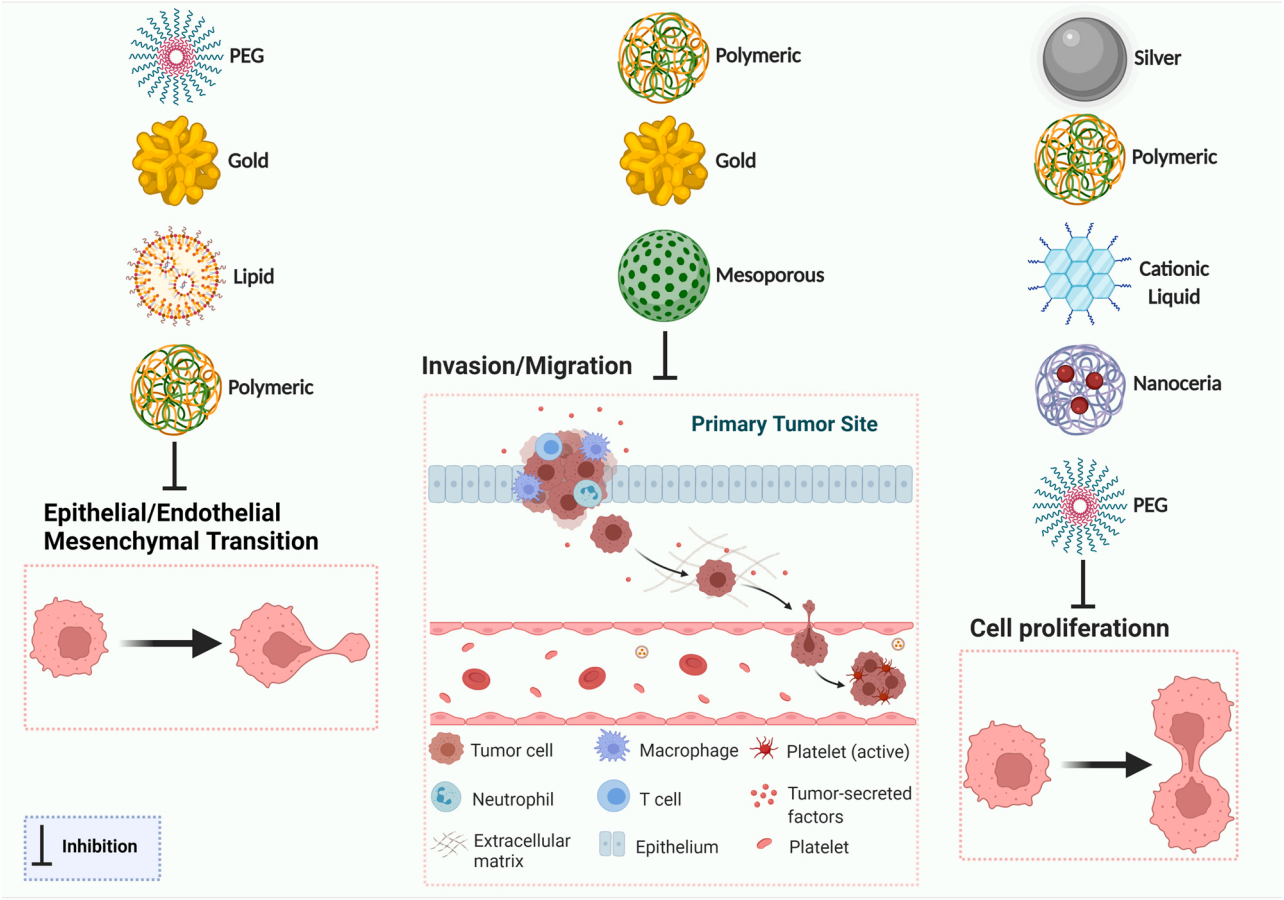

Fig. 2. Description of the principal nanoparticles used to target the main tumor angiogenesis mechanisms. PEG, polyethylene glycol.

vimentin and upregulation of E-cadherin in cancer cells. PEG-coated gold nanoparticles have also been used to overcome EMT-related drug resistance to cold plasma (ionized gas), an emerging treatment strategy [115]. The combined use of gold nanoparticles and cold plasma were shown to reverse EMT in solid tumors in vivo (glioma cells U87MG injected into Balb/c female mice). This effect was associated with a ~50% reduction in tumor volume and weight and was attributed to the suppression of the PI3K/Akt signaling pathway. This resulted in the upregulation of the epithelial marker E-cadherin and the suppression of the mesenchymal associated protein N-cadherin [115].

Therefore, the available evidence supports the efficacy and safety of nano-strategies targeting EMT, particularly gold nanoparticles with or without pharmacological (glucocorticoids, simvastatin, paclitaxel) or non-pharmacological (miRNA, siRNA, radiotherapy) treatment, in melanoma, triple negative breast cancer, colorectal cancer, and lung cancer. This approach is potentially suitable also for reversing the adverse effects of specific therapies on cancer growth and/or for increasing the sensitivity of cancer cells to standard chemotherapeutics.

2.2.4. *Nano-strategies targeting endothelial to mesenchymal transition*

Although critical for the development of the cardiovascular system in the embryo, endothelial to mesenchymal transition (EndMT) is also a major component of cancer progression [116]. During embryonic development, cytokines, mechano-transduction, and other ligands induce a subset of ECs to phenotypically switch back to their original form of mesenchymal cells [117]. This process and its activators are conserved in EndMT associated with tumorigenesis. As with EMT, TGF-β plays a critical role in inducing EndMT through favouring the loss of tight junctions, causing ECs to revert into fibroblast-like cells [117,118]. In tandem, endothelial adhesion proteins are lost while mesenchymal-associated proteins, e.g., vimentin and fibronectin, are upregulated. The resulting mesenchymal cells acquire the ability to differentiate into various mesodermal cell types [77]. Although EndMT is a major contributor to the development of abnormal tumor vasculature and tumor resistance to anti-angiogenic therapy, studies attempting to avert EndMT are limited [116]. It has been suggested that specific nanoparticles may paradoxically act as inducers of EndMT. For example, incubation of HUVECs with a non-cytotoxic concentration of iron oxide NPs (IONPs), resulted in an altered mesenchymal-type morphology [119]. This was associated with the loss of endothelial markers such as VE-cadherin and an increase in migratory capacity [119]. The pro-EndMT effect of IONPs was attributed to the increase in the extracellular concentrations of hydroxyl radicals which, in turn, caused an increase in intracellular ROS. Notably, supplementation with the hydroxyl radical scavenger mannitol and the ROS scavenger ascorbic acid prevented EndMT in vivo. The results of this study highlight the potential unwanted, pro-tumorigenic, effects of specific nanoparticles. However, at the same, they might help to identify safe and effective concomitant treatment strategies that prevent such effects while unmasking the anti-cancer activity of nanoparticles.

Pending further studies investigating the efficacy and safety of nano-strategies targeting EndMT in vivo, a schematic representation of the principal nanoparticles used to target the main mechanisms involved in tumor angiogenesis is described in Fig. 2.

2.3. *Mediators*

As previously discussed, anti-angiogenic therapies target the formation of new blood vessels from the pre-existing vasculature, a key process involved in tumor growth and dissemination [120]. However,

despite the initial expectations, conventional anti-angiogenic agents have often been associated with the development of drug resistance and a relatively hypoxic TME, further favouring tumor invasion and dissemination [18]. The use of nanoparticles can overcome such limitations given their specificity toward the target site [121,122]. Indeed, most available nanoparticles, lipid-based, polymeric, and inorganic, can effectively function as anti-angiogenic drug carriers and be conjugated to various ligands to maximize penetration in specific sites [123,124]. However, some inorganic nanoparticles, such as silver (AgNPs), gold (AuNPs), copper (CuNPs), and mesoporous silica NPs, also possess intrinsic anti-angiogenic properties [124]. This section describes the most promising nano strategies targeting key mediators of angiogenesis (Table 1).

2.3.1. *Nano-strategies targeting vascular endothelial growth factor*

The VEGF represents a family of five growth factors, VEGFA, VEGFB, VEGFC, VEGFD, and placenta growth factor (PIGF), involved in both vasculogenesis and angiogenesis. VEGFs bind to three related receptor tyrosine kinases (RTKs), VEGFR1, VEGFR2, and VEGFR3 [125], the coreceptors neuropilin-1 (NRP1) and neuropilin-2 (NRP2), and heparan sulfate proteoglycans. The VEGF/VEGFRs pathway, besides being a key regulator of physiological angiogenesis, is also the primary driver of tumor angiogenesis and its components have been shown to be overexpressed in a significant number of human cancers [122,125]. Furthermore, from a clinical standpoint, higher VEGFs tissue concentrations have been convincingly shown to predict metastasis and adverse outcomes [126,127].

Several nanotechnological approaches have been developed to specifically target VEGFs (Table 1). In this context, AuNPs, nanoparticles composed of an inorganic gold core encircled by an organic monolayer, have been investigated as drug carriers, photothermal agents, contrast agents, and radiosensitizers [128,129]. Furthermore, gold nanoparticles possess intrinsic anti-angiogenic properties by suppressing the biological activity of the VEGF-A/VEGFR2 axis both in vitro and in vivo [130,131]. This effect has been shown to translate into reduced tumor angiogenesis in a mice xenograft model of liver tumor, H22 cells injected into Balb/c mice, with a concomitant ~66% reduction in tumor weight [132]. A recent study has also described the effects of the combination of gold nanoparticles and sonoporation for enhancing tumor cells radio sensitization in liver cancer [133]. Specifically, gold-nanoparticle-encapsulated microbubbles (AuMBs) were combined with ultrasound, allowing a controlled release of AuNPs along with an increase in their intracellular concentrations. This combination increased the expression of the DNA damage markers pATM and γ-H2AX and the concentrations of the apoptotic marker cleaved PARP1, and reduced cancer cell surviving fractions [133]. The administration of VEGFR2-targeted AuMBs combined with sonoporation and radiotherapy virtually prevented tumor regrowth in Huh7 xenograft models of liver cancer when compared to radiotherapy alone or no treatment [133].

The crosstalk among TME components and ECs is critical in tumor progression, metastasis, and therapy resistance [134,135]. In this context, a recent in vitro study reported that gold nanoparticles inhibit angiogenesis by blocking VEGF-VEGFR2 signaling from TME cells to ECs [135]. In another study, gold nanoparticles enhanced the delivery of cisplatin in colorectal cancer xenograft mice using SW620 cells [136]. At the same time, the treatment reduced the expression of the pro-fibrotic markers CTGF, TGF-β1, VEGF and Col1 through the Aky signaling pathway, resulting in vessel decompression and increased local drug delivery [136]. Since gold nanoparticles offer the advantage of being easily modified or loaded with specific drugs [137], several AuNPs-drug complexes, combining the intrinsic anti-angiogenic properties of gold nanoparticles with targeted drug delivery, have been developed. For example, radiolabelled gold nanoparticles (177Lu-AuNP) were conjugated to the peptide arginine-glycine-aspartic acid (RGD), which selectively antagonizes the function of integrins, proteins overexpressed in ECs during tumor angiogenesis [138]. The triple combination of molecular targeting, radiotherapy, and gold nanoparticles significantly reduced tumor progression over a 23-day treatment. The tumor size was ~27 times smaller than controls and 12-fold and three-fold smaller than that observed with 177Lu-RGD and 177Lu-AuNP, respectively. These effects were associated with reduced metabolic activity and angiogenesis through the reduced VEGF expression in athymic mice bearing alpha(v)beta(3)-integrin-positive C6 gliomas. Importantly, no renal toxicity, assessed both histologically and by measuring serial creatinine and urea concentrations, was observed [138]. A further complex for the co-delivery of the anti-angiogenic drug, angiotensin converting enzyme inhibitor captopril, with VEGF-siRNA loaded gold nanoparticles caused a significant downregulation of VEGF mRNA and protein concentrations. This treatment was associated with a tumor volume that was ~60% smaller compared to captopril/AuNP, and ~75% smaller compared to saline, in a breast cancer animal model consisting of MDA-MB435 tumor-bearing nude mice [139]. However, gold nanoparticles might be not as effective in specific types of cancer. In a recent study, their administration with or without an anti-VEGF antibody failed to affect the GL261-cells-induced progression of glioblastoma multiforme in mice [140]. This lack of effectiveness might be ascribed to the inability of gold nanoparticles to penetrate the blood-brain barrier and GL261-cells adequately [140]. An additional gene therapy system developed for delivering the VEGFC-siRNA using calcium carbonate nanoparticles as a carrier has been shown to effectively inhibit lymphangiogenesis and growth of colorectal cancer in Balb/c mice injected with LoVo cells. The tumor volume was ~38% smaller compared to untreated animals [141]. Mesoporous silica nanoparticles (MSNs) are another category of inorganic NPs that have been widely used for drug delivery in virtue of their pore-rich structure [142]. For example, the use of MSNs carrying doxorubicin hydrochloride (MSNs-DOX) increased the efficacy of the chemotherapy drug against lung cancer both in vitro and in vivo studies (A549 cells injected into female nude mice) [143]. The enhanced anti-metastatic effect of MSNs-DOX, assessed with bioluminescence, was primarily attributed to the suppression of VEGF-mediated angiogenesis [143].

Hybrid nanoparticles are a class of nanomaterials that contain two or more different components, usually inorganic and organic, providing complementary advantages and significant potential in cancer treatment [144,145]. For instance, bioinorganic nanoparticles composed of polyelectrolyte-albumin complex and manganese oxide (A-MnO_2 NPs), combined with ionizing radiation, significantly inhibited tumor growth, ~60%, by downregulating VEGF concentrations in a model of breast cancer consisting of EMT6 cells inoculated into Balb/c mice [146]. The combined treatment enhanced the radiation response by reducing TME abnormalities, such as hypoxia and acidosis, consequently suppressing tumor aggressiveness, metastasis, and resistance to therapies [146]. Lipid-calcium-phosphate nanoparticles (LCP NPs) loaded with siRNA targeting VEGF-A have been used in combination with photodynamic therapy in a xenograft model of human head and neck squamous cell carcinoma (SCC4 and SAS cells inoculated into C57BL/6JNarl mice) [147]. This combination enhanced the therapeutic efficacy of photodynamic therapy, with a ~30% and ~60% reduction in tumor volume compared to photodynamic therapy alone in SCC4 and SAS xenografts, respectively. No kidney or liver toxicity was observed [147]. Another promising approach involves the co-delivery of VEGF-siRNA and the chemotherapy drug paclitaxel, co-encapsulated in vapreotide-modified core-shell nanoparticles [148]. Vapreotide is a ligand with high affinity to the somatostatin receptors, a type of receptor that is overexpressed in many tumor cells [148]. In in vivo studies, this receptor-mediated targeting delivery strategy increased drug penetration and distribution in tumor tissues compared to the non-targeted PEG-NPs [148]. Neovascularization and breast cancer growth in Balb/c mice bearing MCF-7 cells were significantly inhibited by the NPs-induced VEGF silencing, with a ~62% reduction in tumor weight compared to control animals [148]. However, this co-delivery system required relatively high doses of paclitaxel, with risk of toxicity, and the encapsulation efficiency of

paclitaxel was less than 75%. These limitations have been recently overcome with the co-delivery of paclitaxel and anti-VEGF siRNA using organic nanoparticles composed of a tripeptide lipid and sucrose laurate [149]. This led to enhanced biocompatibility, reduced toxicity due to the lower paclitaxel dose required, efficient VEGF inhibition and significant anti-tumor activity, with a ~five-fold reduction in tumor volume compared to paclitaxel-loaded nanoparticles, in a model of lung cancer consisting of NCI-H460 cells inoculated into Balb/c mice [149]. Additional organic co-delivering systems, efficiently providing concomitant VEGF silencing and drug targeting, have been developed using cationic liposomes loaded with VEGF-siRNA and the chemotherapy drug etoposide (ETO) and coated with the polymer PEGylated histidine-grafted chitosan-lipoic acid (PHCL-Lip) [150]. In the orthotopic A549 nude mice model of non-small cell lung cancer, combined treatment with PHCL-Lip/ETO- siVEGF for 30 days exerted superior anti-proliferative effects, assessed with bioluminescence, when compared to monotherapy with either PHCL-Lip/ETO or PHCL-Lip/siVEGF. PHCL-Lip/ETO- siVEGF did not induce significant toxicity, assessed by measuring serial body weight, cell blood counts, biochemical markers of renal and liver function, and histological analysis of the kidney and the spleen [150]. Another organic co-delivering system consisted of polycation liposome-encapsulated calcium phosphate nanoparticles (PLCP) loaded with VEGF-siRNA and doxorubicin [151]. A significant inhibitory effect on angiogenesis and tumor growth were observed with PLCP/VEGF siRNA in an MCF-7 xenograft mouse model of breast cancer. The treatment was associated with a ~68% reduction in tumor volume compared to controls. The effect was event greater, ~93% reduction compared to controls, when combined with doxorubicin. There were no significant changes in body weight, a marker of systemic toxicity, across treatment groups [151].

Within the organic nanoparticles, chitosan or chitosan derivatives offer multiple advantages in cancer treatment, including their preferential accumulation in tumor cells mixed with anti-angiogenic, immunostimulatory, antioxidant, and pro-apoptotic mechanisms [152]. Chitosan nanoparticles are also a suitable tool for the co-delivery of siRNAs, with or without anti-cancer drugs [152]. For instance, bevacizumab treatment in association with VEGF silencing, in the form of tumor-targeted VEGF-siRNA-loaded chitosan nanoparticles, improved the therapeutic effect of bevacizumab in a tumor xenograft mouse model of epidermoid carcinoma using A431 cells without causing toxicity in the kidney, lung, liver, spleen, and heart. Both VEGF-siRNA-loaded chitosan nanoparticles and bevacizumab, used singly, reduced tumor volume by ~47% after 21 days. However, combined treatment resulted in a ~83% reduction in tumor size [153]. A particularly effective anti-cancer application has also been achieved through the intravenous administration of chitosan nanoparticles for the co-delivery of VEGF and Bcl-2 dual-targeted siRNA in an experimental model of prostate cancer, PC-3 tumor bearing mice [154]. After 30-day treatment, the inhibitory effect on tumor growth and angiogenesis of the dual targeted siRNA was superior to that of the individual VEGF, ~57% reduction in tumor volume, and Bcl-2 treatment groups, ~71% reduction in tumor volume [154]. Furthermore, the co-delivery of VEGF and IL-4-siRNA into chitosan nanoparticles led to a ~97% inhibition of tumor growth in a breast cancer model, N-nitroso-N-methylurea injected into the peritoneal cavity of Sprague-Dawley rats, compared to the single chitosan nanoparticles delivering of VEGF-siVEGF or IL-4-siIL-4 [155].

Theranostics is an emerging field that combines diagnostic and therapeutic tools to optimize personalized treatment [156]. In this context, a method based on VEGF-targeted magnetic nanoparticles has been recently investigated in mice bearing breast adenocarcinoma, 4T1 cell line [157]. The intravenous administration of doxorubicin-loaded VEGF-targeted nanoparticles increased the survival rate up to 50%. At the same time, MRI, performed 24 h after the intravenous injection, demonstrated the efficient accumulation of the nanoparticles in the tumor mass [157].

The results of these studies highlight the significant anti-cancer potential of nano-strategies targeting VEGF in a wide range of experimental cancers, including liver cancer, colorectal cancer, breast cancer, gliomas, head and neck cancer, lung cancer, and prostate cancer. The promising results obtaining with different type of nanoparticles and different combinations of treatments (radiotherapy, sonoporation, RGD, VEGF-siRNA, captopril, doxorubicin, paclitaxel, bevacizumab) further support the anti-cancer potential of this approach.

2.3.2. Nano-strategies targeting tumor necrosis factor alpha

Tumor necrosis factor alpha (TNF-α), a pro-inflammatory cytokine involved in various cellular events including cell survival, proliferation, differentiation and death [158], plays a dual role in cancer being either an endogenous tumor promoter or a cancer killer. This apparent paradox is mainly dependent on the opposite effects of low vs. high concentrations of this cytokine, with the latter exerting anti-cancer effects through the stimulation of apoptosis-related pathways and, possibly, immune activation [159]. The complex relationship between TNF-α and cancer is further compounded by the significant association between chronic inflammation and tumor onset, development, and progression [160]. Therefore, being one of the major pro-inflammatory cytokines, TNF-α has been proposed to play a critical role in the interplay between inflammation and carcinogenesis [161]. TNF-α, at relatively low local concentrations, stimulates the growth, proliferation, invasion of cancer cells as well as tumor angiogenesis and metastasis. The effect of TNF-α on tumor angiogenesis is mediated by several angiogenic factors, particularly interleukin-8 and VEGF, and the regulation of VEGF and jagged-1 expression via a JNK- and AP-1-dependent pathway [162]. The role of TNF-α in angiogenesis is supported by the results of seminal studies reporting the suppressing effects of TNF-α antibodies on the formation of new blood vessels [163]. In addition, since TNF-α expression levels are increased in the serum of patients with cancer, this cytokine may also be used as a biomarker of cancer risk, therapy response, and overall prognosis [158]. At the same time, high concentrations of TNF-α can induce cancer cell death however this potential therapeutic application is limited by significant systemic toxicity [158, 164].

A recent study has investigated the combination of magnetic induction hyperthermia with gene therapy in liver cancer (Table 1) [165]. Specifically, the complex SPIONs/TNF-α, composed by the TNF-α gene bound to the surface of superparamagnetic iron oxide nanoparticles (SPIONs), was administered to human liver tumor-transplanted nude mice using Hep G2 cells exposed to an alternating current magnetic field. This combination exerted anti-cancer effects, with a tumor size that was ~60% smaller compared to the two therapies used singly [165]. Gold nanoparticles have also been used as a carrier of TNF-α, overcoming the limitations associated with the toxicity of systemic doses of this cytokine [158,164,166]. Treatment with CYT-6091, a polyethylene glycol-TNF-coated gold nanoparticle, combined with single or fractionated high-dose radiation therapy, significantly reduced tumor interstitial fluid pressure and tumor growth in murine 4T1 breast carcinoma and SCCVII head and neck tumor squamous cell carcinoma models. A 1.3-fold delay in tumor growth compared to radiotherapy and 2.3-fold compared to CYT-6091 alone were observed [166]. The treatment was associated with local vascular damage, red blood cell extravasation, and hemorrhage [166]. In association with radiofrequency ablation (RFA), another type of TNF-coated gold nanoparticle has been used in a rabbit kidney tumor model consisting of VX2 cells. This combination enhanced the effectiveness of RFA, as shown by a ~33% reduction in tumor size compared to the sham group, and the greater cell death zone compared to animals treated with RFA-only or TNF- AuNPs-only [167]. Promising results have also been reported with the combinations, TNF-AuNPs-tumor thermal therapy and TNF-AuNPs-tumor cryosurgery therapy, in models of SCK mammary carcinomas and ELT-3 uterine fibroid tumor, respectively [168,169]. In particular, treatment with TNF-AuNPs-tumor thermal therapy caused a significant reduction in tumor perfusion and a three-day delay for the

tumor volume to increase three-fold compared to control tumors [168]. Organic nanoparticles, such as dendrimer, liposome, and PEG-PHDCA nanoparticles, have also been successfully used for TNF-α gene delivery. For instance, dendrimer nanoparticles, as vehicle for the co-expression of TNF-α and herpes simplex virus-thymidine kinase (HSV1-TK) genes, in combination with radiation therapy, significantly decreased human choroidal melanoma OCM-1 cell proliferation and activated apoptosis and necrosis in vitro [170]. In another study, the association of the stress-inducible expression of TNF-α with hyperthermia, using magnetite cationic liposomes, was investigated [171]. This combination induced a three-fold increase in TNF-α gene expression, with an ~85% reduction in tumor volume vs. hyperthermia alone in nude mice (U251-SP glioma cell line) [171]. Other organic nanoparticles, poly (methoxypolyethyleneglycol cyanoacrylate-co-n-hex adecyl cyanoacrylate) (PEG-PHDCA), have been shown to serve as an effective TNF-α carrier, with prolonged TNF-α half-life and increased distribution and accumulation in tumor tissues [172,173]. Finally, carbon (60)(OH)(20) nanoparticles have shown intrinsic anti-tumor activity in C57BL/6 mice bearing the Lewis lung carcinoma cell line, with a ~38% reduction in tumor weight compared to no treatment. These effects were mediated by the increased production of immune cells that synthesize TNF-α [174].

Alike VEGF, these studies demonstrate the significant anti-cancer potential of nano-strategies targeting TNF-α in a wide range of cancers, particularly liver cancer, breast cancer, head and neck cancer, renal cancer, glioma, and lung cancer. However, further in vivo studies are required to confirm the safety of the local upregulation of this cytokine in neighboring and remote non-cancer tissues.

2.3.3. *Nano-strategies targeting transforming growth factor beta*

TGF-β consists of a family of multifunctional cytokines (TGF-β1, TGF-β2, and TGF-β3) that, through the binding to the transmembrane serine-threonine kinase receptors TGFβR1 and TGFβR2, play a complex role in tumor development [175]. Indeed, the TGF-β signaling pathway can act both as a tumor suppressor and as a promoter of tumor progression and invasion [176,177]. Consistent with its tumor suppressor role, TGF-β signaling is initially downregulated. Then, once carcinogenesis is initiated, it promotes cancer progression [177,178]. Tumor cells and other cellular components of the TME (stromal cells, macrophages and platelets) can actively secrete TGF-β [179]. In addition, since TGF-β is stored in the ECM, a further release of its free form occurs as a consequence of the tumor-associated ECM degradation [176,179]. TGF-β is also a key mediator of tumor-associated angiogenesis by inducing the release of pro-angiogenic factors, such as CTGF and VEGF in epithelial cells and fibroblasts, and the activation of specific ECM components, particularly MMP-2 and MMP-9, in ECs [175]. However, the angiogenic function of TGF-β also depends on the tumor type (cells and microenvironment). For instance, in pancreatic cancer and diffuse-type gastric cancer, TGF-β exerted anti-angiogenic effects through inducing the secretion of the potent angiogenic inhibitor, thrombospondin 1 [175]. The available evidence strongly supports the proposition that the inhibition of TGF-β signaling, alone or in combination with chemotherapy, anti-angiogenic agents, or immunotherapy, represents a promising strategy for cancer treatment [180].

In the contest of nanomedicine, the combination of doxorubicin with the TGF-β receptor inhibitor SB431542, loaded onto bio-responsive nanoparticles constructed by β-cyclodextrin-grafted heparin and pH-sensitive pseudorotaxane, amplified the efficacy of doxorubicin. The tumor inhibition rate increased from 19% to 65% in a breast cancer model consisting of Balb/c mice inoculated with 4T1 and 3T3 cells (Table 1) [181]. Doxorubicin normally exerts cytotoxic effects in tumor cells but does not significantly affect the TME. The latter, however, was effectively targeted by the above-mentioned complex with resulting inhibition of tumor-associated fibroblasts (TAFs) formation and reduced secretion of TGF-β and collagen I [181]. Furthermore, the TME switched from an immunosuppressive to an immunogenic environment, with increased infiltration of T lymphocytes, responsible for anti-tumor immunity and inhibition of breast cancer metastasis [181,182]. Additional effects on the TME, particularly reduction of TAFs activation and TGF-β secretion, have been reported using a modified complex of β-cyclodextrin (β-CD) grafted heparin nanoparticle [183]. Specifically, heparanase-driven sequential release (NLC/H) of β-CD grafted heparin nanoparticles loaded with doxorubicin plus the TGF-β receptor inhibitor SB431542 (S)+ferrocene (Fc) successfully reduced tumor growth, with an inhibitory rate of ~66% compared to ~11.5% with doxorubicin alone, and inhibited breast cancer metastasis (4T1 and 3T3 cells inoculated into Balb/c mice) [183]. The intracellular mechanisms elicited by the NLC/H(D+F+S) NPs complex involved the increased production of ROS and the consequent activation of apoptosis and ferroptosis, an increasingly studied programmed iron-dependent cell death signaling pathway that can stimulate local anti-tumor responses, and a reduced expression of MMP-9 [183]. The downregulation of MMP-9 was associated with the suppressive effect of the treatment on metastasis. Extracellularly, the local release of SB431542 directly targeted TAFs and TGF-β. Reassuringly, three-week treatment with NLC/H(D+F+S) NPs in mice bearing 4T1 cells was not associated with significant toxicity, as indicated by the assessment of body weight and histology of the heart, spleen, liver, lung, and kidney [183]. TME regulation and antitumor immunity improvement have also been reported with the TGF-β pathway/ PD-L1 checkpoint co-inhibition, using pH-responsive clustered NPs, LYClustersiPD-L1, to deliver the TGF-β inhibitor LY2157299 and siRNA targeting PD-L1, in a xenograft model of pancreatic cancer (Panc02 cell line). This treatment significantly increased the local infiltration of $CD8^+$ T cells and the tumor and serum content of the immune surveillance cytokine interferon gamma, and caused a ~90% reduction in tumor weight compared to control animals [184]. The amelioration of antitumor immunity via T-cell activation has also been described in a liver cancer mouse model (H22 cell line) with the co-delivery of unmethylated cytosine-phosphate-guanine (CpG) oligodeoxynucleotides and the TGF-β receptor I inhibitor LY2157299 by polyethylenimine-modified carboxyl-styrene/acrylamide (PS) nano-spheres. The treatment was associated with a 99.7% tumor inhibition rate compared to control animals [185]. Pancreatic cancer is one of the most aggressive tumors, also due to the TME-associated limited access to drugs [186]. TGF-β plays an important role in forming this suppressive TME [187], whereas the KRAS mutation, the hallmark in 90% of patients, is responsible for the low survival rate [188]. A recent study investigated the effects of the sequential-targeting of TGF-β signaling and KRAS mutation in pancreatic cancer [189]. Fraxinellone-loaded CGKRK-modified nanoparticles (Frax-NP-CGKRK) were used to target TGF-β signaling in TME, whereas siRNA-loaded lipid-coated calcium phosphate biomimetic nanoparticles were used to silence the oncogenic KRAS mutation. The treatment significantly increased tumor blood perfusion, normalized the local blood vessel network, reduced tumor volume by ~90% compared to control animals, and doubled the survival of pancreatic tumor-bearing animals (Panc-1 cell line). No significant toxicity was observed [189]. As further confirmed in a recent study in experimental breast cancer, MDA-MB-231 xenografts, the positive effects obtained by combining NPs-delivered gene silencing therapy and TGF-β pathway inhibition may be due to the increased tumor penetration of the nanoparticles promoted by TGF-β inhibition [190]. In this study, polo-like kinase 1 (Plk1) oncogene, a critical modulator of mitosis and cell survival that is overexpressed in cancer stem cells, silencing and TGF-β inhibition with LY364947 was associated with a ~83% reduction in tumor weight and a ~70% reduction in the proportion of cancer stem cells, respectively. Treatment with LY364947 significantly increased vascular permeability and tumor accumulation of poly(ethylene glycol) and poly(D,L-lactide-co-glycolide) nanoparticles [190]. The successful reversal of the immunosuppressive TME phenotype, in a model of desmoplastic melanoma (BPD6 cell line inoculated in female C57BL/6 mice), has also been reported using a nano emulsion intravenous administration to deliver the anti-fibrotic

drug fraxinellone combined with a tumor-specific peptide vaccine [191]. This nano-delivery system decreased tumor volume by ~50% as well as stroma deposition and TAFs formation, responsible for the TME immunosuppression. In addition, the immune response was improved following the increased expression of enhancing anti-tumor immunity factors, concurrently with the downregulation of the immunosuppressive action of TGF-β [191]. An additional nano system to indirectly downregulate TGF-β with consequent TME remodeling and improved anti-tumor efficiency has been developed with lipid-coated hollow mesoporous silica nanoparticle (dHMLB) for the delivery of all-trans retinoic acid, doxorubicin, and interleukin-2. In an experimental model of melanoma, B16F10 tumor bearing mice, the tumor inhibitory rate was ~84.8% compared to ~52.4% with the three agents administered without nanoparticle. A reduced metastatic burden through the accumulation of $CD8^+$ T cells and interferon gamma in the TME and the concomitant reduction of TGF-β was also observed. No significant treatment-associated toxicity was observed in non-cancer tissues [192].

The results of these studies provide robust evidence of the anti-cancer efficacy and safety of nano-strategies downregulating TGF-β, with or without concomitant targeted delivery of chemotherapeutics, in several experimental cancer models, e.g., breast cancer, pancreatic cancer, liver cancer, and melanoma. Given the relatively poor prognosis of patients with pancreatic cancer and the lack of effective therapies, the results of further in vivo studies testing anti-TGF-β strategies are eagerly awaited.

2.3.4. Nano-strategies targeting fibroblast growth factor

Fibroblast growth factors (FGFs) are a family of pleiotropic factors which modulate significant autocrine and paracrine functions through the activation of four tyrosine kinase FGF receptors (FGFR) [193]. The FGF/FGFR signaling pathway plays a ubiquitous role in ECs growth, differentiation, and angiogenesis and in tumor cells and stromal components [194]. The FGF/FGFR pathway activation occurs throughout tumor onset, growth, and progression, and has also been proposed as a tumor escape mechanism in response to anti-angiogenic therapies [195]. Because of the pleiotropic effects of FGF and FGFR family members, the development of FGF/FGFR pathway-targeting drugs may result in complex therapeutic effects. A promising strategy might involve the combined use of agents with both anti-angiogenic and anti-tumor activity as the FGF and VEGF pathways act in synergy, amplifying tumor growth and tumor angiogenesis (Table 1) [194,195]. A diagnostic method for early-stage esophageal cancer has been recently proposed by combining contrast-enhanced computerized X-ray tomography (CECT) with chitosan-Fe_3O_4 nanoparticles, for targeting both FGF and VEGF receptors (CNFV) [196]. This type of tumor is characterized by high invasiveness and metastatic potential and is often diagnosed late [197]. Therefore, early-stage detection can potentially improve prognosis. The CECT-CNFV combination showed higher sensitivity and accuracy than single tomography, representing a promising method for early-stage diagnosis [196]. From a total of 320 patients recruited in this study, 200 were diagnosed with early-stage esophageal cancer [196]. The clinical relevance of this finding is supported by the observation that 76.7% of those diagnosed with early-stage cancer were still alive after 60 months, compared to an average survival of 46.5% [196, 198]. Within the FGF family, FGF-1 and FGF-2 are often upregulated in tumors [194]. In this context, the FGF-binding protein (FGF-BP) facilitates the release of FGF-1 and FGF-2, an essential step for FGF/FGFR signaling activation [199]. Moreover, FGF-BP increases tumorigenicity per se and is highly expressed in specific tumors, e.g., colon carcinoma [200]. Based on the FGF-BP downregulatory effect observed in an in vitro model of colon carcinoma [200], polymer-based nanoparticles for siRNA-FGF-BP delivery were tested in colon carcinoma xenograft-bearing nude mice (cell line LS174T). A significant, ~40%, inhibition of tumor growth was confirmed, supporting the importance of FGF-BP as a therapeutic target [200]. Finally, a promising targeted drug delivering system was developed by chemically linking the biocompatible/biodegradable cholesterol-block-PEG polymer with the truncated bFGF fragments (tbFGF) within paclitaxel-loaded micelles [201]. Because of the high affinity of tbFGF for its receptor (FGFR), which is overexpressed in tumor cells, these conjugated micelles were able to specifically bind FGFR-overexpressing tumor cells releasing paclitaxel into the cytoplasm. Thus, these newly formulated paclitaxel loaded-tbFGF fragment conjugated micelles, besides overcoming the issue of poor solubility with paclitaxel, significantly enhanced the cytotoxic effects of the drug in murine Lewis lung carcinoma cells in vitro [201].

The results of these studies, albeit preliminary, suggest that nano-strategies targeting FGFs might be useful for diagnosis and therapeutic purposes. However, additional in vivo studies are required to confirm these findings, including the potential synergistic anti-cancer effects of combined anti-VEGF nano-strategies.

2.3.5. Nano-strategies targeting platelet-derived growth factor

Platelet-derived growth factor (PDGF) is a pro-angiogenic factor family that consists of four members (PDGF-A, PDGF-B, PDGF-C and PDGF-D) isolated from human platelets [202], which recognize two receptors, PDGFRα and PDGFRβ. PDGF-A, -B, and -C have higher binding affinity for PDGFRα whereas PDGF-B and -D preferentially bind to PDGFRβ [203]. The PDGF/PDGFR signaling pathway, in addition to regulating cell proliferation, migration and invasion [202], plays a crucial role in the development and the progression of various cancers, particularly breast, stomach, prostate, lung, and colon cancer [204]. Indeed, stromal cells such as fibroblasts and myofibroblasts, as well as ECs and pericytes of tumor-associated blood vessels, can express PDGFR [202,205]. PDGFR tissue expression levels are also strongly correlated with fibroblast infiltration in breast cancer [206]. Therefore, the PDGF/PDGFR axis represents a potential target for anti-tumor and anti-angiogenic therapy. PDGF, as well as other growth factors such as FGF2 and TGFβ, activates cancer-associated fibroblasts (CAFs) [207], TME components with a key role in tumor angiogenesis, metastasis and therapeutic resistance [208,209]. Gold nanoparticles, previously described for their intrinsic anti-tumor properties, can also inhibit ovarian CAFs activation by altering the levels of PDGF and other tumor mediators, which secretion is elicited by the interplay between ovarian cancer cells and TME cells [210]. Increased plasma PDGF concentrations can be an indicator of accelerated angiogenesis and metastatic breast tumor growth [211]. In this context, gold nanoparticles have been recently proposed as a sensor for detecting variations in PDGF concentrations in human plasma [212]. A highly specific PDGF-binding DNA-aptamer, immobilized onto AuNPs supported α-cyclodextrin, was used for Square Wave and Cyclic voltammetry electrochemical measurements [212]. The crosstalk between CAFs and cancer cells is crucial in driving myeloma progression (Table 1) [213]. Consequently, a delivering system targeting both cell types simultaneously might provide superior therapeutic benefits than targeting a single cell population. This hypothesis was tested using a dual-targeting drug delivery system obtained by conjugating paclitaxel-loaded PEG-poly (lactic acid) nanoparticles with a PDGFR-β-high affinity cyclic peptide. PDGFR-β is highly overexpressed in CAFs and myeloma cells, providing a dual-target to the delivery system [214]. In in vitro and in vivo experiments, the peptide modification enhanced cell uptake by both CAFs and myeloma cells, compared to unmodified nanoparticles [214]. In mice bearing the multiple myeloma RPMI 8226 cell line, the combined treatment with paclitaxel and cyclic peptide was associated with a ~63% reduction in tumor volume compared to paclitaxel treatment alone [214]. PDGFR-β targeting and the resulting PDGF/PDGFR signaling pathway inhibition allows the combined reduction of TME-associated dense matrix and interstitial fluid pressure (IFP), representing a promising strategy for TME normalization and enhanced delivery of nanomedicines [205,215]. This, however, is also dependent on specific physicochemical properties of the nanoparticles, particularly their size. A recent study emphasized the importance of the

simultaneous optimization of nanoparticle properties and TME normalization treatment to improve anti-tumor effects [215]. The delivery efficiency of paclitaxel-loaded nanoparticles of different sizes, between 23 and 110 nm, in combination with TME normalization treatment based on the PDGFR inhibitor imatinib mesylate, was inversely proportional to the nanoparticle size [215]. Furthermore, in nude mice bearing the lung cancer cell line A549, the combined nano-treatment with paclitaxel and imatinib mesylate was associated with a ~60% reduction in tumor volume compared to ~28% with paclitaxel-only nanoparticles [215].

Albeit promising, the results of these studies require further in vivo confirmation to better establish the efficacy and safety of nano-targeting PDGF in cancer.

2.3.6. Angiopoietins

The angiopoietins (Ang) are a four-member family of vascular growth factors that play a critical role in embryonic and postnatal angiogenesis primarily through their binding to the tyrosine kinase receptor Tie2 [216,217]. Angiopoietin-1 (Ang-1) regulates blood vessel maturation, adhesion, and migration, whereas angiopoietin-2 (Ang-2) promotes cell death and disrupts vascularization. However, in association with VEGF, Ang-2 can also promote neo-vascularization [217]. Under physiological circumstances, Ang-1 expression levels are higher than Ang-2 whereas this ratio is reversed in cancer, with higher Ang-2 expression associated with poor prognosis [218]. Ang-2 expression levels are correlated with stage in lung cancer [219] and are also particularly high in melanoma cells [220] and hepatocellular and endometrial carcinoma-induced angiogenesis [219]. Therefore, Ang-2 represents an attractive candidate cancer target for anti-angiogenic therapies. This proposition is supported by the observation that inhibiting Ang-2 activity, using anti-Ang-2 monoclonal antibodies or Ang-2-specific RNA aptamers, with the consequent loss of interaction between Ang-2 and Tie2, results in tumor stabilization and metastasis inhibition [221,222].

Ang-2-specific nano targeting has been reported in some studies, mostly involving the use of Ang-2-siRNA-loaded chitosan magnetic NPs (CMNPs) for Ang-2-silencing (Table 1) [223–225]. In nude mice bearing human malignant melanoma A375 cells, 24-day treatment with these nanoparticles reduced tumor growth by ~80%. This was associated with a significant reduction in protein CD34, expressed in ECs and a marker of tumor microvessel angiogenesis, and microvessel density. Additional effects included the induction of melanoma cell apoptosis through the mitochondrial apoptotic pathway [225].

Alike PDGF, the preliminary nature of the results of studies investigating angiopoietin nano-targeting require confirmation in other experimental cancer models.

2.3.7. Matrix metalloproteinases

The ability of tumor cells to invade adjacent tissues and metastasize requires the degradation and the remodeling of the ECM, an intricate network of macromolecules (collagens, proteoglycans, elastin, and cell-binding glycoproteins) that functions as a physical barrier to cell migration [226]. The critical enzymes responsible for ECM breakdown, the MMPs [227,228], are able to degrade most of the ECM components, shaping at the same time the metastasis-supporting network of newly formed vessels [227,228]. A total of 23 MMP genes have been identified in humans, many of which are significantly involved in tumor invasion and metastasis, and angiogenesis through the proteolytic release, primarily mediated by MMP-9, of VEGF from the tumor matrix [227,228]. Whilst the exact functional mechanisms involved in the link between MMPs and angiogenesis are not fully established, there is evidence that HIF-1α simulates the expression of both MMP-9 and VEGF. Furthermore, VEGF can stimulate the expression of MMP-9 and other MMPs in stromal cells and TECs. Conversely, some MMPs, particularly MMP-2, MMP-14, and MMP-13 can enhance VEGF expression and secretion [229]. The close interplay between MMPs, VEGF, and angiogenesis is further confirmed by the observation that MMP blockade suppresses VEGF production and reduces the volume of the newly formed vasculature in squamous cell carcinoma xenograft models using HaCaT-ras A-5RT3 cells [230].

A favorable impact on nano-vectors drug delivery has been observed using stimuli-responsive nanomaterials that release their load upon exposure to specific stimuli, e.g., enzymes, temperature, pH, heat, and electrical fields [231]. MMPs, specifically MMP-2 and MMP-9, important modulators of cell receptors and growth factor activity, might be specifically exploited for this goal [231,232].

Various types of MMPs-responsive nano systems have been developed both for diagnostic and therapeutic purposes in cancer (Table 1). For instance, a promising tool to predict the enhanced permeability and retention (EPR) effect in cancer patients has been recently developed using MMP-2-responsive PEG-NPs [233]. The EPR effect describes the preferential accumulation of nanomedicines or macromolecular drugs in the tumor sites, as a result of leaky vascular structures and dysfunctional lymphatic drainage, compared to normal tissues [234]. MMP-2-responsive PEG-NPs have been conjugated with a fluorescent dye linked to a synthetic substrate that can be cleaved by MMP-2, which is overexpressed in the tumor ECM [233]. Upon tumor accumulation, the fluorescent dye is released from the PEG and excreted in the urine. Therefore, assessing the fluorescent signal in the urine can be considered as marker of the degree of NP tumor accumulation. This approach is supported by the results of experiments that showed a significantly higher excretion of this dye in the urine of mice bearing colon cancer CT26 cells compared to control animals [233]. MMP-2-responsive chimeric polymersomes have been recently shown to represent an efficient tool against colorectal cancer [235]. This responsive system was developed by linking PEG to polylactide (PLA) through the synthetic peptide PVGLIG, which can be selectively cleaved by tumor-associated MMP-2. The resulting chimeric polymersomes were then loaded with the hydrophobic antineoplastic drug SN38 and finally conjugated with the synthetic DNA aptamer (AS1411) to provide guided drug delivery [235]. The biodegradable targeted chimeric polymersomes provided an efficient and controlled drug delivery system as, bearing both the cleavable peptide sequence and targeting ligand, they increased the overall encapsulated drug therapeutic index in mice bearing C26 cells without evidence of systemic toxicity [235]. Other MMPs-responsive nanoparticles have demonstrated excellent anti-cancer efficiency both in vitro and in vivo. For instance, gelatin- and albumin-based nanoparticles sensitive to changes in MMP-2 concentrations and redox potential have been used for targeted delivery of paclitaxel. Fourteen-day treatment with this BSA/Gel-SS-PTX/PTX-SS-COOH nanoparticle in B16 melanoma cell bearing mice significantly reduced tumor volume, ~7.5 times, compared to controls. While the effect of BSA/Gel-SS-PTX/PTX-SS-COOH nanoparticles on tumor size was similar to conventional paclitaxel, the body weight slightly increased with the former whereas it significantly decreased with the latter, reflecting the significant toxicity of systemic paclitaxel [236]. MMP-2-triggered gelatin-based NPs loaded with photodynamic therapy (5-amino-levulinic acid) and chemotherapeutic agent (doxorubicin), Ge-DOX-5-ALA/NPs, reduced tumor volume by ~73% compared to saline treatment in S180 sarcoma cell-bearing mice, without overt systemic toxicity [237]. Maximization of drug efficacy and minimization of side effects have also been reported with another nanoscale micelle system, HEKMs, which demonstrated enhanced cellular targeting and drug internalization through TME-regulated shape-changing mechanisms [238]. Under physiological conditions, HEKMs self-assembled into cylindrical nanorods with a prolonged retention time in the circulation. By contrast, in the TME, where MMP-2 is highly expressed, due to their MMP-2-responsive element, HEKMs transformed into nanospheres with high tumor tissue penetration ability. In nude mice bearing the SK-BR-3 breast cancer cell line, HEKM-doxorubicin virtually suppressed tumor growth compared to doxorubicin alone. Furthermore, the elimination half-life was 11.5 h and 2.6 h with doxorubicin-loaded HEKMs

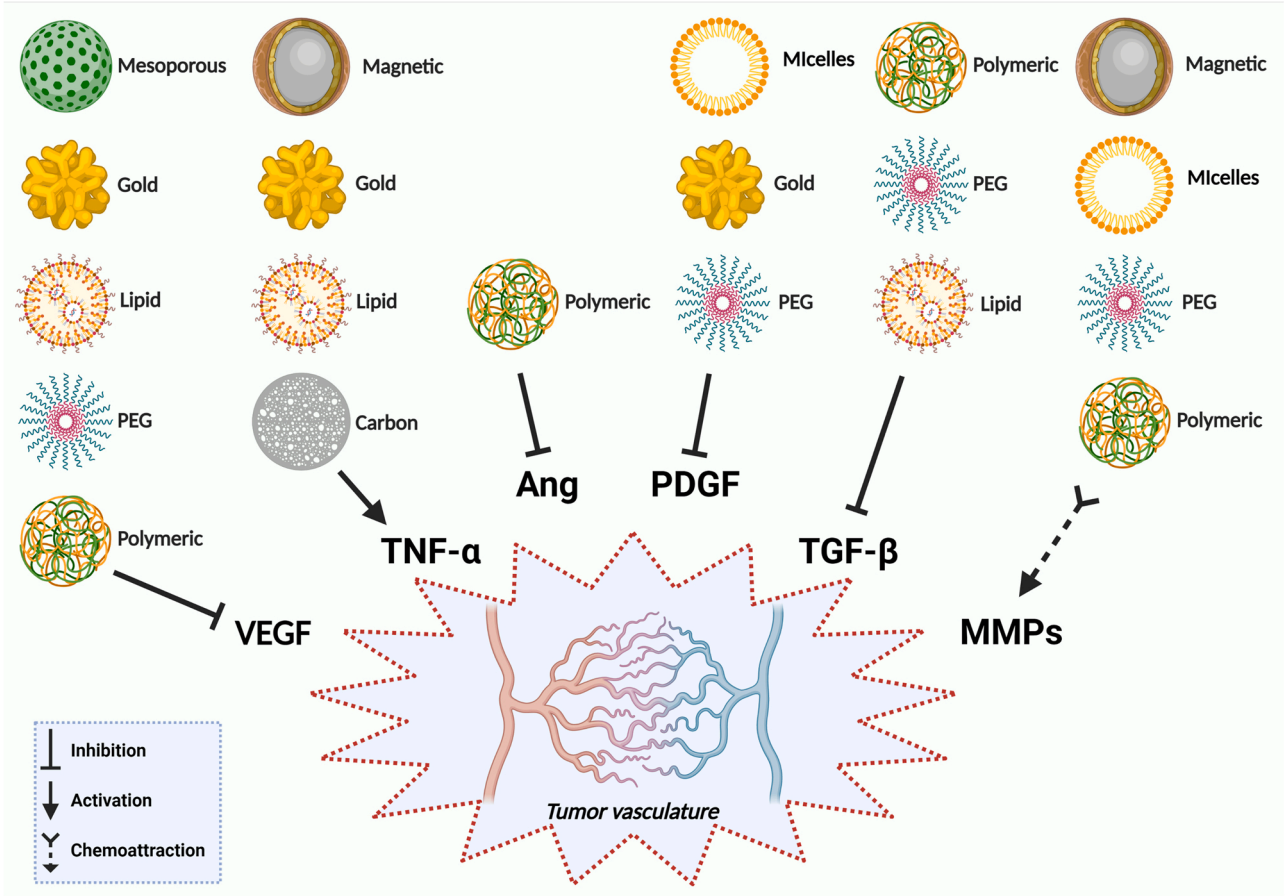

Fig. 3. Description of the principal nanoparticles used to target the key mediators of tumor angiogenesis. PEG, polyethylene glycol.

and free doxorubicin, respectively [238]. Similarly, size-controllable-MMP-2-responsive nanoparticles coated with the CD47 peptide and doxorubicin exhibited excellent tumor-targeting ability. This was reflected in a ~25% reduction in tumor volume compared to doxorubicin alone in Balb/c nude mice bearing HT-1080 fibrosarcoma, characterized by a high expression of MMP-2. The tumor penetration ability of DOX-CD47 NPs was primarily due to the decreased sequestration by macrophages and their MMP-2-mediated disassembling into poly(amidoamine) dendrimers [239].

MMP-2 targeting (direct or indirect) as a therapeutic approach for cancer treatment has also been described. For instance, the oral administration of gallium nanoparticles (GaNPs, 1 mg/Kg, 5 times/week for 6 weeks) in combination with low levels of gamma radiation (IR) caused a significant reduction in MMP-2 and TGF-β protein levels in diethylnitrosamine (DEN)-induced hepatocellular carcinoma (HCC) in rats [240]. As previously discussed, MMP-9 also plays a crucial role in EGF/EGFR-mediated malignant progression and metastasis of triple negative breast cancer [241]. On the other hand, gallic acid can downregulate MMP-9 expression in cancer cells [242]. In this context, an increased inhibition of EGF/EGFR-mediated MMP-9 expression and an enhanced anti-tumor activity with the use of gallic acid-conjugated AuNPs has been recently reported in triple negative breast cancer MDA-MB-231 cells in vitro [243]. A similar reduction in MMP-9 expression has also reported using gold nanoparticles in conjugation with the antioxidant natural compound resveratrol [244]. Finally, by concomitantly suppressing VEGF and MMP-9 expression, the combination of nanosized As_2O_3/Fe_3O_4 particles with magnetic fluid hyperthermia showed a significant inhibitory effect on the mass (88% reduction) and the volume (92% reduction) of mouse xenograft cervical tumors (HeLa cell line) [245]. A schematic representation of the principal nanoparticles used to target the key mediators of tumor angiogenesis is described in Fig. 3.

The results of these studies strongly suggest that nano-targeting MMP-2 and MMP-9, with or without combined treatments with chemotherapeutics, e.g., paclitaxel and doxorubicin, exerts significant anti-cancer effects, particularly in experimental colorectal cancer, melanoma, sarcoma, breast cancer, liver cancer, and cervical cancer. Therefore, matrix metalloproteinases appear to be one of the most promising targets for suppressing tumor invasion and neovascularization with specific nano-strategies.

3. General considerations and future directions

Significant advances have been made over the last 20–30 years in identifying the critical mechanisms that regulate vascular remodeling in solid cancers, particularly those involved in creating new blood vessels and vascular networks and the close interactions between these structures and other components of the TME. This knowledge has been of paramount importance for discovering new classes of anti-cancer agents, i.e., anti-angiogenic drugs, as well as a better understanding of the mechanisms responsible for the dissemination and metastasis of cancer cells and the resistance to drug treatment.

The significant issues with tackling cancer dissemination and drug resistance, in particular, have stimulated new areas of research focused on novel strategies to enhance the penetration and the dissemination of anti-cancer therapies within the TME, minimizing at the same time the risk of toxicity in healthy organs and tissues resulting from systemic exposure. The field of nanomedicine, particularly over the last decade, has enormously facilitated the development of effective delivery systems that are designed not only to specifically target cancer cells but also to

Table 2
Issues requiring additional investigation in pre-clinical studies.

- Additional factors, e.g., proteins, haemodynamic parameters, and local endothelial characteristics, ensuring the effective delivery of nanotherapeutics to solid tumors.
- Robust biomarkers of enhanced permeability and retention for personalized nanomedicine.
- Toxic effects of nanomaterials and/or combined therapies in cancer and non-cancer tissues over longer follow-up observation periods.
- Potential cross-reactivity of specific nano probes with non-cancer tissues with local inflammatory state.
- Appropriate prediction of pharmacokinetic characteristics in humans from different animal species.
- New animal models of cancer that more closely reproduce all aspects of human cancer, including metastasis.
- Selection of the most appropriate dose and route of administration in humans.
- Additional toxic effects of nano therapies in presence of specific co-morbidities.

'normalize' the structure and the function of newly formed blood vessels, reducing local hypoxia and hypo-perfusion and creating at the same time a more favorable immunological TME phenotype. The significantly lower doses of chemotherapeutics used with nano strategies have translated, at least in animal studies, into the lack of significant toxicity in non-cancer tissues, further highlighting the potential clinical advantages of such approach.

Overall, the results of the studies assessed in this review suggest that nano-strategies consisting of various combinations of nanoparticles, chemotherapeutics and other agents targeting specific modulators of tumor vascular biology, particularly EMT, VEGF, TNF-α, TGF-β, and MMPs, exert significant anti-cancer effects, with minimal systemic toxicity, in a wide range of solid cancers. Notably, anti-cancer effects have also been reported in cancer types, particularly liver, lung, ovarian and pancreatic cancer, that are associated with a relatively poor prognosis given the paucity of effective therapies. Therefore, a significant amount of research is anticipated to focus on the effects of the described nano-strategies in these types of cancers. Such studies, however, will also need to address several potential concerns before routine clinical use (Table 2). For example, the efficacy and safety of treatments have been investigated over a relatively short period, a few days, or weeks, in virtually all studies. A longer follow-up observation is warranted to rule out the development of local or systemic toxicity to specific nanomaterials and other components of the designed nano strategies and to determine their degradation, metabolic activity, and interactions with the immune system. In this context, the availability of robust biomarkers of enhanced permeability and retention might further assist in identifying specific cancers and patient groups that are most likely to respond to nanotherapeutics. At the same time, the requirement for specific nano therapies to be administered chronically in some human cancers will have to be adequately met by scaling up production and availability and identifying the most appropriate route of administration by the manufacturer. Furthermore, while the development of specific nano probes that are sensitive to pH and redox changes typical of the TME represent an attractive option for targeted drug delivery, it should be emphasized that such changes can also occur in non-cancer tissues, for example in the context of other inflammatory states, such as infections, autoimmune conditions, and other types of tissue injury [246,247]. This issue is particularly relevant given the frequent coexistence of such conditions in cancer patients, particularly those in the older age group [248]. Further studies that also include animal models of cancer co-morbidities will shed light on the possible unwanted penetration and accumulation of nano therapies in non-cancer tissues and the potential clinical consequences of this phenomenon.

These important issues notwithstanding, the use of nanomedicine strategies that target several critical components involved in the pathophysiology of vascular remodeling in cancer is facing an exciting phase, particularly over the next 5–10 years, which will hopefully lead to the widespread introduction of this therapeutic paradigm in the routine care of cancer patients.

4. Conclusions

Over the last 20 years, there have been major advances in the identification of the key mechanisms that regulate the process of vascular remodeling in cancer and, consequently, cancer cell migration and metastasis. This has stimulated an enormous amount of research to design better therapeutic agents and more efficient and safe methods of targeted drug delivery. In particular, the nano targeting of several components involved in vascular remodeling has led to exciting developments in the formulation of treatments that combine specific nanomaterials with gene therapy, chemotherapeutics, and probes that are sensitive to environmental changes in the TME. The evidence discussed in this review suggests that nano-targeting specific messengers and modulators of tumor vascular biology, particularly EMT, VEGF, TNF-α, TGF-β, and MMPs, is particularly promising in terms of anti-cancer effects and safety endpoints in several solid cancers, including those that are currently characterized by rapid progression and poor survival. The results of these studies are likely to drive the next phase of pre-clinical and clinical development of nanomedicine and, eventually, its routine clinical use in patients with cancer.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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