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Antiangiogenic anticancer strategy based on nanoparticulate systems

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Introduction: Angiogenesis is a process that provides a blood supply for cancer cells. The discovery that the blockade of this blood supply results in the inhibition of cancer cell growth has been applied in cancer treatment. This antiangiogenic strategy is mainly directed at the inhibition of the binding process between proangiogenic growth factors and their receptors or the inhibition of the activity of proteolytic enzymes of the extracellular matrix. The toxicity of some antiangiogenic agents, such as small-molecule inhibitors, and the instability of antiangiogenic proteins require their formulation in an appropriate delivery system. On the other hand, active drug targeting to selective markers expressed on tumor vasculature could improve antiangiogenic treatment.

Areas covered: The present review focuses on nanoparticulate systems (nanoparticles, liposomes, polymeric micelles, etc.) because their properties could enable both the targeting of endothelial cells and the efficient delivery of antiangiogenic agents. The most important properties of nanoparticles that influence both processes, such as their size, charge and surface modification, are also discussed. Various examples illustrating the targeting ability of nanoparticles are reported, in particular conjugated nanoparticles targeting VEGF and its receptors, fibroblast growth factor and its receptors, EGFRs, MMPs, tubulin function and so on.

Expert opinion: Targeting of nanoparticles (e.g., by tumor-penetrating peptides) allows the co-administration of antiangiogenic and anticancer drugs, facilitates drug penetration into extravascular tumor tissue and improves the therapeutic effect at reduced drug doses.

Keywords: angiogenesis, antiangiogenic agents, gene therapy, nanoparticles, targeted drug delivery

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1. Introduction

It is well established that in order for a tumor formation to grow beyond a size of about 2 mm^3, it needs to develop an autonomic network of newly formed blood vessels. Angiogenesis is the formation of new blood vessels from pre-existing vessels. Angiogenesis is controlled by a delicate balance between endogenous angiogenic stimulators and antiangiogenic factors (e.g., thrombospondin (TSP)-1, somatostatin, endostatin). Many tumor cells seem to upregulate one or other angiogenic stimulators, such as growth factors, for example, VEGF and fibroblast growth factor (FGF). The antiangiogenic anticancer strategy is targeting of the blockade of blood supply for cancer cells resulting in inhibition of their growth [1].

Antiangiogenic agents can be classified into several categories, namely: i) inhibitors of growth factors (including VEGF, FGF, platelet-derived growth factor (PDGF)), ii) inhibitors of proteolytic enzymes of the extracellular matrix

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Article highlights.

- The blockade of angiogenesis cuts blood supply for cancer cells and further inhibits their growth. Antiangiogenic agents have some limitations (e.g., instability, poor solubility, toxicity) that could be overcome by their formulation in nanoparticulate systems.
- Various antiangiogenic agents have been incorporated into nanoparticles (the term is used for polymeric nanoparticles, liposomes, polymeric micelles, etc.) and efficiently inhibit growth factors or their receptors, thus, suppressing angiogenesis.
- Other antiangiogenic agents (e.g., mAbs, antiangiogenic peptides) have been conjugated to nanoparticles providing an efficient selective nanoparticle targeting, which enhances antitumor effect of loaded anticancer drugs.
- Nanoparticle-mediated gene delivery results in sustained gene expression and hence better efficacy of antiangiogenic genes.

This box summarizes key points contained in the article.

and iii) miscellaneous antiangiogenic compounds including inhibitors of other targets related to the extracellular matrix, diverse endogenous inhibitors of angiogenesis and modulators of cellular adhesion molecules. Among these groups, the inhibitors of proangiogenic growth factors are intensively investigated and some of them have entered clinical trials. Antiangiogenic agents such as mAbs and small-molecule inhibitors could block the activities of the growth factors resulting in inhibition of angiogenesis and tumor growth. However, their administration should be carefully considered because of toxicity and instability (e.g., antiangiogenic proteins) of many antiangiogenic agents. Thus, vehicles that could provide sustained delivery and higher stability of antiangiogenic agents are required.

2. Nanoparticles properties

The present review is focused on nanoparticulate systems (nanoparticles, liposomes, polymeric micelles, etc.) as their properties could enable both the targeting of endothelial cells and efficient delivery of antiangiogenic agents. The most important properties of nanoparticles that influence both processes are their size, charge and surface modification. The small size of nanoparticles enables long circulation time, nanoparticle extravasation and cellular internalization. It is known that the smaller nanoparticles (optimal size < 100 nm) are able to circulate longer due to the possibility of avoiding the capture by the phagocytic cells of the reticuloendothelial system. In order to prolong circulation time, nanoparticle surface could be modified by attachment of hydrophilic polymers such as PEGs [2]. The formation of flexible hydrophilic layer on nanoparticle surface repeals adsorption of opsonins that further prevents phagocytosis. For antiangiogenic treatment, the long circulation is important taking into account that the endothelium of tumor vessels is accessible to circulating compounds, and the vessels could be regarded as a gateway to the tumor interior for compounds accumulated in the vessels. A recent study has reported that the incorporation of melittin into nanoparticles prolongs the melittin circulation time, thereby increasing probability of accumulation in the tumor and targeted binding to angiogenic cells [3].

On the other hand, nanoparticle size and surface properties are key factors for the targeting ability of nanoparticles. Passive targeting of nanoparticles occurred mainly due to the facilitated nanoparticle penetration through the leaky tumor vasculature and the limited lymphatic drainage. Both phenomena contribute for the enhanced permeability and retention (EPR) effect [4]. Nanoparticle size is the main feature that contributes to nanoparticle extravasation into the tumor site through the EPR effect. In fact, nanoparticles larger than 200 nm often accumulate in the extracellular space, whereas those lower than 200 nm passively extravasate into the tumor site [5]. Sengupta *et al.* have designed nanocell delivery system (approximate size 200 nm) that preferentially accumulated into tumor by the EPR effect [6]. The nanocell system enables a temporal release of two drugs: the outer pegylated-lipid layer first releases antiangiogenic agent combretastatin, causing a vascular shutdown, and the inner PLGA-matrix, which is trapped inside the tumor, releases a chemotherapeutic agent doxorubicin. The administration of the nanocell system resulted in improved therapeutic index and reduced toxicity. The surface charge is another factor that could influence nanoparticle targeting. It could be suggested that the positively charged nanoparticles would be able to interact with negatively charged vascular glycocalyx due to the electrostatic attraction. A study of Campbell *et al.* reported that the charge of liposomes affected their partitioning between the vascular and extravascular compartments [7]. The authors observed that cationic liposomes (mean diameter of 150 nm) accumulated more intensively in tumor vessels compared with the electroneutral liposomes.

Passive targeting is highly dependent on tumor structure and the degree of angiogenesis that could limit the efficacy of nanoparticle administration. In this view, active nanoparticle targeting to selective markers expressed on tumor vasculature could improve antiangiogenic treatment. Active targeting has been achieved by targeting various receptors, integrins and other angiogenic factors, as discussed in the following sections. Functionalization of nanoparticles with ligands having affinity to receptors localized on angiogenic endothelial cells could result in selective drug delivery (**Table 1**). For example, peptide ligands containing arginine-glycine-aspartic acid (RGD) or asparagine-glycine-arginine (NGR) motifs efficiently target $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors that are overexpressed in angiogenic vessels [8]. RGD-targeted nanoparticles containing doxorubicin inhibited angiogenesis by 70%, whereas minimal response was observed when testing RGD-NP without doxorubicin or RGD peptide alone [9]. Targeted delivery of

Table 1. Various examples representing active targeting of nanoparticulate systems.

Drug delivery systems	Drug	Ligands	Target	Ref.
Liposomes	Doxorubicin	RGDfK peptide	$\alpha_v\beta_3$ Integrin	[9]
Liposomes	Doxorubicin	NGR peptide Anti-GD2 antibodies	Integrin tumor cells	[11]
Nanoparticles	Abraxane	iRGD peptide	Integrin neuropilin-1	[12]
Nanoparticles	Abraxane	CREKA	Clotted plasma proteins in tumor vessels	[13]
		LyP-1	p32 Expressing tumor cells	
Micelles	sFlt-1 expressing pDNA	RGD peptide	$\alpha_v\beta_3$ And $\alpha_v\beta_5$ integrins	[24,25]
Liposomes	Doxorubicin	RGD peptide	$\alpha_v\beta_3$ And $\alpha_v\beta_5$ integrins	[26]
Nanoparticles	siRNA inhibiting VEGFR-2 expression	RGD peptide	$\alpha_v\beta_3$ And $\alpha_v\beta_5$ integrins	[30]
Liposomes	Doxorubicin	Cetuximab	EGFR	[59]
Nanoparticles	Gemcitabine	Cetuximab	EGFR	[62]
Liposomes	Doxorubicin	CTT peptide	MMP-2 and MMP-9	[70]

CREKA: Pentapeptide (Cys-Arg-glutamic acid-Lys-Ala); CTT: Cys-Thr-Thr-His-Trp-Gly-Phe-Thr-Leu-Cys; LyP-1: Cyclic 9-amino-acid peptide (Cys-Gly-Gln-Lys-Arg-Thr-Arg-Gly-Cys); RGD: Arg-Gly-Asp.

doxorubicin to the tumor vasculature provided a 15-fold increase in the efficacy of the drug without the side effects associated with the administration of the free drug. Another study has evaluated the antiangiogenic effect of RGD-targeted nanoparticles loaded with antagomir targeting miR-132, anti-miR-132 [10]. As microRNA miR-132 acts as an angiogenic switch by targeting p120RasGAP, the targeted nanoparticle delivery of anti-miR-132 restored p120RasGAP expression in the endothelial cells and suppressed angiogenesis. Pastorino *et al*. have reported sequential administration of doxorubicin loaded liposomes targeting either tumor vasculature via the NGR peptide or tumor cells via anti-GD2 mAbs [11]. The authors reported that the NGR-targeted liposomes bind to and kill angiogenic vessels and the tumor cells that these vessels support. Then, anti-GD2-targeted liposomes provide direct cell kill, including cytotoxicity against cells that are at the tumor periphery and independent of the tumor vasculature.

Integrin targeting has been further optimized by nanoparticle conjugation with novel peptide moieties possessing better affinity to integrins. The conjugation with an iRGD peptide, an RGD peptide containing a CendR motif, demonstrates greater selectivity and capability not only to bind to the vessels, but also to penetrate into the extravascular tumor parenchyma [12]. The mechanism of targeting is associated with the affinity of RGD motif to integrins, followed by binding of CendR fragment to neuropilin-1 receptor responsible for tissue penetration. Abraxane is a clinically approved paclitaxel-albumin nanoparticle transported by passive targeting. Coating of abraxane with iRGD peptide facilitated nanoparticles penetration in tumor tissue, resulting in significantly higher activity than that of untargeted abraxane. Conjugation of abraxane with other tumor-homing peptides (CREKA (Cys-Arg-glutamic acid-Lys-Ala) and Lyp-1 (Cys-Gly-Gln-Lys-Arg-Thr-Arg-Gly-Cys)) also improved tumor treatment [13]. CREKA-abraxane treatment resulted in a modest inhibition of tumor growth compared to untargeted abraxane, while LyP-1-abraxane produced significant inhibition of tumor growth. Thus, the main benefits of such active nanoparticle targeting would be an improved intracellular uptake by the target cells, improved drug effect and lower toxicity [14-16].

3. Nanoparticles targeting vascular endothelial factor and its receptors

It is well established that VEGF signaling plays a pivotal role in blood vessel formation and is implicated in all stages of angiogenesis. Hence, the inhibition of VEGF signaling pathways represents an attractive therapeutic target in a wide range of tumor types, and the disruption of the VEGF has become a dominant strategy for the angiogenesis-related treatment of cancer. VEGFRs are membrane receptors with intracellular tyrosine kinase domains and extracellular ligand-binding domains. Several receptor subtypes have been identified and designated VEGFR-1, -2 and -3, respectively. The VEGFR-1-mediated cell signaling is crucial for tumor growth and metastasis, including the induction of MMPs. The other subtype VEGFR-2 is expressed in endothelial cells and is the principal receptor through which VEGFs exert their mitogenic, chemotactic and vascular permeabilizing effects on the host vasculature (**Figure 1**). Activation of VEGFR-3 promotes lymphangiogenesis.

The blockade of VEGF leads to improved oxygenation and delivery of chemotherapy to tumor cells and inhibition of the abnormal angiogenesis [17,18]. Inhibition of VEGF-induced angiogenesis could be achieved by: i) direct targeting of antiangiogenic loaded nanoparticles to VEGFRs (VEGFR-1 and VEGFR-2), ii) targeting VEGF itself and/or iii) blocking other elements of downstream signaling pathways (**Figure 2**). The inhibitors could be mAbs that are typically directed against receptor tyrosine kinases or their ligands and small-molecules targeting specific kinases.

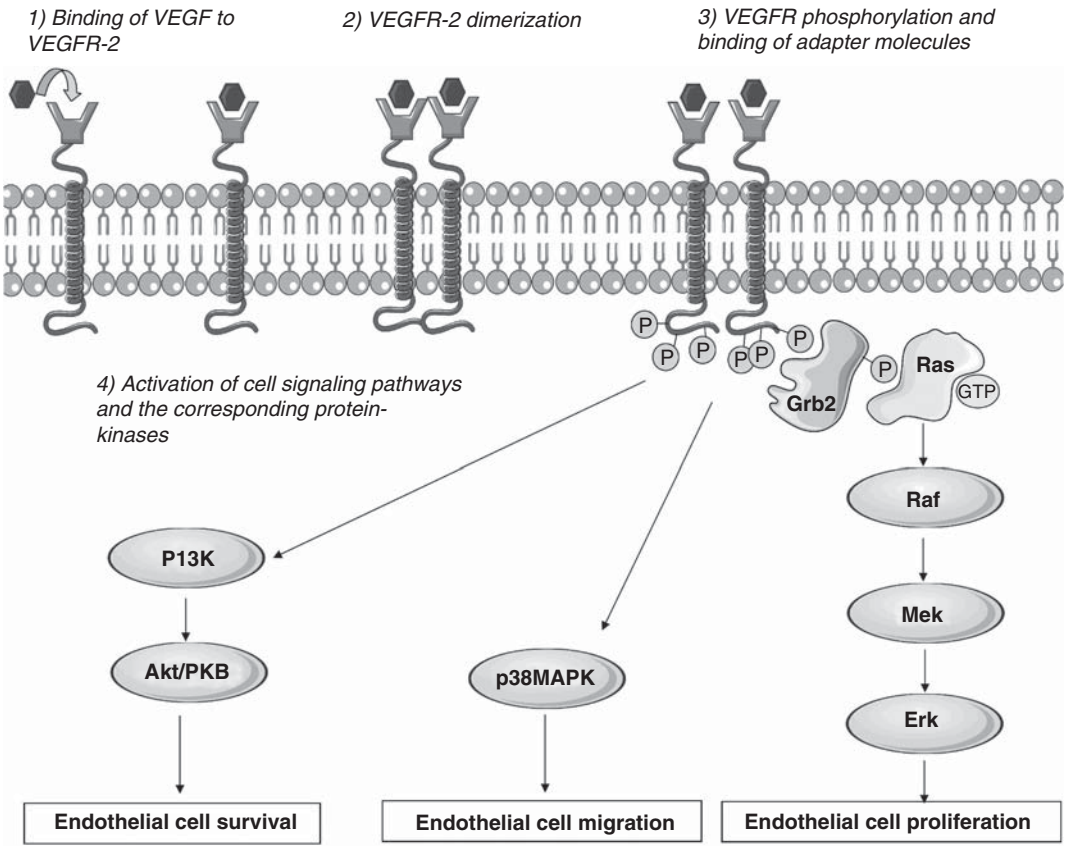

Figure 1. Schematic representation of VEGF-induced cell signaling events in endothelial cells via binding to the VEGFR-2 receptor subtype. Binding of VEGF leads to receptor dimerization and activation of the intracellular tyrosine kinase domains, leading to phosphorylation of the dimerized receptors. The phosphorylation affords interactions with the adapter molecules Grb2 and Ras, initiating a cascade of events leading to activation of MAPKs and eventually to recruitment of transcription factors (not shown) leading to increased endothelial cell survival, migration and proliferation.

Akt/PKB: Specific serine/threonine protein kinase; Erk: Extracellular signal-regulated kinase; Grb2: Growth factor receptor-bound protein 2; Mek: MAPK kinase; Ras: Rat sarcoma G-protein.

3.1 Nanoparticles incorporating antibodies or soluble VEGFRs

Antibodies and soluble VEGFRs bind to VEGF ligand and block its binding to VEGFR which inhibits the proangiogenic VEGF cascade [19]. For example, the humanized antibody bevacizumab binds VEGF with high affinity and neutralizes all human VEGFA isoforms and bioactive proteolytic fragments [19]. Some antibodies are able to bind to VEGFRs (e.g., ramucirumab). The mAb 2C3 (Peregrine Pharmaceuticals, Inc., Tustin, CA, USA) has been designed to inhibit VEGFR-2 activation by human VEGF [20]. Recently, combined gold nanoparticles (~ 5 nm) were loaded with antiangiogenic molecule, VEGF antibody-2C3 (AbVF) and anticancer drug gemcitabine [21]. The functional activity of the individual components in the nanocomposites was tested on HUVEC and 786-O cells and showed retention of functional activity of both VEGF antibody and gemcitabine.

The soluble form of the extracellular domain of VEGFR-1 (sFlt-1) binds to VEGF with the same affinity and equivalent specificity as that of the original receptor, but inhibits its signal transduction [22]. Thus, sFlt-1 could be considered a potent exogenous agent for antiangiogenic therapy. The efficient delivery of antiangiogenic genes to tumors appears to be an important tack for the therapy. The modification of the delivery system with appropriate ligands has been reported as an alternative approach, for example, coupling with RGD because of its affinity to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors (**Figure 3**) [8]. Conjugation of RGD peptide to the cationic polymer (branched PEI with a PEG spacer) efficiently transferred therapeutic pCMV-sFlt-1 gene to angiogenic endothelial cells, but not to the non-angiogenic cells [23]. Moreover, PEI-*g*-PEG-RGD–pCMV-sFlt-1 complexes (150 nm) delivered pCMV-sFlt-1 to tumors more efficiently than PEI-*g*-PEG after systemic administration into subcutaneous tumor-bearing mice. As

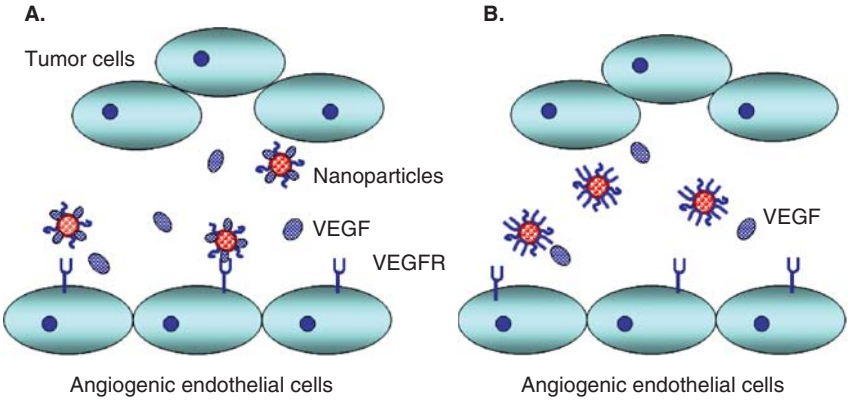

Figure 2. Schematic illustration of nanoparticles targeting angiogenic process. A) Long-circulating stealth nanoparticles coupled with targeting ligands (e.g., antibodies) bind to VEGFR (e.g., VEGFR-1, VEGFR-2) and block its binding to VEGF, which inhibits the proangiogenic VEGF cascade. B) Long-circulating stealth nanoparticles coupled with targeting ligands bind to VEGF derived from tumor cells and block its binding to VEGFR (e.g., VEGFR-1, VEGFR-2). Nanoparticles could be loaded with antitumor drug in the nanoparticle core.

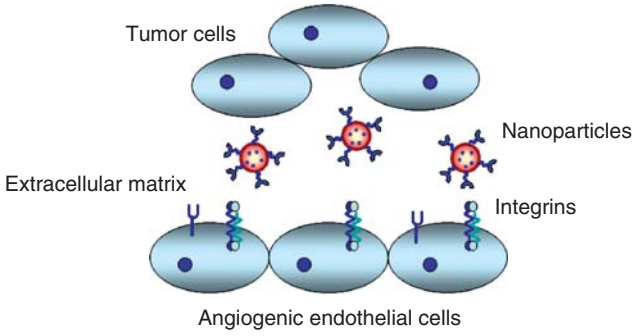

Figure 3. A schematic illustration of the interaction between RGD-conjugated nanoparticles and integrin receptors, in particular $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins.
RGD: Arg-Gly-Asp.

a result, tumor growth was inhibited in the PEI-*g*-PEG-RGD/pCMV-sFlt-1 group but not in PEI-*g*-PEG/pCMV-sFlt-1 suggesting that delivery of pCMV-sFlt-1 using peptide modified carrier is more effective for antiangiogenic gene therapy. A recent study has reported the therapeutic effect of sFlt-1 expressing pDNA complexed into crosslinked polyplex micelles evaluated after systemic administration to BxPC3 human pancreas adenocarcinoma tumor bearing mice [24]. The polyplex micelles were formed by complexation of pDNA and thiolated PEG-poly(L-lysine) block co-polymer, and further modified by coupling with RGD peptide. Vascular density measurements showed that systemically injected polyplex micelles demonstrated significant inhibition of tumor growth up to day 18 due to antiangiogenic effect [25]. The effect of doxorubicin-loaded liposomes and doxorubicin-loaded RGD-grafted liposomes has illustrated the importance of RGD ligands [26]. The RGD-grafted liposomes inhibited tumor growth in mice bearing a doxorubicin-sensitive C26 colon sarcoma, while the non-modified did not inhibited growth. Thus, the therapeutic effect of RGD-grafted liposomes was considered to be mediated by target doxorubicin delivery to proliferating endothelial cells rather than the tumor cells.

Kommareddy and Amiji have described antiangiogenic nanoparticle platform consisting of PEG-modified thiolated gelatin nanoparticles loaded with plasmid DNA encoding sFlt-1 [27]. PEG-modified thiolated gelatin nanoparticles were effective in transfection with sFlt-1 expressing plasmid DNA *in vivo* and showed significant suppression of tumor growth in MDAMB-435 tumor-bearing mice. Microvessel density analysis revealed that the expressed sFlt-1 was able to suppress angiogenesis.

The Raf-1 protein, encoded by the c-raf-1 gene, is a serine–threonine kinase that functions as a regulator of cell growth, proliferation and, according to some reports, could regulate angiogenesis [28]. The expression of activated Raf-1 is associated with upregulation of VEGF production, and a dominant-negative mutant Raf-1 inhibits angiogenesis in response to either FGF or VEGF. Hood *et al.* designed a dominant negative mutant form of the Raf gene that was defective and when delivered into endothelial cells would shut down the normal Raf-1 kinase activity [28]. Further, the mutant Raf gene was loaded in cationic nanoparticles coupled to integrin $\alpha_v\beta_3$ targeting ligands in order to deliver the gene selectively to angiogenic vessels in tumor-bearing mice. Systemic injection of these nanoparticles into mice resulted in apoptosis of the tumor-associated endothelium, leading to regression of established primary and metastatic tumors. Clinical evaluation of liposomal c-raf-1 antisense oligonucleotide formulation in patients has been reported [29]. The liposomes have been considered more advantageous regarding oligonucleotide stability and its efficient intratumoral delivery. Hypersensitivity reactions and dose-dependent thrombocytopenia limited tolerance of the liposomal formulation. The authors concluded that

future clinical evaluation of this approach will depend on modification of the liposome composition.

RNA interference-based antiangiogenic approaches that target VEGF, such as siRNA, have been designed. However, therapeutic application of siRNA requires sufficient stabilization of the RNA against nuclease degradation as well as cytoplasmic delivery to target cells. Formulation of siRNA into polymeric delivery system could provide higher stability of siRNA, while surface modification with appropriate ligand could ensure targeting. Schiffelers *et al*. have developed self-assembled nanoparticles due to the electrostatic interactions between negatively charged siRNA and cationic polyethyleneimine [30]. The nanoparticles were protected by exposed steric polymer (PEG) and targeted by attachment of RGD peptide ligands. These nanoparticles were designed to target tumor neovasculature expressing integrins and used to deliver siRNA inhibiting VEGFR-2 expression and thereby tumor angiogenesis. Intravenous administration into tumor-bearing mice gave selective tumor uptake, siRNA sequence-specific inhibition of protein expression within the tumor and inhibition of both tumor angiogenesis and growth rate. The results suggest achievement of two levels of targeting: tumor tissue selective delivery via the nanoparticle ligand and gene pathway selectivity via the siRNA oligonucleotide. In another study, VEGF siRNA was conjugated to PEG and the conjugate formed micelles by interacting with cationic polyethylenimine (PEI) as a core forming agent [31]. The experiments showed that VEGF siRNA–PEG/PEI micelles showed greater stability than naked VEGF siRNA against enzymatic degradation. VEGF siRNA–PEG/PEI micelles effectively silenced VEGF gene expression in prostate carcinoma cells (PC-3) up to 96.5% under an optimized formulation condition. The same group has reported conjugation of luteinizing hormone-releasing hormone (LHRH) peptide analog to siRNA-PEG and formation of polyelectrolyte micelles by interaction with PEI [32]. For LHRH receptor overexpressing ovarian cancer cells (A2780), the micelles with LHRH exhibited enhanced cellular uptake compared to those without LHRH, resulting in increased VEGF gene silencing efficiency via receptor-mediated endocytosis.

3.2 Nanoparticles incorporating small-molecules inhibitors

Semaxinib (SU5416) is inhibitor of VEGFR-2 tyrosine kinase; in particular, it suppresses VEGF-mediated angiogenesis *in vitro* and *in vivo* through the inhibition of autophosphorylation of VEGFR-2 by blocking the AMP-binding site within the kinase domain of the receptor [33]. Because of its hydrophobic nature, SU5416 was dissolved in Cremophor EL that induced toxicity in patients in a clinical trial [34]. Thus, incorporation of SU5416 into nanoparticulate system could overcome solubility problem and promote neovasculature targeting of SU5416. Katanasaka *et al*. have incorporated SU5416 into liposomes (approximate size of 130 nm) modified with a peptide possessing high affinity to angiogenic vessels (Ala-Pro-Arg-Pro-Gly) [35]. The authors discovered that the liposomal SU5416 did not induce hemolysis *in vitro* compared to free SU5416. *In vivo* study in Colon26 NL-17 carcinoma cell-bearing mice showed that the modified liposomes significantly suppressed tumor growth compared with free SU5416 without affecting the body weight of the mice. The results suggested that APRPG-modified liposomes may enhance antiangiogenic activity through targeted delivery of SU5416 to angiogenic endothelial cells *in vivo*.

Sorafenib (Nexavar®, Bayer Health Care Pharmaceuticals, Montville, NJ, USA) and sunitinib (Sutent®, Pfizer, New York, NY, USA) are tyrosine kinase inhibitors approved as antitumor treatments. It has been shown that sorafenib inhibits tumor cell proliferation and tumor angiogenesis. A recent study has examined whether liposomal sorafenib as well as sunitinib are substrates for transport by RLIP76 and compared the signaling effects of RLIP76 antisense with kinase inhibitors used in treatment of renal cell carcinoma [36]. RLIP76 (RALBP1 gene product) is a multi-functional transporter protein of glutathione conjugate and some anticancer drugs [37]. Inhibition of RLIP76 function by antibody or its depletion by siRNA or antisense DNA caused marked and sustained regression of established human kidney xenografts of Caki-2 cells in nude mouse. The results showed that sorafenib and sunitinib are substrates for transport by RLIP76 and that kinase inhibition by RLIP76 depletion is more widely apparent in a number of renal carcinoma cell lines [38].

Retinoids modulate cell growth and differentiation and inhibit angiogenesis and endothelial cell migration [39]. Some studies have reported that retinoids downregulate VEGF production [40]. Park *et al*. have designed self-assembled nanoparticles composed of a hydrophobic inner core containing aggregated retinoic acid molecules and a hydrophilic heparin shell [41]. Nanoparticles have been modified by coupling of folic acid as targeting ligands. The studies have showed a pronounced inhibition of vessel formation by heparin–retinoic acid conjugated nanoparticles as well as greater cytotoxicity against folate receptor-positive cells compared to free retinoic acid.

4. Nanoparticles targeting FGF and its receptors

FGFs constitute a family of potent growth and angiogenic factors abundant in normal and malignantly transformed cells [42]. Basic FGF (FGF-2) is involved in the process of angiogenesis and represents the target area for antiangiogenic therapy. FGF-2 mediates its biological activity by binding to specific cell-surface receptors and heparan sulfate proteoglycans (HSPG). HSPG contribute to the binding of FGF-2 to high-affinity receptors in different ways, in particular stabilizing the FGF-2–FGF receptor complex, protecting FGF-2 from degradation or facilitating FGF-2 oligomerization [43].

Thus, angiogenesis inhibitors could influence FGF-2 activity by interfering at the level of HSPG–FGF-2–FGF receptor interactions.

Studies with heparin and heparin derivatives showed that their antiangiogenic efficacy might be due to its binding mainly to FGF-2 and inhibiting its binding to the receptors [44]. The drawback of unmodified heparin as antiangiogenic drug is that it possesses anticoagulant activity and binds to a wide array of physiological molecules. This promoted the development of synthetic heparin derivatives and heparin-like molecules (e.g., pentosan polysulfate) able to bind to the specific domain of FGF-2. The study of Plum *et al*. has described an antiangiogenic effect of liposomes containing a peptide targeting heparin binding domain of FGF-2 [45]. Mice treated with the liposomes containing heparin binding peptide generated a specific antibody response to FGF-2, blocked neovascularization in a gelfoam sponge model of angiogenesis and inhibited experimental metastasis by > 90% in two tumor models, the B16BL6 melanoma and the Lewis lung carcinoma. Another approach could be modification of vehicles with heparin or derivatized heparin. Silver and gold nanoparticles were conjugated with diaminopyridinyl-derivatized heparin polysaccharides in order to evaluate their antiangiogenic effect [46]. Both types of conjugated nanoparticles exhibited lower toxicity and effective inhibition of FGF-2-induced angiogenesis, with an enhanced antiangiogenic efficacy with the conjugation to the derivatized heparin as compared to glucose conjugation. Synergistic effect could be considered taking into account the antiangiogenic properties of gold and silver nanoparticles themselves [47,48]. It has been reported that gold nanoparticles bind to heparin-binding domain of heparin-binding growth factors, in particular vascular permeability factor VPF/VEGF165 and FGF-2 [47]. Thus, gold/silver nanoparticles appear to be an alternative antiangiogenic therapy due to their potential to target multiple pathways by disrupting VPF/VEGF165 and FGF-2 activities.

Suramin is a polysulfonated naphthylurea with potential antineoplastic activity. Suramin inhibits mainly the binding of FGF-2 to its receptors but also blocks the binding of various growth factors, including VEGF, IGF-I, EGF, PDGF and TGF-β, to their receptors, thereby inhibiting endothelial cell proliferation and migration [49-53]. The narrow therapeutic window and toxicity of suramin require development of optimal drug delivery vehicle. As clinical reports suggest that paclitaxel and suramin have additive effect on treating solid tumors, the idea for their combined controlled delivery seems attractive [54]. A recent study has reported the examination of core/shell microspheres loaded with paclitaxel and suramin for treating of brain tumors [55]. Sequential release of suramin and paclitaxel provided initial presence of suramin that efficiently prevented the excess growth of tumor cells in the initial stage. Further, the subsequent controlled release of paclitaxel induced the apoptosis of tumor cells. *In vivo* tumor inhibition study against subcutaneous U87 glioma in BALB/c nude mice showed that the animals treated with these microparticles showed significant decreases in the number of tumor cells on day 21 post-tumor inoculation compared with placebo control.

TSP-1 and -2 are members of TSP family. They are endogenous proteins able to inhibit angiogenesis. TSP-1 binds and modulates the activity of different mediators of angiogenesis, such as angiogenic factors (FGF-2, VEGF, HGF, PDGF), cytokines and proteases [56]. TSP-1 inhibits angiogenesis directly by interaction with specific receptors on endothelial cells. Thus, TSPs (TSP-1 and -2) could be explored as antiangiogenic agents because of their potential to suppress angiogenic process by different mechanisms (indirect and direct). Aspartimide analogs of natural sequences of TSP-1 and -2 have been evaluated for their antiangiogenic effect as well for their capacity as ligands allowing adhesion on endothelial cells [57]. Both analogs supported the adhesion, but only the analog of TSP-2 has demonstrated inhibition of angiogenesis. Doxorubicin loaded liposomes have been modified with TSP-2 analog and their administration decreased by 58% the HT29 tumor growth in nude mice. The authors concluded that the improvement in the doxorubicin antitumor effect was due to the antiangiogenic effect of aspartimide analog of TSP-2.

5. Nanoparticles targeting EGFRs

Human EGFRs (HER1, HER2, HER3 and HER4) are transmembrane tyrosine kinase receptors that regulate cell growth and survival, adhesion, migration, differentiation and other cell responses. Overexpression of HER2 in human tumor cells is closely associated with increased angiogenesis and expression of VEGF.

5.1 Nanoparticles incorporating antibodies

The FDA and European Medicines Agency have approved two mAbs that bind HER and have shown some antitumor activity in various cancer cell lines and tumor xenograft models, cetuximab (Erbitux®, Imclone, Inc., Branchburg, NJ, USA) and panitumumab (Vectibix®, Amgen, Inc., Thousand Oaks, CA, USA). They have indirect antiangiogenic properties through the inhibition of VEGF secretion. It has been reported that the inhibition of HER with cetuximab has an antiangiogenic effect [58]. In order to achieve specific targeting, cetuximab was either covalently or non-covalently (e.g., using affinity between folate–liposomes and folate–folate binding protein coupled to cetuximab) coupled to liposomes [59,60]. In both cases, specific and efficient targeting to EGFR was observed. Mamot *et al*. have loaded into immunoliposomes (cetuximab-conjugated) anticancer drugs (doxorubicin, epirubicin and vinorelbine) and have observed superior antitumor effect compared to free drugs or drug incorporated into non-targeted liposomes [61]. Thus, conjugation with a specific EGFR-antibody could provide efficient and targeted drug delivery of anticancer compounds to tumors that overexpress the EGFR. Recently, gold nanoparticles (5 nm) were conjugated with cetuximab as a targeting agent and gemcitabine as an anticancer drug

(Au-C225-Gem) [62]. *In vitro* targeting ability of the nanoparticles was evaluated by following nanoparticle uptake from three pancreatic cancer cell lines (PANC-1, AsPC-1 and MIA Paca2) with variable EGFR expression. PANC-1 and MIA Paca-2 cells showed better specificity of targeting compared with AsPC-1, although it has high HER expression. The authors postulated that it could be due to the fact that both PANC-1 and MIA Paca-2 are primary cell lines, whereas AsPC-1 is a metastatic cell line. Significant tumor growth inhibition was found in mice treated with Au-C225-Gem compared with the non-targeted gold–gemcitabine conjugate, which clearly revealed targeting efficiency achieved by cetuximab conjugation.

Trastuzumab (Herceptin) is another humanized mAb which binds to the extracellular segment of the HER2 and inhibits tumor cell growth and VEGF expression [63]. The mechanisms by which trastuzumab induces some of its effect are disruption of receptor dimerization, increased endocytotic destruction of the receptor, inhibition of shedding of extracellular domain and immune activation. Lee *et al*. have designed cationic micellar nanoparticles as carriers to co-deliver paclitaxel and herceptin [64]. The aim of their study was to achieve targeted delivery of paclitaxel to HER2 and enhanced cytotoxicity through synergistic activities. Anticancer effects of nanoparticles were investigated in human breast cancer cell lines with varying degrees of HER2 expression level, MCF7, T47D and BT474. The co-delivery of herceptin increased the cytotoxicity of paclitaxel and this enhancement showed a dependency on their HER2 expression levels. Significantly higher cellular uptake of the nanoparticles complexed with herceptin in HER2-overexpressing BT474 cells as compared to HER2-negative HEK293 cells suggested their targeting capacity. In addition, the nanoparticles delivered herceptin much more efficiently than BioPorter, a commercially available lipid-based protein carrier, and displayed a much higher anticancer effectiveness.

Nanobodies are the smallest functional antigen-binding immunoglobulin fragments possessing several attractive characteristics, for example, better solubility and similar degree of specificity and affinity towards their antigen as Fabs, having affinities in the low nanomolar to picomolar range. These properties as well as possibility of being easily engineered and produced open new opportunity for the achievement of high specific targeting. A recent study has reported the effects of multivalent system consisting of EGFR-antagonist nanobodies EGa1 grafted to the surface of pegylated liposomes (average size of 120 – 130 nm) via maleimide linker [65]. The liposomes-grafted EGa1 induced massive EGFR sequestration from the cell surface (90%) versus 30 – 40% reduction induced by monovalent EGa1 (not grafted to liposomes) and a mixture of non-grafted liposomes and EGa1 (both serving as controls). Further, the efficient EGFR internalization mediated by EGa1-liposomes led to receptor degradation (i.e., downregulation) and inhibition of tumor cell proliferation while no degradation was detected with the monovalent nanobody. EGFR downregulation was observed *in vivo* in tumors of mice intravenously injected with EGa1-liposomes, indicating that this multivalent system blocks ligand binding to the receptor and simultaneously induces the downregulation of EGFR.

Li *et al*. have reported intravenous administration of self-assembled protamine nanoparticles bearing siRNA [66]. Nanoparticles were modified with PEG phospholipid derivatized with anisamide ligand for targeting sigma receptor expressing B16F10 tumor. Three daily injections (1.2 mg/kg) of siRNA formulated in the targeted nanoparticles silenced the EGFR in the tumor and induced tumor cell apoptosis. Forty percent tumor growth inhibition was achieved by treatment with targeted nanoparticles. In another study, a mixture of siRNA against MDM2, and VEGF was formulated into targeted nanoparticles [67]. The results of the combined delivery by the targeted nanoparticles showed that the corresponding oncogenes were simultaneously silenced, the mass of lung metastasis greatly reduced and animal survival significantly prolonged with a relatively low and nontoxic dose.

5.2 Nanoparticles incorporating small-molecule inhibitors of the EGFR

Erlotinib is a quinazoline derivative which is a selective EGFR antagonist. In order to avoid the side effects of long therapy with erlotinib, polymeric poly(lactide-co-glycolide) nanoparticles were developed [68]. Oral administration of free erlotinib and erlotinib-loaded PLGA nanoparticles in rats showed significant damage of the internal organs of animals treated with free drug, whereas those treated with encapsulated erlotinib were similar to the healthy organs. The results revealed the low risk associated with the application of encapsulated erlotinib.

6. Nanoparticles targeting MMPs

In response to tumor-induced or other angiogenic stimuli, endothelial proteases initiate the breakdown of the surrounding extracellular matrix, which allows the migration of proliferating endothelial cells and their growth to spontaneously form capillary-like structures. MMPs are a family of multifunctional endopeptidases capable of degrading the basement membrane and extracellular matrix, thus contributing to tissue remodeling and cell migration. MMPs can be divided into subgroups, one of which is constituted by the type IV collagenases or gelatinases, MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B). Elevated or unregulated expression of gelatinases and other MMPs can contribute to the pathogenesis of several diseases, including tumor angiogenesis and metastasis [69]. Thus, a selective targeting of MMP-2 and -9 could be considered new approach in antiangiogenesis. A recent study has reported preparation of liposomes conjugated with a cyclic peptide CTT (Cys-Thr-Thr-His-Trp-Gly-Phe-Thr-Leu-Cys) [70]. Conjugation of liposomes with CTT enhanced three to fourfold cellular uptake of liposomes by gelatinase-expressing cells. Augmented killing (approximately fourfold) of U937 leukemia and HT1080 sarcoma cells was obtained by the CTT-conjugated

doxorubicin-loaded liposomes, compared with control liposomes (without targeting peptide). Thus, using CTT as a homing peptide resulted in both selective targeting and enhanced uptake of anticancer-loaded liposomes by MMP-2 and -9 expressing cells.

Another study has reported the development of MMP-specific PEGylated peptide–doxorubicin conjugate micelles [71]. The conjugates were formed using peptides cleaved specifically by MMPs, namely, Gly-Pro-Leu-Gly-Val and Gly-Pro-Leu-Gly-Val-Arg-Gly. In order to increase antitumor activity, free doxorubicin was physically loaded into PEGylated peptide–DOX conjugate micelles. Animals treated with doxorubicin-loaded PEGylated peptide–doxorubicin conjugate micelles inhibited tumor growth up to about 72% compared to the control. The authors concluded that the significant tumor inhibition effect of doxorubicin-loaded PEGylated peptide–doxorubicin conjugate micelles resulted from the peptide specificity for MMPs and the additive effect of the loaded doxorubicin.

7. Nanoparticles targeting tubulin function

Microtubules constitute one of the most important cellular targets of anticancer drugs and especially of the cytotoxic natural products. These drugs bind to several sites of the tubulin molecule suppressing microtubule dynamics and turnover, and consequently block mitosis at the metaphase/anaphase transition and induce cell death. Most of these agents (e.g., vinblastin, paclitaxel, podophyllotoxin) are typical cytotoxic antineoplastic agents. However, some microtubule-targeted agents are able to shut down the existing vasculature at tumors because of depolymerization of the microtubule cytoskeleton at the endothelial cells (vascular-targeting agents). Among them, the most efficient at damaging tumor vasculature are those binding the colchicine site, for example, the combretastatins. The combretastatins are natural products isolated from the African willow tree *Combretum caffrum*. Combretastatin binds to tubulin causing cytoskeletal and morphological changes in endothelial cells [72]. These changes increase vascular permeability and disrupt tumor blood flow.

Combretastatin-loaded RGD-targeted liposomes were combined with radiation therapy [73]. The modification of liposomes with RGD ligands allowed combretastatin targeting to $\alpha_v\beta_3$ integrin while ionizing radiation was considered to upregulate $\alpha_v\beta_3$ integrin. C57BL mice bearing a transplanted B16-F10 melanoma were treated with combined therapy (single dose of 5-Gy radiation and single dose of combretastatin-loaded liposomes), only radiation or free drug (drug dose of almost six times that used in the liposomes). The results showed no significant increase in the volume of the tumors treated with combined therapy during the initial 6 days post-treatment, while the other treatment groups exhibited exponential growth curves after day 3. Preliminary investigations of the group have indicated that this treatment may also be effective in controlling tumor volume in spontaneous mammary ($MMTV^+$) tumors.

An endogenous metabolite of estrogen, 2-methoxyestradiol (2-ME), and its analogs are known as antiangiogenic agents [74]. According to the literature, the mechanism of its action is associated with the ability of 2-ME to bind to tubulin and disrupt normal microtubule function through altering microtubule stability [75]. In addition, 2-ME inhibits the proangiogenic transcription factor hypoxia-inducible factor 1-α, which leads to reduced expression of some angiogenic growth factors (e.g., VEGF) [76]. Du *et al*. have evaluated the possibility of 2-ME specific delivery to lungs by intravenous application of liposomes [77]. Target efficiency of 2-ME-loaded liposomes to rat lungs was significantly improved compared with 2-ME solution. The study demonstrated that liposomes may serve as a passive targeting system for 2-ME.

8. Other antiangiogenic approaches

8.1 Targeting the p53-dependent cell signaling pathways

Protein p53 (known also as cellular tumor antigen) is a tumor suppressor that plays a role in apoptosis, genetic stability and inhibition of angiogenesis [78,79]. Protein p53 functions by binding to p53 DNA recognition sequences and regulates transcription of growth-regulatory genes. The protein is continually produced and degraded in the cell although its mutation under different factors may occur [80]. Mutation of the p53 gene results in loss of p53 function and in oncogenic functions. Thus, the restoring of endogenous p53 function could be considered efficient anticancer strategy. Gautam *et al*. have evaluated the antiangiogenic effect of polyethyleneimine–p53 DNA complexes formulated for aerosol delivery [81]. The authors have reported that the p53 transfection leads to an upregulation of the antiangiogenic factor TSP-1 in the lung tissue and the serum of the mice. Furthermore, they have observed a downregulation of VEGF in the lung tissue and serum of the B16-F10 tumor-bearing mice treated with PEI–p53 DNA complexes, compared with untreated tumor-bearing animals. The investigators suggested that aerosol delivery of PEI–p53 complexes leads to inhibition of B16-F10 lung metastases, in part by suppression of angiogenesis. In another study, the same group investigated the effect of sequential aerosol delivery of PEI–p53 DNA complexes and dilauroylphosphatidylcholine liposome formulation of 9-nitrocamptothecin (9NC-DLPC) [82]. The authors found that sequential delivery of p53 and 9NC by aerosol arrested the growth of established B16-F10 melanoma pulmonary metastases in experimental mice although the doses of p53 gene and 9NC in the combination group were reduced at least twofold as compared with single agent studies. The potential of wt-p53 DNA-loaded PLGA nanoparticles (approximate size of 200 nm) as a non-viral gene expression vector has been evaluated [83]. Greater anti-proliferative activity *in vitro* was reported for nanoparticles

as compared to that with naked DNA and DNA–liposome complex, probably due to the sustained intracellular DNA delivery and gene expression. In addition, a single-dose intratumoral administration of wt-p53 DNA-loaded nanoparticles demonstrated significant inhibition of tumor growth in MDAMB-435-induced subcutaneous breast cancer mouse model. The authors considered that the inhibition was attributed to higher apoptosis of tumor cells and the induction of TSP-1 that inhibited tumor angiogenesis.

8.2 Nanoparticles incorporating endothelial cell proliferation inhibitors

Angiostatin and endostatin are endogenous proteins with potent antiangiogenic function that were found to be secreted by some solid tumors and are believed to be the mediators by which large primary solid tumors inhibit the growth or spread of distant metastases. Angiostatin is a protein whose sequence is identical with the first four triple loop structures of plasminogen. Endostatin is produced by hemangioendothelioma which is a COOH-terminal fragment of collagen XVIII. Endostatin is a broad spectrum inhibitor that inhibits 65 different tumor types and modifies 12% of the human genome to downregulate pathological angiogenesis without side effects [84]. However, antiangiogenic therapy with angiostatin and endostatin in cancer requires prolonged administration of recombinant protein *in vivo*. In this view, gene therapy transfer of angiostatin and endostatin could be considered an alternative method to deliver these inhibitors. It has been shown that liposomes complexed to plasmids encoding angiostatin or endostatin effectively reduced angiogenesis using an *in vivo* Matrigel assay [85]. Both types of liposomes significantly reduced tumor size when injected intratumorally compared to the untreated group. The liposomes complexed to plasmid encoding endostatin reduced tumor growth in the nude mice when applied intravenously by nearly 40% compared to either empty liposomes or untreated controls. Therapeutic potential of endostatin-coding plasmid (Endo cDNA) complexed to cationic liposomes has been evaluated in an orthotopic osteosarcoma model in rats [86]. Systemic administration of Endo cDNA-complexed liposomes encoding a secreted form of murine endostatin slowed tumor growth and delayed formation of metastases in rats. Histopathological analysis performed on tumor tissue from the Endo cDNA-liposomes treated group revealed an increase in apoptotic tumor cells and necrotic area, a phenomenon not observed in the tumor tissues of animals treated with cDNA-liposomes (empty plasmid without the endostatin gene complexed to cationic liposomes). The authors concluded that endostatin gene was specifically transferred into tumor endothelial cells and displayed an antiangiogenic effect in the model. Circulating levels of endostatin that are too high or too low are inactive, which point to the necessity of optimized drug delivery of endostatin [87]. Poly(lactic-co-glycolic acid) nanoparticles coupled with a synthetic ligand specific for selectin have been loaded with both paclitaxel (at low concentration) and endostatin [88]. Nanoparticles have been examined for their antiangiogenic efficacy in HUVEC culture *in vitro* and rat aorta tissue culture *ex vivo* models. An enhanced anti-proliferative effect on HUVECs and heightened antiangiogenic action on rat aorta ring cultures was observed for the drug-loaded nanoparticles compared to the free drugs. The authors concluded that co-loading with both drugs exhibited a synergetic antiangiogenic effect.

The fumagillin family of natural products is known to inhibit angiogenesis through irreversible inhibition of human type 2 methionine aminopeptidase [89]. TNP-470 is a low molecular synthetic analog of fumagillin known as a broad spectrum angiogenesis inhibitor [90]. The administration of TNP-470 should be carefully considered due to its strong potency and toxicity, poor pharmacokinetic profile and poor solubility [91]. Thus, an entrapment of TNP-470 into nanoparticulate system could improve the specificity of drug delivery and reduce the side effects. Satchi-Fainaro *et al.* have synthesized a water-soluble conjugate of *N*-(2-hydroxypropyl)methacrylamide (HPMA) co-polymer and TNP-470 [92]. HPMA co-polymer–TNP-470 substantially enhanced and prolonged the activity of TNP-470 *in vivo* in tumor and hepatectomy models. Polymer conjugation prevented TNP-470 from crossing the BBB and decreased its accumulation in normal organs, thereby avoiding drug-related toxicities. Later, Benny *et al.* have conjugated TNP-470 to monomethoxy–PEG–polylactic acid to form nanopolymeric micelles for oral administration [93]. Micelles significantly inhibited tumor growth, without causing neurological impairment in tumor-bearing mice. The authors concluded that the micelles could be chronically administered for cancer therapy or metastasis prevention.

8.3 Inhibition of integrins

Integrin are heterodimeric transmembrane receptors that are composed of two transmembrane glycoproteins (α and β units). The integrins are > 20 types, but $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are found to be overexpressed on tumor angiogenic endothelial cells [94]. As described above, modification of nanoparticulate systems with appropriate peptide ligands (e.g., those possessing RGD motif) represents opportunity for achievement of selective delivery of antitumor drugs to tumor cells due to their affinity to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins (**Figure 3**) [8]. On the other hand, inhibition of these integrins by anti-integrin agents has been shown to suppress angiogenesis [95]. The most investigated integrin inhibitors are mAbs that target the extracellular domain of the heterodimeric receptor (e.g., the humanized mAb etaracizumab) and synthetic peptides that contain RGD sequence (e.g., cilengitide) [96]. The main problem associated with administration of antiangiogenic proteins is their instability and short half-life. Thus, a delivery system that could ensure protein protection and sustained delivery is required. According to recent study, the attachment of RGD peptides to nanoparticles resulted in an extension of the peptide blood half-life from 13 to 180 min, illustrating that RGD-nanoparticle system could provide more desirable

properties of RGD peptide [97]. Another study has reported that RGD-peptide encapsulated in glycol chitosan nanoparticles markedly inhibited FGF-2-induced angiogenesis and decreased hemoglobin content in Matrigel plugs [98]. Further, intratumoral administration of RGD-glycol chitosan nanoparticles significantly decreased tumor growth and microvessel density compared to native RGD peptide injected either intravenously or intratumorally, indicating the efficiency of nanoparticles as an RGD vehicle.

8.4 IL delivery

IL-12 is an immunomodulatory cytokine produced primarily by antigen-presenting cells, which play an important role in promoting T_H1-type immune response and cell-mediated immunity. It has been reported that IL-12 possesses antiangiogenic activity which could be exerted through activation of IFN-γ to upregulate IFN-inducible protein-10, an antiangiogenic cytokine [99]. However, IL-12 protein delivery is associated with its instability as well as cytotoxicity after systemic administration in rodents and human clinical trials. These problems could be overcome by gene delivery carried out by appropriate nanoparticulate vehicles allowing specific IL-12 gene delivery to the tumor site. Recently, asialofetuin targeted nanoparticles formulated by blending PLGA with cationic lipid 1,2-dioleoyl-3-(trimethylammonium) propane (DOTAP) were loaded with therapeutic gene IL-12 [100]. Tumor-bearing animals treated with these nanoparticles showed tumor growth inhibition, leading to a complete tumor regression in 75% of the treated mice. High levels of IL-12 and IFN-γ were detected in the sera of treated animals. Another study has reported the development of biodegradable cationic core-shell nanoparticles allowing simultaneous delivery of paclitaxel and IL-12-encoded plasmid to the same cells [101]. The results showed that co-delivery of drug and gene suppressed cancer growth more efficiently than the delivery either of paclitaxel or plasmid in a 4T1 mouse breast cancer model. PEI–DNA complexes carrying IL-12 gene and mannosylated chitosan nanoparticles have been reported as efficient gene delivery vehicles able to suppress tumor growth and angiogenesis [102,103].

The human melanoma differentiation associated gene-7 (mda-7), also known as IL-24, possesses tumor suppressor, antiangiogenic and cytokine properties. Intratumoral administration of Ad-mda-7 (adenovirus-mediated gene transfer) to lung tumor xenografts results in growth suppression via induction of apoptosis and antiangiogenic mechanisms. In this view, Ramesh *et al.* have developed non-viral DOTAP–cholesterol nanoparticles and have evaluated nanoparticle-mediated delivery of mda-7 gene to primary and disseminated lung tumors *in vivo* [104]. The authors found that the nanoparticles efficiently delivered the mda-7 gene to human lung tumor xenografts, resulting in suppression of tumor growth in both primary and metastatic lung tumors. Furthermore, tumor vascularization was reduced in mda-7 treated tumors. The growth was also inhibited in murine syngenic tumors treated with the mda-7 loaded nanoparticles. Thus, the study has demonstrated the capacity of these non-viral nanoparticles for the development of mda-7 treatments for primary and disseminated cancers.

IL-8 is a potent proangiogenic cytokine that is overexpressed in most human cancers. It promotes tumor growth, angiogenesis and metastasis in murine models of several cancers. Blocking of IL-8 activity by mAbs or other approaches could decrease tumor growth. The therapeutic efficacy of using liposome-encapsulated siRNAs to silence IL-8 gene expression was examined in orthotopic mouse models of ovarian cancer [105]. The results demonstrated that siRNA-mediated IL-8 gene silencing decreased tumor growth through antiangiogenic mechanisms.

8.5 Glucocorticoid delivery

Glucocorticoids (GCs) have inhibitory actions on solid tumor growth due to suppressive effects on tumor angiogenesis and inflammation. Targeted delivery of GC to tumor tissue is required because antitumor effect is achieved with substantially higher doses than the doses needed to treat inflammatory disease, which provokes their side effects. Intravenous administration of prednisolone phosphate loaded long-circulating liposomes in mice inhibited tumor growth by 85% compared to treatment with free prednisolone [106]. The authors found that the angiogenesis inhibition occurred by reduction of the intratumoral production of some proangiogenic factors, in particular FGF-2, IL-1α, IL-1β, IL-9, G-CSF, GS-CSF and GM-CSF, and direct inhibition of endothelial cell proliferation. On average, the effect of prednisolone delivered by liposomes on the levels of proangiogenic factors was 25% higher that the effect of free prednisolone indicating the importance of liposomal vehicle. The effects of liposomal formulations loaded with other GCs (budesonide, dexamethasone and methylprednisolone) on the production of angiogenic/inflammatory factors *in vivo* in the B16.F10 murine were investigated [107]. All formulations inhibited tumor growth in a different degree that correlated with their efficacy to suppress tumor angiogenesis and inflammation.

9. Expert opinion

The ability to specifically deliver therapeutic agents to selected cell types while minimizing systemic toxicity is the main goal of nanoparticulate drug delivery systems. The important properties of nanoparticles such as long circulation, targeted drug delivery and drug protection could solve some of the problems of antiangiogenic agents, for example, instability and short half-lifes *in vivo* of antiangiogenic proteins, poor solubility and toxicity of small-molecule inhibitors of angiogenesis. Substitution of large antibodies by encapsulation of antibody fragments (e.g., nanobodies) opens new direction in their application. Antiangiogenic gene therapy also could be improved by nanoparticle formulations. Nanoparticle-mediated gene delivery would result in sustained gene expression, and hence better efficacy with a therapeutic gene.

Independent of the significant improvement in antiangiogenic nanoparticle delivery, the limited clinical success of agents targeted at a single signaling cascade (e.g., VEGF-dependent downstream events) has to be taken into account. Even if therapies directed against VEGF are effective initially, tumors may escape from inhibition after a time as they mutate to express other angiogenic growth factors. This situation significantly decreases the single utility of indirect angiogenesis inhibitors that block the activity of a certain proangiogenic factor. This acquired drug resistance induced by tumor cells could be overcome by co-encapsulation of indirect angiogenesis inhibitors with a multi-drug resistance modulator.

More interesting, some antiangiogenic agents are capable of blocking two or more proangiogenic factors or some crucial mediator of angiogenesis that gets turned on by multiple growth factors. This knowledge could provide a new therapeutic approach taking into account that tumors produce > 20 different growth factors able to induce angiogenesis. The complexity of the angiogenesis process and its regulation throw the crosstalk of multiple cell signaling pathways indicating that multi-targeted agents, acting on different elements of the process, appear to be more promising for further development. Thus, targeting multiple angiogenic pathways rather than a single pathway could be a more effective mode of treatment.

Another opportunity is the development of novel nanoparticles able to deliver anticancer and antiangiogenic drug. Because of various structures of nanoparticles, it is possible to encapsulate drugs with different activities (or physicochemical properties). Core/shell nanoparticles are very attractive carriers for incorporation of both drugs. Varying core/shell properties (e.g., polymer blending) offer the possibility for achieving sequential drug release. In addition, nanoparticle shells could be modified resulting in high selectivity of drug delivery. In such a system, an antiangiogenic drug could provide either active targeting of nanoparticles or inhibition of growth factor or its receptors. This combined administration will not only block the angiogenesis but also will sensitize the tumor cells to chemotherapy due to the normalization of tumor vasculature. In addition, the conjugation of nanoparticles with tumor-penetrating peptides (e.g., iRGD) allows co-administered drugs to penetrate into extravascular tumor tissue. Thus, the administration of multifunctional nanoparticles could improve therapeutic effect at reduced drug doses and consequently to be less toxic.

Thus, the emerging antiangiogenic agents and especially those with complex pharmacology (involving antiangiogenic effects, together with cytotoxic and antivascular effects on established tumor vasculature) are expected to benefit from drug targeting using nanoscale delivery systems.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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