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Passive targeting of nanoparticles to cancer: A comprehensive review of the literature

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Abstract. Cancer remains the one of the most common causes of mortality in humans; thus, cancer treatment is currently a major focus of investigation. Researchers worldwide have been searching for the optimal treatment (the 'magic bullet') that will selectively target cancer, without afflicting significant morbidity. Recent advances in cancer nanotechnology have raised exciting opportunities for specific drug delivery by an emerging class of nanotherapeutics that may be targeted to neoplastic cells, thereby offering a major advantage over conventional chemotherapeutic agents. There are two ways by which targeting of nanoparticles may be achieved, namely passive and active targeting. The aim of this study was to provide a comprehensive review of the literature focusing on passive targeting.

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1. Introduction

Cancer remains the one of the most common causes of mortality in humans; thus, cancer treatment is currently a major focus of investigation (1). Intense efforts aimed at improving conventional treatment did not achieve the desired goal and there has been little advancement on the overall cancer survival landscape. Chemotherapeutics used in cancer management have one prime purpose, which is eliminating malignant cells, a task usually achieved with high efficacy but little precision (2). Patients subjected to treatment paradigms with such non-specific toxic compounds commonly develop severe side effects that may be debilitating in their own right (3,4). Ehrlich introduced the concept of the 'magic bullet' at the turn of the 20th century (5). Since then, researchers worldwide have been searching for an optimal treatment that selectively targets cancer without afflicting significant morbidity. Recent advances in cancer nanotechnology have raised exciting opportunities for specific drug delivery by an emerging class of nanotherapeutics that may be targeted to neoplastic cells, thereby offering a major advantage over conventional chemotherapeutic agents (4,6). There are two ways by which targeting of nanoparticles may be achieved, namely passive and active targeting.

Passive targeting facilitates deposition of nanovectors within the tumor microenvironment, owing to distinctive characteristics inherent to the tumor milieu, not normally present in healthy tissues (5). The delivery of nanoparticles is determined by factors associated with the tumor microvasculature, in addition to factors inherent to the nanoparticle itself, such as size, shape and surface charge (5). Targeting strategies have taken a step further to enhance the selective uptake of nanoparticles into the tumor cells. Biorecognition molecules have been attached to the surface of the nanovectors to target specific markers that are overexpressed by the neoplastic cells. These strategies have been awarded the appellation 'active targeting', which exhibits a higher specificity and efficacy in achieving the desired goal (6). The aim of this study was to provide an overview of the factors orchestrated in the passive targeting of nanoparticles to tumors. In deference to the traditional views, we also aimed to investigate the various strategies that may be adopted to maximize the benefits of this approach.

2. Passive targeting

In passive targeting, macromolecules including nanoparticles accumulate preferentially in the neoplastic tissues as a result of the enhanced permeability and retention (EPR) phenomenon, first described by Maeda and Matsumura (7,8). The EPR is based on the nanometer size range of the nanoparticles and two fundamental characteristics of the neoplastic tissues, namely, the leaky vasculature and impaired lymphatic drainage.

3. Nanometer size range

In 1986, Maeda *et al* (9) observed that intravenous administration of Evans blue dye, which binds to plasma albumin, resulted in selective concentration in tumor tissues. The tumor concentration of blue albumin mounted to ~10-fold that in the blood at 145 h. This phenomenon was also demonstrated with radio-labeled plasma proteins, including transferrin (90 kDa) and IgG (160 kDa), whereas smaller proteins, such as neocarzinostatin (12 kDa) and ovomucoid (29 kDa), did not accumulate within tumors (1,8,10). Subsequent studies have confirmed that macromolecules with a molecular weight above the renal threshold (40 kDa) tend to accumulate preferentially in neoplastic tissues upon intravenous administration (1,11). This unique phenomenon of preferential accumulation of macromolecules is the resultant effect of the abnormal vasculature and impaired lymphatic drainage within neoplastic tissues.

4. Abnormal tumor vasculature

Once a malignant tumor grows to >2-3 mm^3 in size, the delivery of oxygen and nutrients becomes diffusion-limited and the formation of new blood vessels becomes essential to meet the ever increasing demands of the rapidly growing malignant cells (3). This is accomplished through the release of angiogenic factors by the neoplastic tissue aiming to increase the microvasculature within the tumor in order to sustain further growth (3). The resultant imbalance of angiogenic factors and matrix metalloproteinases (MMPs) within neoplastic tissues results in highly disorganized vessels, which are dilated, with numerous pores and wide gap junctions between endothelial cells (12). The perivascular cells and basement membrane are absent or defective (1,13). Furthermore, tumor vessels frequently lack the smooth muscle layer that normally surrounds endothelial cells (14). The normal vasculature is endowed with tight junctions that are impermeable to molecules sized >2-4 nm, thus keeping the nanoparticles within the circulation; however, the leaky vasculature of neoplastic tissue allows macromolecules with a diameter of ≥600 nm to extravasate into the neoplastic tissues. Since tumors do not have a well-developed lymphatic system, these extravasated nanoparticles tend to stagnate within the neoplastic tissue (12,15). This phenomenon of leaky vasculature and impaired lymphatic drainage has been referred to as the EPR effect (7,8).

5. Factors affecting enhanced permeability and retention

Architectural abnormalities of the neoplastic vessels and blood pressure. In normal blood vessels, the smooth muscle layer is essential for mediating a vasogenic response to vascular mediators and, hence, for maintaining a constant blood flow to an organ. By contrast, the microvasculature in neoplastic tissues lacks these smooth muscle cells; therefore, these vessels are in a state of permanent vasodilation and non-responsiveness to physiological stimuli regulating blood flow (16). These aberrant neoplastic vessels result in abnormal transport dynamics of fluid and solutes across tumor vessels, which may be exploited to further accentuate the EPR effect (1).

Suzuki *et al* (17) demonstrated that elevating the mean arterial blood pressure by infusion of angiotensin II resulted in an ~5.7-fold selective increase in blood flow in tumor tissue, without an associated increase in normal tissue. Li *et al* (18) later verified that angiotensin II-induced hypertension augments the EPR effect. Raising the systolic blood pressure in tumor-bearing rats by angiotensin II infusion resulted in a 2-6-fold selective increase in tumor blood flow volume, depending on the blood pressure attained. In addition to the increased blood flow, the authors of that study observed a preferential accumulation of drugs with a molecular mass of ~80 kDa within the tumor tissue. Moreover, drug accumulation in normal organs, such as kidney and bone marrow, was reduced to 60-80%. Tight endothelial gap junctions and normal vasogenic response to angiotensin in healthy tissues permit less transvascular transfer of macromolecules. By contrast, lack of a vasogenic response due to the deficient vascular smooth muscle layer in the neoplastic blood vessels results in an increased intratumoral blood flow in responose to a systemic elevation of the blood pressure. An increased blood flow and a leaky vasculature result in accumulation of macromolecular drugs in neoplastic tissues. Similar results have been observed upon systemic administration of macromolecular drugs to patients with several solid tumors under an angiotensin II-induced hypertensive state (13,19). While low-molecular weight anticancer drugs have a dose-limiting toxicity, using macromolecular agents under a hypertensive state achieved a >5-fold higher concentration of anticancer drugs in the tumor, even though the hypertension was maintained only for ~20 min (13,19).

Vasogenic mediators. The tumor microvasculature is orchestrated by a number of local mediators, including bradykinin, nitric oxide (NO), peroxynitrite, MMPs, vascular endothelial growth factor (VEGF) and prostaglandins (PGs). These mediators have been investigated in an attempt to potentiate the EPR and, hence, achieve better drug targeting to the neoplastic tissue (Table I) (20).

Bradykinin. The Hageman factor (factor XII) of the coagulation cascade is the chief protease of the kallikrein-kinin system. The activation of factor XII is followed by activation of prekallikrein to kallikrein. Kallikrein generates bradykinin directly from kininogen (21). Bradykinin receptors have been identified in various human and rodent solid tumors (22,23) and it was demonstrated that the bradykinin-generating cascade is activated in neoplastic tissues (24). Bradykinin is present at high levels in the peritoneal and pleural fluids of humans and animals with cancer. The crucial role of bradykinin in the extravasation of plasma components into the peritoneal or pleural cavity was further demonstrated by the inhibition of kallikrein (19). Therefore, bradykinin, a key factor controlling

Table I. A summary of the effects of different mediators on the microvasculature of solid tumors.

Mediator	Effect	(Refs.)
Angiotensin II	Raises the systolic blood pressure, thereby increasing blood flow	(27-31)
	Lack of normal vasogenic response to angiotensin increases the permeability of tumor vessels	
Bradykinin	Controls vascular permeability	(13,37)
	Activates NO production through eNOS	(40,41)
ACE inhibitors (e.g., enalapril)	Enhance vascular permeability effects of bradykinin	(33)
	ACE inhibitors increase delivery of macromolecular drugs to tumors, even under normotensive conditions	(3,33,45,46)
Nitric oxide	Induces dilation of tumor vessels	(20,33)
	Promotes angiogenesis	
	Increases vascular permeability	
	Indirect activation of MMPs	(49)
Peroxynitrite and MMPs	Degrade ECM and enhance angiogenesis and metastasis	(58,59)
	Enhance vascular permeability	(20,32,60)
Vascular endothelial growth factor	Up to 30-fold increase in vascular permeability in neoplastic tissues	(33,47)
Prostaglandins	PGE2 and PGI2 enhance vascular permeability	(62,63)
	PGI2 analogues enhance the EPR effect in tumors up to 3-fold	(64)

NO, nitric oxide; eNOS, endothelial nitric oxide synthase; ACE, angiotensin-converting enzyme; MMPs, matrix metalloproteinases; ECM, extracellular matrix; PG, prostaglandin; EPR, enhanced permeability and retention.

vascular permeability, is an important mediator controlling the EPR effect in neoplastic tissues (11,24). Bradykinin is also known to activate NO production via the activation of endothelial NO synthase (eNOS) (25). NO production contributes to the angiogenic properties of VEGF in human endothelial cells (26), also referred to as vascular permeability factor (VPF) (27).

Bradykinin is degraded by several peptidases, particularly angiotensin-converting enzyme (ACE) (21). Inhibition of ACE is expected to increase the local concentration of bradykinin and, hence, increase the vascular permeability of the tumor. It was reported that ACE inhibitors, such as enalapril and temocapril, potentiate the EPR effect (28,29). More importantly, ACE inhibitors increase the delivery of macromolecular drugs to tumors, even under normotensive conditions (2). ACE inhibitors are non-toxic, without major adverse effects in healthy individuals and are only active in hypertensive patients. Therefore, ACE inhibitors may act selectively at tumor sites in normotensive patients with neoplasia to potentiate the EPR effect (11).

NO. NO is generated from L-arginine and oxygen by three isoforms of NOS. Inducible NOS, the most potent isoform, is produced in macrophages and neutrophils, which are known to extensively infiltrate tumor tissues (11,21,30). NO is a well-known mediator of vasodilation, angiogenesis and extravasation (13,21). It has been demonstrated that enhanced vascular permeability in solid tumors is mediated by NO and inhibited by NO scavengers and NO synthase inhibitors (19,31). As a mediator affecting tumor vascular permeability, NO is expected to play a critical role in enhancing the EPR effect in solid tumors (21). Apart from exerting a direct effect on EPR, NO reacts rapidly with superoxide anion, which is predominantly produced by leukocytes, to generate peroxynitrite. The formed peroxynitrite, in turn, activates MMP precursors (proMMPs) into MMPs (32), which may also contribute to the EPR effect (21,32).

When the NO-releasing agent isosorbide dinitrate was infused into the local tumor feeding artery and angiotensin II was concomitantly injected systemically, the site-specific delivery of SMANCS-Lipiodol was enhanced, supporting the hypothesis that NO enhances the EPR effect (13). An analogy between hypoxic solid tumor and ischemic cardiac tissue in angina pectoris has been described (33). Nitroglycerin used in the management of angina pectoris liberates nitrite through the action of denitrase, which is then converted to NO in the ischemic tissues by nitrite reductase (11,33). This pharmacological benefit of nitroglycerin has also been validated *in vivo*, in a mouse tumor model (34). Topical application of nitroglycerin ointment to the skin of mice with breast cancer resulted in an increased blood flow only in the neoplastic tissue, thereby increasing macromolecular drug delivery to the tumor. Clinical evaluations of nitroglycerin used in combination with conventional low-molecular weight anticancer agents were recently undertaken by Yasuda *et al* (35,36) and Siemens *et al* (37); both studies reported significant clinical improvement in therapeutic response, indicating that NO clearly benefits patients undergoing chemotherapy (11).

Peroxynitrite and MMPs. Neoplastic tissues synthesize NO, which reacts with superoxide anion generated by the recruited inflammatory cells, generating peroxynitrite (30). ProMMPs react with peroxynitrite and are activated to MMPs (13). MMPs are known to facilitate cancer metastasis by degrading the extracellular matrix and to enhance angiogenesis, thus

supporting the growth of solid tumors (38,39). It has been demonstrated that MMPs also enhance the vascular permeability of solid tumors in mice and this effect was shown to be suppressed by MMP inhibitors (13,20,40). Several MMP inhibitors have been developed over the last two decades; however, none of these inhibitors was applicable clinically. The first reason for this failure may be that some tumor cells remain viable and, therefore, may resume growth when the drug treatment is discontinued. The second reason may be that MMPs are proteases vital for cellular metabolism and high doses of MMP inhibitors cause toxicity. This has led to the termination of the development of a number of anti-MMP drugs (13).

VEGF. VEGF, formerly known as VPF, has been shown to be 2- to 30-fold higher in neoplastic compared to normal tissues, with the exception of the lung (21,30). In addition to being a mitogen for endothelial cells, VEGF plays a pivotal role in the induction of vascular permeability (13). Intradermal injection of VEGF has been shown to significantly enhance the extravasation of Evans blue dye in a dose-dependent manner, thereby highlighting its important role in enhancing the EPR effect (21,30).

PGs. PGs, particularly PGE2, are important mediators of vascular permeability. PGE2 is generated via cyclooxygenase (COX) isozymes, such as COX-2, which is markedly elevated in tumors. The suppression of vascular permeability in sarcoma 180 and other solid tumors by COX inhibitors, such as indomethacin and salicylic acid, provides solid evidence for the role of PGs in enhancing vascular permeability (1,19). A PGI2 analogue, beraprost sodium, exhibits a significantly longer half-life *in vivo* (>1 h) compared to PGI2, which only lasts for a few seconds. Tanaka *et al* (41) demonstrated that PGI2 analogues may enhance the EPR effect by 2- to 3-fold, thereby providing a useful strategy for macromolecule delivery.

6. Perquisites for enhanced permeability and retention

Nanoparticles may attain high concentrations within the neoplastic tissue via the EPR effect only if they are able to evade the reticuloendothelial system (RES) and resist renal clearance by virtue of their macromolecular size, thereby remaining in the circulation for ≥6 h (1).

Evasion of the RES. Upon intravenous administration, nanoparticles are rapidly recognized as foreign particles and are opsonized by the adsorption of plasma proteins. The opsonized nanoparticles interact with specific receptors on Kupffer cells in the liver and macrophages in the spleen and are thus rapidly eliminated from the systemic circulation (42). The rapid clearance of the nanoparticles from the systemic circulation by the RES results in a short circulation half-life, which is not adequate to permit the accumulation of the nanoparticles within the neoplastic tissues (15,42,43). In order to prolong the half-life of nanoparticles in the circulation, they have to be converted to stealth nanoparticles, thereby evading opsonization and uptake by the RES cells. Evasion of opsonization is based on the physicochemical concept of steric repulsion, by grafting polyethylene glycol (PEG) residues or polysaccharides onto the nanoparticle surface (43). The presence of such macromolecules creates a 'steric stabilization', which provides a protective hydrophilic layer on the surface of nanoparticles, preventing aggregation between the particles themselves, as well as their interaction with blood components (44). This masking effect confers nanoparticles with the ability to evade the cells of the RES (45,46). PEG is the most widely used material for surface modification, as it is non-toxic, non-immunogenic and has been approved by the United States Food and Drug Administration for oral and parenteral applications in humans (43). PEGylated nanoparticles exhibit a circulation half-life of 2-24 h in mice and rats and as long as 45 h in humans, thus providing the nanoparticles with sufficient time to reach their target tissue (45).

Macromolecular size. The EPR effect is a molecular weight-dependent phenomenon. Molecules exhibiting a molecular weight below the renal clearance threshold are rapidly eliminated from the circulation. However, a drug has to remain in the circulation for ≥6 h to be able to accumulate in the neoplastic tissue by the EPR effect (20). Therefore, the EPR effect is particular to macromolecules with an apparent molecular size of >40-50 kDa (20,47).

7. Concluding remarks

With the rapid emergence of novel nanoparticulate devices, there comes a pressing need for greater precision in delivering drugs to neoplastic cancer cells, whilst salvaging the surrounding healthy tissues. The tumor microvasculature represents the epicenter of the concept of passive targeting of nanoparticles. Mediators regulating blood pressure and vascular caliber may be controlled to shift the balance towards a more inviting tumor environment for nanoparticles. Skeptics point to the fact that passive targeting facilitates the efficient localization of nanoparticles in the tumor interstitium, but cannot further promote their uptake by cancer cells. This uptake may be achieved by actively targeting nanoparticles to receptors overexpressed on target cancer cells. It is our view that the two strategies must be synchronized in order to achieve maximum benefit from future nanodesigned 'magic bullets'.

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