ELSEVIER

Exploiting the enhanced permeability and retention effect for tumor targeting

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Of the tumor targeting strategies, the enhanced permeability and retention (EPR) effect of macromolecules is a key mechanism for solid tumor targeting, and considered a gold standard for novel drug design. In this review, we discuss various endogenous factors that can positively impact the EPR effect in tumor tissues. Further, we discuss ways to augment the EPR effect by use of exogenous agents, as well as practical methods available in the clinical setting. Some innovative examples developed by researchers to combat cancer by the EPR mechanism are also discussed.

Cancer has been, and still remains, one of the most dreaded diseases and a major threat to human life. Most of the conventional cancer chemotherapy – the standard treatment – is not always successful, even after 50 years of research, although lymphocytic leukemia and Hodgkin's lymphoma are treated rather successfully in this way [1]. Conventional chemotherapy delivers the toxic anticancer agent indiscriminately, to tumors or normal organs and tissues. Therefore, we need to devise cancer-selective drug delivery to avoid undesirable systemic side-effects. One way of tackling these problems is to deliver anticancer drugs selectively to the tumor site. Among the most effective strategies, in terms of drug delivery, is exploiting the anatomical and pathophysiological abnormalities of tumor tissue, particularly the tumor vasculature, utilizing the enhanced permeability and retention (EPR) effect [2]. By harnessing this unique characteristic (EPR effect) of solid tumors, the selective delivery of macromolecular anticancer drugs to the tumor site, with pin-pointed accuracy, has become a reality. This approach can be compared to the 'magic bullet' concept put forward by Paul Ehrlich at the turn of the 20th century. This review discusses the concept of the EPR effect and the factors influencing the EPR effect, as well as demonstrating examples of macromolecular tumor targeting and suggesting possible ways to further enhance this effect *in vivo*.

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Enhanced permeability and retention effect: theory, principles and consequence

The theory behind enhanced permeability and retention, pathophysiology and anatomy of tumor vasculature

When tumor cells multiply, cluster together and reach a size of 2–3 mm angiogenesis is induced, to cater for the ever-increasing nutrition and oxygen demands of the growing tumor [3]. This neovasculature differs greatly from that of normal tissues in microscopic anatomical architecture [4]. For instance, the blood vessels in the tumor are irregular in shape, dilated, leaky or defective, and the endothelial cells are poorly aligned or disorganized with large fenestrations. Also, the perivascular cells and the basement membrane, or the smooth-muscle layer, are frequently absent or abnormal in the vascular wall. Tumor vessels have a wide lumen, whereas tumor tissues have poor lymphatic drainage [2,4–7]. This anatomical defectiveness, along with functional abnormalities, results in extensive leakage of blood plasma components, such as macromolecules, nanoparticles and lipidic particles, into the tumor tissue. Moreover, the slow venous return in tumor tissue [8,9] and the poor lymphatic clearance mean that macromolecules are retained in the tumor, whereas extravasation into tumor interstitium continues. This phenomenon, termed the EPR effect, was described by us almost 20 years ago, and is the basis for the selective targeting of macromolecular drugs to the site of solid tumors [2]. It is possible to achieve very high local concentrations of polymeric drugs at the tumor site, for instance 10–50-fold higher than in normal tissue within 1–2 days. More recently,

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2006.07.005

polymer conjugates, micellar or liposomal drugs of anticancer agents, and antibody conjugates are based on this mechanism and the EPR effect is becoming a gold standard of such drug design [10,11]. Interestingly, the EPR effect does not apply to low-molecular-weight drugs because of their rapid diffusion into the circulating blood followed by renal clearance [6,12–14].

Factors affecting the enhanced permeability and retention effect

Vascular endothelial growth factor

Vascular permeability factor (VPF) [15], identical to vascular endothelial growth factor (VEGF), has a significant role in tumor angiogenesis [16,17]. In addition to being a mitogen for endothelial cells, it plays a pivotal role in tumor growth and, perhaps, in metastasis because of its induction of vascular permeability [15]. Increased vascular permeability will lead to an enhanced extravasation of macromolecules. Besides VEGF, the EPR effect is further amplified by numerous other vascular mediators described below and listed in Table 1 [18–24].

Bradykinin and prostaglandins

Bradykinin (BK) and prostaglandins (PGs) play an important role in enhanced vascular permeability in inflammatory and tumor tissue, and thus sustain tumor growth. We extensively studied the role of BK in infection, inflammation and cancer [18–21,25–28], and reported that the signaling cascade upstream of BK is upregulated in tumor compartments and is involved in the EPR effect of tumors [28]. Moreover, overexpression of BK receptors in various human and rodent solid tumors has been observed [27–29].

PGs are synthesized by cyclooxygenase (COX)-1 and -2, and PGE_2 is produced in human and experimental tumor models [30,31]. It was found that the COX inhibitor indomethacin significantly repressed vascular permeability in sarcoma S-180 and other tumor models [18]. Beraprost Na®, the prostacyclin PGI_2 agonist, enhanced the EPR effect 2-fold, which led to a decrease in tumor blood flow of almost 70% [32]. These vascular mediators did not affect the blood flow much in healthy organs (only ~10%), for example the kidney and the liver.

Nitric oxide

Nitric oxide (NO) is a well known mediator of vasodilatation, hypotension, angiogenesis, cell proliferation and extravasation (EPR effect). For instance, NO synthesized from L-arginine by NO synthase (NOS) induces vascular permeability in tumors [18,19,24]. Consequently, inhibition of NO generation, for example using the NOS inhibitor N^{ω}-monomethyl-L-arginine (NMMG), was found to suppress vascular permeability and, thereby, the EPR effect and, hence, tumor growth [18,23,24]. It was found that extravasation, assessed by an intravenous (i.v.) injection of Evans blue, could be suppressed by NO scavengers and NOS inhibitor (see Figure 1), which confirms that NO is responsible for vascular permeability in solid tumors [22,24].

Peroxynitrite and matrix metalloproteinases (collagenases)

Peroxynitrite ($ONOO^-$), which is formed by a rapid reaction between superoxide radical ($O_2^{\bullet -}$) and NO, is extensively produced in tumor and inflammatory tissues, where it activates promatrix-metalloproteinases (proMMPs) [23,33–35]. On the one hand, MMPs are known to facilitate cancer metastasis by

TABLE 1

Factors responsible for the enhanced permeability and retention effect of macromolecules in solid tumors

Anatomical factors
Extensive angiogenesis and high vascular density
Lack of smooth-muscle layer, pericytes, sporadic blood flow → passive dilatation of vessels in the angiotensin-II (AT-II)- induced hypertensive state → more leakage
Defective vascular architecture → extensive leakage
Meager lymphatic clearance → enhanced retention of macromolecular drugs and lipidic particles in the interstitium of tumors
Slow venous return → accumulation of macromolecules in the interstitium
Generation of permeability-enhancing factors as follows
Vascular endothelial growth factor (VEGF, or VPF)
Bradykinin (BK) and/or ^{3}hydroxypropyl BK
Nitric oxide (NO)
Peroxynitrite ($ONOO^-$), a reaction product of superoxide radical and NO
Prostaglandins (PGs)
Matrix metalloproteinases (MMPs) → proMMP is activated by $ONOO^-$
Other proteinases (e.g. kallikrein system) → involved in various protease cascades
Other cytokines (e.g. tumor necrosis factor, interleukin-2) → facilitate enhanced permeability and retention (EPR) effect

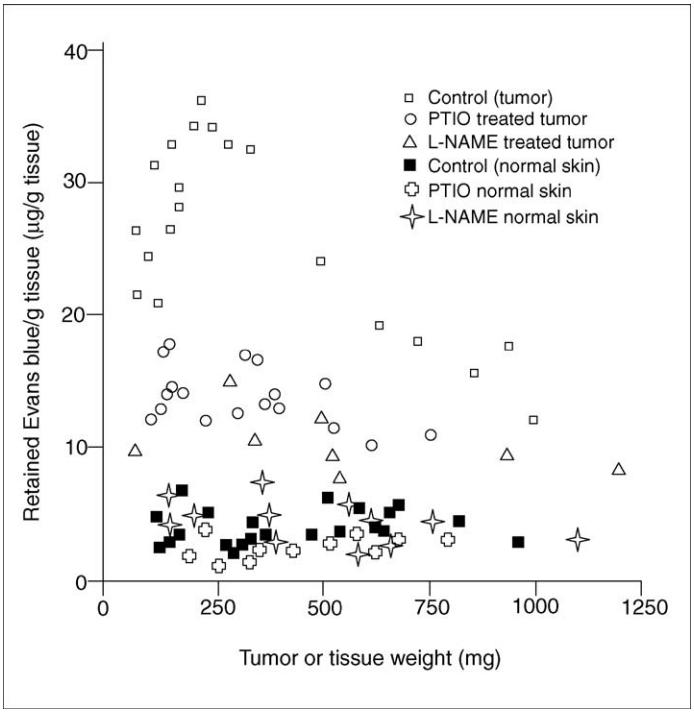

FIGURE 1

Vascular permeability (or enhanced permeability and retention effect) as seen by Evans-blue–albumin complex uptake (quantified by absorbance) in sarcoma S-180 tumors of various sizes in mice. Mice were administered with a nitric oxide (NO) scavenger 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO) orally, or the NOS inhibitor L-N^{ω}-nitro-L-arginine methylester (L-NAME) intraperitoneally (i.p.). Dose: PTIO, 125 mg/kg (×4) in 8 h; L-NAME, 1.05 mg/kg (×4) in 8 h. Note that the enhanced permeability and retention (EPR) effect is suppressed by PTIO and L-NAME (a lower uptake of the Evans-blue–albumin complex, compared with control tumor). Further note that the larger the tumor size the lesser the contribution of NO to the EPR effect, implicating the importance of NO in the early phase of tumor growth. See Refs [18,20] for details.

degrading the extracellular matrix, whereas, on the other hand, they also enhance the EPR effect, which helps to support the growing tumor. The EPR effect was found to be inhibited by many MMP inhibitors [18,23]. Several MMP inhibitors were developed over the past 10–15 years; however, none of their clinical development was successful. The first reason for this failure could be that a fraction of tumor cells remains viable, and can therefore resume growth when the drug treatment is suspended. The second reason could be that MMPs are vital proteases in cellular metabolism, and high doses of MMP inhibitors cause toxicity. This has led to the termination of the development of many anti-MMP drugs.

Contribution of anatomical and microscopic defects of tumor vasculature to the enhanced permeability and retention effect

Recently, it was shown that dextran (as large as 2000 kDa), conjugated to a dye (cascade blue), readily entered and stained the tumor interstitium within 30 min of exposure [36]. A similar result was reported almost 20 years ago and, more recently, by us [2,6]. Lactobacillus bacteria, ~2 μm in size, were found to localize more in tumor tissue than normal tissue after i.v. injection [37]. Similarly, polymeric drugs with a molecular weight above the renal threshold, namely >40 kDa, accumulate in tumor tissue for prolonged time periods [2,6,12,38]. The rate of accumulation of the macromolecules and lipids in tumors is not proportional to their clearance rate because of the impaired lymphatic system [12,13,39–43]. Another prerequisite for the EPR effect is that the plasma concentration of the drug, as measured by the area under the time-concentration curve (AUC), must remain high for >6 h in mice and rats [2,6,12,41].

In other studies on the transport of macromolecules into tumor tissues, it was demonstrated that the vascular pore size of the LS174T tumor, a human colon adenocarcinoma, could be as large as 400 nm [44]. Taken together, these studies indicate that abnormalities and disfunction of tumor vasculature leads to the EPR effect, which can be harnessed to deliver macromolecular drugs that will extravasate into the tumor tissue and stay there for long periods of time. In this context, the term 'passive targeting' does not imply intratumoral retention, in contrast to the EPR effect.

Augmentation of drug delivery to tumors by modulating the enhanced permeability and retention effect

Angiotensin-II-induced hypertension

The vascular density of many, if not all, tumors is higher than that of normal tissues. Further, tumor blood vessels lack a smooth-muscle layer, which plays a vital role in regulating blood pressure and flow. In normal blood vessels, the smooth-muscle layer responds to vascular mediators such as BK, acetylcholine, NO and calcium via receptors on vascular smooth-muscle cells, helping to maintain constant blood flow volume. In normal tissues, when hypertension is induced by infusing angiotensin-II (AT-II) i.v., constriction of the smooth-muscle layer results in higher blood pressure and higher flow rate. Interestingly however, the blood flow volume remains constant [5,45–47]. By contrast, in AT-II-induced hypertension, tumor blood vessels cannot regulate the blood flow volume because of the absence of the smooth-muscle layer. Consequently, blood flow volume will increase in proportion to elevated blood pressure. In tumor-bearing rats, raising the systolic blood pressure by infusing AT-II caused a selective increase in tumor blood flow volume, ~2–6 times depending on the blood pressure attained [5]. Therefore, we anticipated that the induction of the hypertensive state would augment the EPR effect and, hence, the delivery of macromolecular drugs. In fact, when ^{51}Cr-labeled styrene-co-maleic-acid polymer (SMA) conjugated to neocarzinostatin (NCS), known as SMANCS, or ^{51}Cr-albumin was injected i.v. in rats, after the systolic blood pressure was raised from 100 to 150 mmHg using AT-II for 15 min, there was a 1.3–3.0-fold increase in accumulation of macromolecular drugs in tumor tissue [41] (see Figure 2). At the same time, a significant reduction in the amount of drug delivered to healthy organs (e.g. the kidney and the bone marrow) was observed because of vasoconstriction and tighter endothelial gap junctions, which curbed extravasation of polymeric drugs. Similar results were obtained with SMANCS–Lipiodol®, administered under the AT-II-induced hypertensive state, in many solid tumors in patients including hepatocellular carcinoma (HCC), metastatic liver cancer, renal cancer, cholangiocarcinoma, pancreatic cancer, among others [46].

It is known that low-molecular-weight anticancer agents should only be administered at the recommended dose because of dose-limiting toxicity. However, using macromolecular drugs in subjects under the hypertensive state, in animal models as well as in the clinic, it was possible to achieve a >5-fold higher concentration of anticancer drugs in the tumor compared with normotensive conditions, even though the hypertension was maintained only for ~20 min [41,46] (Maeda, H. *et al.* upublished results).

Use of nitric-oxide-releasing agent and angiotensin-converting-enzyme inhibitors

Not only can vasoconstrictors (AT-II) be used to enhance vascular permeability, vasodilators are also suited to increase the EPR effect by widening the endothelial gaps of tumor-feeding arteries. In one such case, in humans, the NO-releasing agent isosorbide dinitrate (ISDN; Nitrol®) was infused into the local tumor-feeding artery using a catheter (local), whereas concomitantly AT-II was injected systemically. This permitted enhanced opening of the feeding artery, and more drug entered the tumor. This technique further enhanced the site-specific delivery of SMANCS–Lipiodol® to the tumor. In this context, PGI_2 agonist (Beraprost Na®) was also found to be useful, as discussed previously [32].

Also discussed earlier, BK is upregulated in many tumors; it is also degraded by angiotensin-converting enzyme (ACE). Thus, it is conceivable that inhibition of ACE will increase the local concentration of BK in the tumor. In experiments, the use of ACE inhibitors, such as temocapril, elevated the BK level and, hence, the EPR effect [26,47]. More importantly, even under normotensive conditions, ACE inhibitors facilitated the increased delivery of macromolecular drugs to tumors [47].

Pro-enhanced permeability and retention effect of anticancer agents affecting blood vessels

While analyzing the EPR effect of macromolecular anticancer agents, it was important to record the influence that the vascular mediators or the drugs themselves had on the EPR effect. We have observed that proinflammatory anticancer agents that generate superoxide radical and NO (or activate proMMP to MMP, for

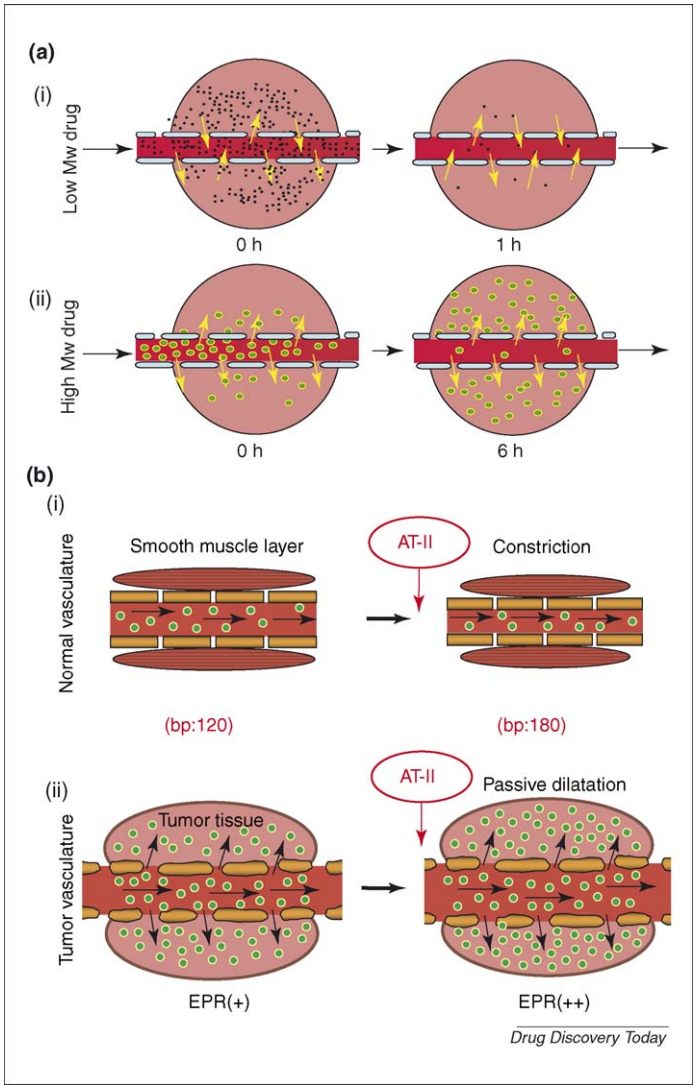

FIGURE 2

Diagrammatic representation of the enhanced permeability and retention effect. (a) Shows the diffusion of a low molecular weight (Mw) drug (black spots) in (i) and a high Mw drug (green spots with yellow circles) in (ii), from the blood vessels into the interstitium of tumor tissue (large pink circles). Note that low Mw drugs can diffuse freely in and out of the tumor blood vessels because of their small size and, hence, the effective concentration of the drug in the tumor diminishes after 1 h when the drug concentration in plasma becomes low (a; i), whereas the high Mw drug cannot diffuse back into the blood stream because of its large size. Thus, there is progressive accumulation of macromolecular drug in the tumor tissues with time by the enhanced permeability and retention (EPR) effect (a; ii). In **(b)**, we show a diagrammatic representation of vascular leakage in response to angiotensin-II (AT-II)-induced hypertension and the subsequent contraction of the smooth-muscle layer. Note that normal blood vessels (b; i) will constrict in the hypertensive state (restricting the extravasation of macromolecular drugs to normal tissues or organs owing to tighter endothelial gap junctions), whereas in tumor blood vessels (b; ii) there will be passive dilatation, because of the absence of a smooth-muscle layer, leading to enhanced extravasation of macromolecular drugs and, hence, augmentation of the EPR effect. Abbreviation: bp, blood pressure (systolic blood pressure is represented in this Figure).

example SMANCS, anthracyclins, mitomycin C and nitrosourea) induce enhanced vascular permeability and, hence, a pro-EPR effect [19,48,49]. In this context, we observed an enhanced EPR effect for SMANCS in treated patients. It was recently reported that certain anticancer drugs, such as doxorubicin, upregulated VEGF, resulting in increased vascular permeability [50].

Advantages of polymer conjugates with regard to the enhanced permeability and retention effect

Prolonging half-life, stealth-character and reduced antigenicity

Because accumulation of macromolecules by the EPR effect is a progressive phenomenon, it is essential that the drugs are stable in plasma for long time periods. In addition to prolonging the half-life in plasma of low-molecular-weight drugs or proteins, polymer conjugation also guides the drugs to their target by the EPR effect [51]. It also confers stealth character and suppresses antigenicity of the proteinaceous drugs, as well as diminishing uptake by the reticuloendothelial system (RES) or macrophages (Mϕ). Therefore, the half-life of polymeric drugs in the blood circulation can be extended greatly [6,52–54].

Immunopotentiation or biological-response modifier

Polymer conjugates, such as SMANCS for instance, have displayed various immunomodulating properties: Mϕ activation [55–59]; induction of interferon-γ production [56,59]; and augmentation of natural killer (NK) cells [57,59], stimulating antitumor immunity to tumors in mice. Similar findings were also reported for pyran copolymer and other anionic polymers that can induce interferons and cytokines [60,61].

Enhancing solubility, cellular uptake and stability

It is well known that many promising drugs cannot be used because of their poor water solubility. The conjugation of such drugs to water-soluble polymers often confers water solubility [53,54,62]. Micellar encapsulation can afford similar benefits [40,63]. Moreover, many proteins and drugs that have poor intestinal absorption can be rendered orally active by conjugation with a hydrophobic polymer in combination with an oily formulation [64]. Further, the stability (and shelf-life) of the drug or protein could be remarkably improved by using an oily formulation [65].

Receptor-mediated drug targeting

Besides EPR-based targeting, we can enhance intracellular uptake of polymeric drugs by utilizing receptor-mediated internalization. To this end, ligands (or targeting moieties) can be attached to the polymer backbone, which will act as a secondary uptake mechanism following EPR-based primary accumulation. Epitope-dependent targeting based on antibody conjugates also initially depends on EPR-based primary targeting [11]. An example of ligand-dependent targeting is galactosamine (targeting moiety) attached to poly[*N*-(2-hydroxypropyl)methacrylamide] (HPMA) copolymer bearing doxorubicin, targeted to the liver (known as PK2) [66]. There are several other examples of receptor-mediated drug delivery reported elsewhere [10,11,67,68].

Patient compliance and quality of life

Owing to the prolonged retention of polymeric drugs by the EPR effect and enhanced plasma half-life, polymer-conjugated drugs require less-frequent administration compared with free drugs, which is a great benefit to patients [69]. One such example is the use of polyethylene glycol (PEG)–L-asparaginase (Oncaspar®) in the

treatment of lymphocytic leukemia [70], or PEG–adenosine deaminase [71].

Limitations and a word of caution on the use of polymer conjugates

With many researchers working with polymer drug conjugates, micellar drugs and nanoparticles (on the basis of EPR effect for targeted delivery), it is essential to be aware of the fate of the polymer used (i.e. its molecular weight, solubility, biocompatibility, biodegradability and clearance from the body). For instance, the basic unit of the polymer used should preferably be smaller than the size of the renal excretion threshold (<40 kDa), or biodegradable. Hydrophobic polymers, if resistant to degradation *in vivo*, are mostly eliminated via the bile, although they occasionally localize in the skin. Therefore, the total amount of polymers to be administered could be important. Some polymers could illicit an immune response or allergic reactions, more so in second or subsequent injections, although these side-effects can be controlled by corticosteroid and antihistamine.

When one devises micromachines or nanocapsules, or drugs encapsulated in carbon nanotubes (CNTs), the ultimate fate and specific site of accumulation must be clearly studied. For instance, many anticancer agents generate oxygen free-radicals in aqueous systems, and CNTs containing such agents, or transition metals, will behave in a similar way to asbestos, which can cause mesothelioma [72,73]. Also, large materials (when administered i.v.) will be preferentially deposited in the lung, because the first-pass effect will capture such particles in the lung owing to physiological, anatomical reasons. However, under the adequate precautions, there are many synthetic and natural polymers that are totally safe materials for drug carriers.

Polymeric drugs in tumor-targeted delivery based on the enhanced permeability and retention effect

The SMANCS–polymer conjugate of copoly(styrene maleic-acid) with neocarzinostatin

The first prototype macromolecular anticancer agent, SMANCS, developed in our group, was approved for clinical use in Japan in 1993. The plasma half-life of SMANCS is ~20 times longer than that of native NCS [2,14,38,46]. Further, NCS increased lipid solubility after conjugation with SMA, which enabled administration of SMANCS together with the lipid contrast agent Lipiodol®. SMANCS–Lipiodol® (injected via the tumor-feeding artery) resulted in drug retention >2000-fold higher in tumor tissue than in blood plasma, and the drug remained in the tumor for several weeks [7,14,38]. Under these circumstances, accurate quantification of the drug (in lipid contrast agent) that accumulates in the tumor becomes possible using an X-ray computed tomography (CT) scan [74]. The uptake of SMANCS–Lipiodol® in the tumor is particularly high because the arterial injection into the tumor-feeding artery benefits from the powerful first-pass effect, in addition to the EPR effect. Consequently, 90% of the patients with HCC treated by this procedure (Seldinger's method) exhibited remarkable tumor regression and a simultaneous decrease in α-fetoprotein levels (a specific tumor marker for hepatoma) [14,38].

Further, SMANCS–Lipiodol®, given intra-arterially under an AT-II-induced hypertensive state, showed a more intense drug uptake and clear tumor staining, and a faster rate of tumor regression compared with administration in the normotensive state [38]. Moreover, it was observed that a lower dose of drug was enough for an adequate therapeutic effect. This was because increased hydrodynamic pressure facilitated the opening up of the gaps between the endothelial cells, and extravasation of the drug occurred at the tumor vasculature only (not in the normal vessels, see earlier section).

PEG-conjugated oxidoreductases

Production of reactive oxygen species (ROS) at the tumor site is another cancer-selective-toxicity approach. One such agent, xanthine oxidase (XO), catalyzes the oxidation of purine and produces ROS, $O_2^{\bullet -}$ and H_2O_2. However, the high binding affinity of native cationic XO to blood vessels causes systemic vascular damage. To overcome this shortfall, XO was chemically conjugated to PEG [75]. Similarly, another ROS-producing redox enzyme, D-amino-acid oxidase (DAO), was conjugated to PEG for tumor-targeting, taking advantage of the EPR effect [76]. When these conjugates were injected (i.v.) into tumor-bearing mice, much higher accumulation of the conjugates was seen in the tumor, as compared with native enzymes. The *in vivo* half-life and the AUC of PEG–DAO increased 2.6- and 2.9-fold, respectively, compared with native DAO. Accumulation of PEG–DAO was found in tumors, but not in healthy organs [76]. The advantage of this method is based on the fact that tumors are highly vulnerable to oxygen-radical stress owing to downregulation of catalase [76], and DAO is an endogenous enzyme and our healthy body tissue can scavenge H_2O_2 effectively (catalase-mediated), in contrast to tumor tissue. Thus, these results demonstrate the EPR effect and the tumor-selective antitumor effect of PEG–XO and PEG–DAO by the generation of ROS, therefore warranting further investigation.

PK1 a HPMA copolymer–doxorubicin conjugate

PK1 is a HPMA polymer conjugated to doxorubicin with a tetrapeptide linker, having an apparent molecular weight of 30 kDa. This polymer conjugate is stable in circulation, but cleaved by cathepsin B following endocytic uptake after EPR-mediated accumulation in tumor insterstitium. By using the polymer conjugated to doxorubicin, a 70-times higher concentration of doxorubicin was found in mouse melanomas, compared with healthy tissues [10,77]. The maximum tolerable dose (MTD) of the conjugate increased ten times, compared with that of the free-drug (non-conjugated). In Phase I trials, the MTD of PK1 was 320 mg/m^2 (doxorubicin-equivalent), which was 4–5-fold greater than the safe dose of free-drug. Despite cumulative doses up to 1680 mg/m^2 (doxorubicin-equivalent), no cardiotoxicity (a side-effect typical of antracyclines) was observed. Antitumor activity in chemotherapy-resistant or refractory patients (including breast cancer) was also seen with the use of PK1 at doses of 80–320 mg/m^2 (doxorubicin equivalent), consistent with tumor-targeting by the EPR effect. PK1 is currently in Phase II trials for breast, colon and non-small-cell lung cancer (NSCLC) [10,77].

PEGylated liposomes: targeting tumor vasculature

Liposomes have been used as carriers for the targeted delivery of anticancer drugs and several of them are already used clinically [78]. PEG-conjugated stealth liposomes have a long plasma half-

life and can avoid uptake by RES. In the clinical setting, liposomal antracycline Doxil® and DaunoXome® were successfully targeted to tumors by EPR. Targeted liposome (PEG–APRPG) conjugated to distearoyl-phosphatidyl-ethanolamine (DSPE) showed enhanced accumulation in tumors *in vivo* (when tested in colon 26 NL-17 carcinoma-bearing mice) [79]. An adriamycin-encapsulated liposome modified with PEG–APRPG showed more-efficient tumor growth-suppression than liposome modified with PEG only [80], indicating that the secondary targeting via APRPG (targeting moiety) can enhance the primary targeting by the EPR effect.

SMA anthracyclines: a new micellar platform technology

Using an amphiphilic SMA polymer, micelles containing doxorubicin or pirarubicin were constructed through a noncovalent interaction between SMA and the drugs [40,63]. The resulting micelles showed high solubility in water and were stable upon lyophilization, freeze–thawing and/or sonication (30 kHz). An *in vivo* antitumor assay of SMA–pirarubicin (at doses of 20 mg/kg in mice bearing sarcoma S-180 tumor) revealed complete tumor eradication in 100% of tested animals, and all survived for >1 year [40]. The free-drug-equivalent concentration of pirarubicin in the tumor after administration of SMA–pirarubicin was 13-times higher than the concentration observed after administration of the free-drug alone. The plasma AUC of SMA–pirarubicin was >200-fold higher than non-conjugated pirarubicin *in vivo*. Similar results were observed with SMA–doxorubicin micelles [63]. This remarkable result can also be attributed to the EPR effect of these micellar drugs.

Conclusion

The EPR effect is commonly observed in most solid tumors, either primary or metastatic in nature. The EPR effect is modulated or mediated by various factors produced by tumor cells, infiltrating leukocytes or even tumor-surrounding normal cells.

Obviously, many vascular mediators, such as BK, PGs and NO, also affect normal blood vessels near tumor tissue. Also, vascular density and microanatomical defects play important roles in this phenomenon. The EPR effect will confer most macromolecular and liposomal or micellar drugs with selective tumor-targeting characteristics, and once delivered to the tumor these macromolecular drugs will remain in the tumor tissues for long periods of time (days to months). Even targeting drugs that are conjugated to monoclonal antibodies will rely on access to tumor tissue, mediated by the EPR effect. The EPR effect can be further augmented by elevating the systemic blood pressure using AT-II. Consequently, the delivery of macromolecular drugs will be facilitated. The next step after EPR-driven tumor delivery will be to access target molecules in tumor cells, after liberation of drugs from their carriers. Intracellular access of drugs to molecular targets is frequently limited by the release rate or uptake rate of macromolecules by endocytosis, or by receptor-mediated uptake into the cells. Diffusion-dependent uptake of drugs can also take place after liberation from the carriers, although at a slower rate. However, dividing tumor cells are known to have a more active endocytic uptake than non-dividing normal cells.

Thus far, the EPR effect is one of the most common characteristics that differentiate a tumor from normal healthy tissue. A judicious choice of drug carriers (which can exploit the EPR effect) and targeting moieties (which can guide the drugs to, or into, the cells) will be a good combination for better therapy. Augmentation of EPR-dependent uptake to a tumor is further possible by the use of an AT-II-induced hypertensive state, and this will be applicable for a wider range of solid tumors.

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