Chapter 4
Enhanced Vascular Permeability in Solid Tumors: A Promise for Anticancer Nanomedicine

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Abstract Tumor vessels are structurally and functionally abnormal. The heterogeneity, irregularity as well as the leakiness of the tight junctions of tumor vasculatures are potential targets for anti-cancer therapy. Early in 1980s Yasuhiro Matsumura and Hiroshi Maeda described the enhanced permeability and retention (EPR) effect. EPR effect is a unique tumor vascular phenomenon; central to it is the abnormal structure and function of endothelial tight junctions in the developing irregular tumor vasculatures that allows for selective concentration of Nanosize molecules in tumor tissues. Nanomedicine that emerged in parallel to the recent advance in Nanotechnology can concentrate in tumors due to EPR effect. The main advantage offered by the EPR effect for Nanomedicine is superior pharmacokinetics with prolonged drug circulatory half-life and improved biodistribution to tumor tissues. The EPR effect served as the bridge through which Nanotechnology found appropriate application in treatment of cancer. In this chapter, we discuss the principles and factors involved in EPR mechanism, the opportunities, and the challenges that face this cancer treatment strategy.

Keywords EPR effect • Tight junctions • Nanomedicine • Half-life • Targeted anticancer therapy • Macromolecular drugs • Tumor model • In vivo • Biodistribution

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4.1 Introduction

Cancer remains a major challenge to the human kind, both the scientific community and cancer patients. By 2011, more than 3.8 million research articles have been published with cancer as a keyword. Conversely, WHO has predicted that global cancer mortality will increase by 50% by the year 2020, claiming the lives of 50 million patients worldwide (World Health Organization 2003). Clearly, this indicates the need for the scientific community to devise new strategies for more effective cancer management.

A major focus in cancer research concerns the unique characteristics of tumor cells or tumor tissues. Understanding these characteristics will aid development of strategies for selective destruction of abnormal cancer cells without harm to normal structures and hence less toxicity to the patients. Tumor vasculature is an ideal target for such strategies because it demonstrates extensive abnormalities clearly defining them from vessels in normal tissues or organs. Such features of vascular abnormalities rely largely on the alteration of the properties of tight junctions. Compared with the vasculature of normal tissue where the endothelium forms a continuous monolayer and diffusion of plasma solutes or extravasation of inflammatory cells is controlled, to a considerable extent, by tight junction permeation (Satoh et al. 1996). The tumor vasculature is markedly disorganized, heterotypic and highly permeable to solutes and potential metastatic cells (Bazzoni and Dejana 2004). Subsequently, tumor vasculature exhibit different fluid and molecular transport dynamics to meet an ever-increasing demand for nutrients and oxygen to the cancer cells. Among these hallmark characteristics is the enhanced permeability and retention (EPR) effect of macromolecular agents in solid tumors, or the EPR effects, which was described more than 20 years ago (Noguchi et al. 1998; Matsumura and Maeda 1986; Iwai et al. 1984). By means of the EPR effects, accumulation of macromolecules at the interstitium of tumor tissues is facilitated. One can take advantage of this macromolecular accumulation, via the EPR effect, for the delivery of macromolecular drugs or Nano-carriers. In this chapter, we will first describe the basic mechanism and the factors that contribute to it, followed by the strategies that can be used to further enhance this effect in solid tumors and finally discuss the challenges that face this cancer treatment strategy.

4.2 Historical Prospective

Paul Ehrlich at the turn of the twentieth century coined the term “magic bullet”. Even though the magic bullet was coined to describe a specific antibiotic to target disease causing organism, the term caught the attention of cancer researchers, who sought methods to translate this concept in cancer treatment. In 1971, Judah Folkman described in the *New England Journal of Medicine* how survival of tumor tissues was dependent on its ability to develop its own new blood supply (Folkman 1971).

This finding established the first steps towards exploiting tumor vasculature for anticancer treatment. In the same year Folkman also described a factor that can enhance tumor vascular development which is now known as Vascular Endothelial Growth Factor (VEGF) (Folkman et al. 1971). In 1973, Hans-Inge Peterson et al. conducted a systemic study to evaluate the uptake and later retention of labeled albumin and fibrinogen in a transplantable rat tumor compared to various normal tissues of the rat. They found high uptake and retention of both proteins in tumor as compared to normal tissues and attributed this phenomenon to high tumor capillary permeability for large protein molecules (Peterson and Appelgren 1973). In early 1980s Hiroshi Maeda invented the first known anticancer Nanomedicine Styrene co-maleic acid conjugated NeoCarzinoStatin (SMANCS). The drug design was mainly influenced by the then newly developing theory of enhanced tumor permeability (Iwai et al. 1984). Maeda and his colleagues further studied this phenomenon and extensively analyzed the different factors that contribute to it for over 20 years. Maeda's EPR principle now constitutes the golden standard for the design of many anticancer Nanocarriers.

4.3 Role of Tight Junction in the Vasculature

The main functions attributed to tight junctions are the regulation of the paracellular permeability or barrier function and the formation of a paracellular seal or fence preventing diffusion of lipids and proteins to maintain cell polarity. Evidence for the barrier functions of tight junctions was shown by early electrophysiological approaches and tracer barrier assays, suggesting the existence of paracellular aqueous pores or channels within the tight junctions (Gumbiner 1993; Diamond 1977; Claude 1978). Additional studies have shown the large contribution of the paracellular permeability to ions and solutes (perm-selectivity), compared to the transcellular transport in epithelial cells and endothelial cells, is largely dependent on the nature of the cells involved and can be modified in an organ-specific manner (Van Itallie et al. 2003). Movement of material across cell membranes occurs in an energy dependent manner and involves a specific channel, pump or transporter. In contrast, the paracellular transport results from the passive movement of material down an electrochemical gradient created by the active transcellular transport or by an external driving force (Van Itallie and Anderson 2006).

Tight junctions are intercellular topical junctional complexes found in epithelial and endothelial cells (Gow et al. 1999). They are one of the two main adhesive structures responsible for cell-cell contact along the lateral membrane in endothelial cells, the other being adherens junctions. Whereas the tight junctions control the paracellular pathway, the adherens junctions maintain cellular proximity. In addition to cell-cell adhesion, another type of junction, the gap junction mediates cell-cell communication (Yin and Green 2004; Perez-Moreno et al. 2003; Matter et al. 2005; Farquhar and Palade 1963). Tight junctions in the endothelium control different features of vascular homeostasis and show considerable variability across the

vascular tree (Bazzoni and Dejana 2004). The variability of the composition of the tight junctions affects the permeability to plasma solutes as well as leukocyte extravasation and infiltration into areas of inflammation. In large arteries, tight junctions are abundant and well developed, creating a dynamic seal characterized by a low permeability. Within the microvasculature, tight junctions are more abundant in the arterioles compared to the capillaries, but both display high transendothelial resistance (Aird 2007). In contrast, post-capillary venules display poorly organized tight junctions which allow dynamic trafficking of circulating cells and plasma proteins (Aird 2007). The tight junction permeability is also influenced by the abundance of receptors such as histamine, serotonin and bradykinin at the site of extravasation (Aird 2007). The abundance of tight junctions as well as their structural organization and composition has been implicated in the establishment of tightly regulated barriers such as the blood brain barrier (BBB) or blood retina barrier (BRB) where a strict control of the fluid dynamic is required to restrict the transport of solutes (Huber et al. 2001). The blood brain barrier has involved highly specialized endothelial cells. Tight junctions separate the apical and the basal membrane domain leading to polarization of the endothelial cells and restriction of the paracellular pathway (Coisne and Engelhardt 2011). Disruption of the structure and organization of the blood brain barrier tight junctions has been implicated in development and propagation of various tumors (Feng et al. 2011a; Fazakas et al. 2011; Davies 2002).

4.4 Tumor Vascular Permeability, the EPR Concept

As tumor cells reach the size of 150–200 um, they start developing their own blood supply and become dependent on neovasculature for their nutritional and oxygen supply (Wu et al. 1998). Normal vasculature networks consist of arterioles, capillaries and venules and form a well-organized network with dichotomous branching and hierarchic order (Herbert and Stainier 2011). Newly formed tumor vessels are usually abnormal in form and architecture. They are dilated, saccular, poorly aligned and heterogeneous. Tumor vessels have defective endothelial cells with wide fenestration, lacking smooth muscle layer, or enervation with wider lumen, and impaired functional receptors for angiotensin II. Furthermore, tumor tissues usually lack effective lymphatic drainage (Noguchi et al. 1998; Folkman et al. 1971; Folkman 1971). Interestingly, in brain tumors similar to solid tumors, blood vessels characteristically lose their blood-brain barrier properties resulting in an increase vascular permeability (Liebner et al. 2000). All these factors lead to abnormal molecular and fluid transport dynamics especially for Nanosize anticancer drugs. The EPR effect is even more pronounced by pathophysiological factors involved in enhancement of extravasation of macromolecules (larger than 7 nm) in solid tumor tissues. For instance, vascular endothelial growth factor VEGF, bradykinin, nitric oxide/peroxynitrite, prostaglandins, tumor necrosis factor and others (Wu et al. 1998;

Senger et al. 1983; Peterson and Appelgren 1973; Matsumura et al. 1988; Maeda et al. 1996; Folkman et al. 1971; Folkman 1971; Doi et al. 1996, 1999). The EPR effect can be clearly demonstrated in rodent tumors by the intravenous injection of Evans blue dye (EBD), which complexes with albumin and produces an intense blue color in tumor tissues in great contrast with normal colored surrounding tissues (Matsumura and Maeda 1986). Sat and Duncan have used HPMA copolymer-doxorubicin conjugate (PK1) as a probe to investigate the extent of the EPR effect in different tumor models, and found that many mouse and human xenograft tumors displayed clear tumor size-dependent EPR-mediated targeting (from ~20% dose/g of tumor tissue in small tumors to 1–5% dose/g in large tumors). This result was consistent with their previous reports describing the accumulation and retention of ^{125}I-labelled HPMA copolymer in B16- F10 and sarcoma 180 tumors (Seymour et al. 1995; Duncan 1999). Ohkouchi K et al., used a system composed of a Walker 256 solid tumor with a supplying artery and a draining vein to study the extravasation characteristics of mitomycin C-dextran conjugates, where they were able to show the enhanced vascular permeability of macromolecular drugs in solid tumors (Ohkouchi et al. 1990).

To accomplish the EPR effect-based cancer drug targeting, the plasma concentration of the drug, generally measured by AUC, must remain high, preferably for more than 6 h (Matsumura and Maeda 1986). Consequently, extravasations into tumor tissue increase progressively with time in a matter of several hours or days. Subsequently, the release of the active component or principle from Nano – carrier would proceed in tumor tissue to attain therapeutic concentration. For example, SMANCS in Lipiodol (SMANCS/Lipiodol) when administered interarterialy, is cleared very slowly from tumor (clearance takes several weeks). The activity of SMANCS was detected at 20–30 μg/g tumor tissues even 2–3 months after arterial injection of 1 mg/ml (SMANCS/Lipiodol) (Konno et al. 1984). This remaining activity was more than 100 times of the minimal inhibitory concentration against tumor cells in culture (i.e. SMANCS exhibited a minimum effective concentration below 0.05 ng/ml). This remarkable tumor selective accumulation of SMANCS resulted in unprecedented antitumor effect in treating patients with hepatocellular carcinoma in Japan.

4.5 Factors Contributing to Tumor Vascular Permeability

In 1907 E. Goldmann (1908) wrote the following in Lancet describing tumor vasculature “The normal blood vessels of the organs in which the tumor is developing are disturbed by chaotic growth, there is a dilatation and spiraling of the affected vessels, marked capillary budding and new vessel formation, particularly at the advanced border”. Essentially this phrase by this early pioneer summarizes our current knowledge of the gross vascular abnormalities in many human tumors. Following are the main factors that are attributed to these vascular abnormalities;

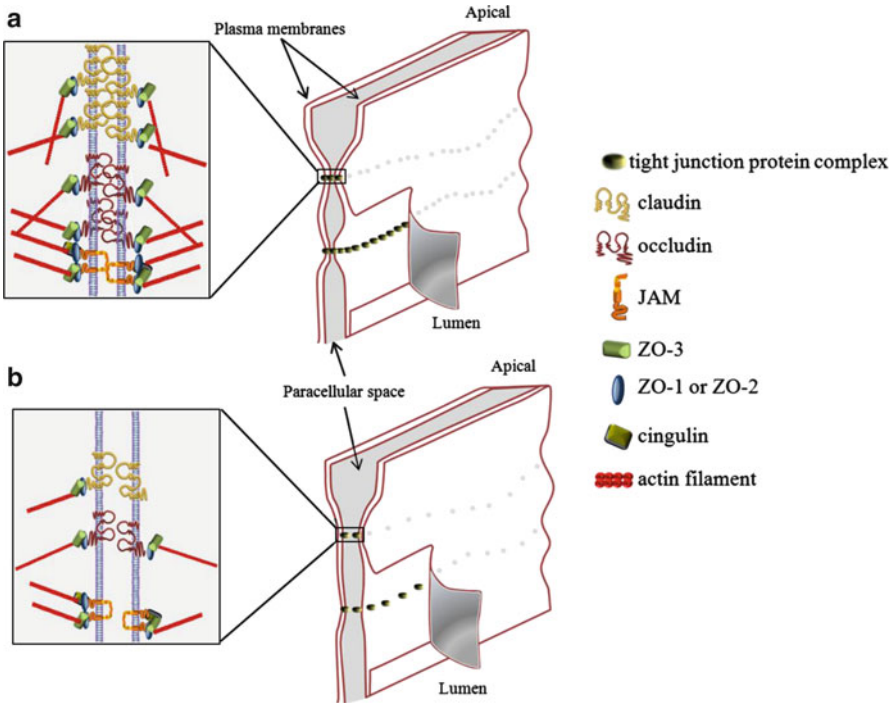

Fig. 4.1 Schematic representation of the tight junction organization between endothelial cells from a normal blood vessel (**a**) and from a tumor associated blood vessel (**b**). Tight junctions are the most apical structure and govern the paracellular permeability of endothelial cells. They are composed of both transmenbrane proteins like claudins, occludin and junctional adhesion molecules (*JAMs*), and intracellular molecules implicated in scaffolding, cytoskeletal attachment, trafficking and cell signaling. In tumors, the localization of these proteins at the endothelial tight junction is affected either by a decrease expression, increase degradation or mislocalization leading to increase permeability of the tight junction

4.5.1 Alteration of Tight Junction in Tumor Vessel

Tight junctions are the most apical structure and govern the paracellular permeability of endothelial cells (Tsukita et al. 1996). Aberration of the tight junction functions is a key event during neoangiogenesis of the tumor and may further promote the formation of metastases (See Fig. 4.1). There are more than 40 proteins identified within the tight junctions (Yamazaki et al. 2008; Tsukita et al. 2008; Schneeberger and Lynch 2004), and the interactions and assembly between these components remains largely unknown. Tight junctions are composed of both transmenbrane proteins like claudins, occludin (Furuse et al. 1993) and junctional adhesion molecules (JAMs) (Bazzoni 2003), and intracellular molecules implicated in scaffolding, cytoskeletal attachment, trafficking and cell signaling (González-Mariscal et al. 2003). Several cytokines involved in cancer cell proliferation and metastasis (Wu and Zhou 2010;

Table 4.1 Modification of tight junction protein complex in tumour associated endothelium

Cancer type		Protein	Change in expression	References
Gliosbalastoma	Hyperplastic vessel	Claudin-5, occludin	Down	Liebner et al. (2000)
Glioblastoma	Interendothelial junction	Claudin 1	Down	Liebner et al. (2000)
Angiosarcomas		Claudin-5	Down	Miettinen et al. (2011)
Hemangioendotheliomas		Claudin-5	Down	Miettinen et al. (2011)
Glioblastoma multiforme		Claudin-3	Down	Wolburg et al. (2003)
Astrocytoma		Occludin	Down	Papadopoulos et al. (2001)
Astrocytoma		ZO-1	Down	Sawada et al. (2000)
Melanoma		Claudin-1	Down	Cohn et al. (2005)
Hepatocarcinoma	Sinusoidal endothelium	Claudin-5	Down	Sakaguchi et al. (2008)
Endometrial atypical hyperplasia		Claudin-3, -4	Up	Pan et al. (2007)
Endometrioid adenocarcinoma		Claudin-3, -4	Up	Pan et al. (2007)
Acute Leukemia		Occludin, ZO-1, claudin-5	Down	Feng et al. (2011b)
Testicular carcinoma		ZO-1 and Z0-2	Down	Fink et al. (2006)

Lichtenberger et al. 2010; Hosoda et al. 2011) affect the expression of proteins essential for the tight junction barrier function in endothelial cells (Table 4.1). Among those, hepatocytes growth factor/scatter factor (HGF/SF) is a known angiogenic cytokines associated with tumor growth and metastasis (Junbo et al. 1999; Gupta et al. 2008). HGF/SF decreases the expression of occludin and promote a decrease of the transendothelial resistance and increase of the paracellular permeability (Jiang et al. 1999; Martin et al. 2002). Additional studies performed with different cytokines such as Interferon γ (Oshima et al. 2001), Tumor necrosis factor (TNF) -α (Wachtel et al. 2001) and VEGF (Wang et al. 2001) were also associated with a decrease in the expression of occludin and affected the transendothelial resistance. Most of the studies performed to determine the role of occludin were done in epithelial cells, nevertheless occludin knock-out mice have a complex phenotype, but they do not appear to have a defect of the intestinal transport or barrier function (Saitou et al. 2000; Schulzke et al. 2005). A compensatory mechanism could be involved as tricellulin, a tight junction protein localized at tricellular junctions, may provide functional redundancy (Ikenouchi et al. 2005). Interestingly, the expression of occludin in the endothelium correlates with the number of the claudin strands (Saitou et al. 1997), the permeability and homeostasis along the vascular tree (Hirase et al. 1997).

Furthermore, additional studies showed that HGF/SF also decreased the expression of other proteins involved in the endothelial tight junction complex such as ZO-1, claudin-1, promoting a decrease of the transendothelial resistance and stimulating

the invasion of a metastatic breast cancer cell line MDA-MB-231 (Martin et al. 2002). While ZO-1 appears to be critical for claudin localization and initiation of claudin polymerization (Itoh et al. 1999), claudin-1 is a member of the large PMP22/EMP/MP20/claudin mammalian superfamily. This family of proteins has emerged as the most critical protein complex for defining tight junction selectivity. Claudins are essential for the correct assembly and functions of the tight junctions (Krause et al. 2008). Immunohistological analysis performed on hepatocellular carcinoma specimens showed a decrease expression of the endothelial tight junction protein claudin-5. The protein is found in larger amount in the endothelial cells (Tsukita et al. 2001). In claudin-5 deficient mice, a size-selective increase in the permeability of the tight junctions in blood vessels was observed with the blood brain barrier appearing to be leaky for small size particles (<800 Da) but no deficiency was observed for the transportation of larger molecules, suggesting that claudins can create variables pore sizes (Nitta et al. 2003). Moreover in vitro work showed a selectivity of the porosity depending on the claudins expressed (Van Itallie et al. 2008). Claudin-5 expression was found to be down-regulated in hepatocellular carcinoma and this may promote leakiness of blood vessels (Sakaguchi et al. 2008). In cutaneous melanoma, a decrease of claudin-1 expression in the endothelium was associated with the acquisition of the metastatic phenotype (Cohn et al. 2005). In glioblastoma multiform, the increase in microvascular permeability correlates with a modification of the ratio of expression of proteins involved in tight junction organization. Namely, claudin-1 expression was decreased while claudin-5 expression was not changed; such modification resulted in the alteration of tight junction particles distribution (Liebner et al. 2000).

All together, these data suggest the implication of the tight junction in the development and propagation of cancer. As the molecular mechanism and the structure of the tight junction of the tumor vasculature are progressively understood, these studies highlight the specificity of the tight junction components involved and their potential as a new target for drug delivery.

4.5.2 Anatomical Irregularities of Tumor Neoangiogenesis

The increased vascular density has been repeatedly documented in animal models; however, its role in human tumors has been debated. Many investigators have established the presence of high vascular density in human cancers and correlated the vascular density with tumor metastasis, which can be linked to poor patient outcome. In the first study of this type, Weidner et al. (1991) showed a direct correlation between the vascular density (number of vessels per high-powered field) and the likelihood of metastasis in human breast cancer patients. This finding is not limited to breast cancer but has been extended to several other tumors, including carcinoma of the prostate (Weidner et al. 1993; Brawer 1996), lung (Yamazaki et al. 1994; Angeletti et al. 1996), stomach (Maeda et al. 1995), cervix (Wiggins et al. 1995), ovary (Hollingsworth et al. 1995), and in squamous cell carcinoma of the

head and neck (Gasparini et al. 1993). Thus, for many tumors, increased vascular density is indicative of increased metastasis and decreased survival. High vascular density can ensure continuous blood supply to the rapidly growing and metabolically demanding tumor cells. Further it provides a channel for communication between the external body environment with its cellular and chemical signals (e.g. macrophages, and growth factors). Eventually tumor cells utilize this extensive vascular network to extravasate into the blood stream in order to establish secondary niche in distant organs. Interestingly, a recent study shows that inhibition of claudin-5 expression suppresses angiogenesis both in vitro and in vivo in a retinal neovascularization model (Chen et al. 2011), suggesting a role of claudin-5 in promoting neovascularization and more specifically, sprouting of blood vessels during development and in pathological conditions(Chen et al. 2011). This study suggests that regulation of claudin-5 expression and localization by post translational modification involving the Wnt pathway, a pathway known to promote tumor development (Dejana 2010; Giles et al. 2003), potentially affects the permeability of the tight junction, but may also promote tumor angiogenesis and vascular density.

Even though this hypervasculature seems to be to the advantage of rapidly growing tumor cells, it can be utilized to deliver anticancer drugs with high affinity to tumor cells. In addition to high vascular density in tumor tissues, vascular pore size in tumor vasculatures exhibits drastic difference from normal blood vessels. Yuan et al. (1995) measured the size of tumor vessels pores in LS 147 human colon adenocarcinoma implanted in dorsal skin fold chamber in severe combined immunodeficiency (SCID) mice and its relation to macromolecular transport into tumor tissues. They demonstrated that the tumor vascular pore could be as large as 0.4 um in diameters. In another study by Hashizume et al. (2000) using electron microscopy, they were able to identify structural abnormalities in the endothelium of tumor blood vessels due to intercellular openings having a mean diameter of 1.7 μm (range, 0.3–4.7 μm) and transcellular holes with a mean diameter 0.6 μm in mouse mammary carcinomas. These large vascular pores can, in part, explain the ability of tumor cells of several um diameter, to squeeze itself in its journey for metastasis.

Fortunately, this large pores can provide selective targeting opportunity for Nano-size drug carriers (7–100 nM), as carriers with this size will escape clearance through globular filtration, which have a threshold of 5–7 nm. Thus, they can concentrate into tumor tissues with its wide fenestration more preferentially than small size molecules of classical anticancer chemotherapy. The third element in EPR anatomical abnormality triad is the lack of functional tumor lymphatics. According to EPR theory, molecules of size larger than 5–7 nm cannot pass through the renal glomerular cells, hence it can concentrate preferentially where the endothelial gaps in small blood vessels allows for its extravasation. This means tumor tissue is a prime target for this concentration due to the wide gaps present in its ill-developed vasculature as described above. Further, the lack of functional lymphatics will lead to the accumulation (retention) of these extravasated Nanosize molecules. This notion of the absence of lymphatics in tumor was derived from the long held belief that tumors lack lymphatic vessels. In 1955 Irving Zeidman et al. injected

radiolabeled gold and berlin blue dye tracer substance into the same afferent lymphatic that previously carried VX-II tumor-cell emboli to the lymph node, thereby assuring injection of the proper lymphatic vessels. This experiment showed that tumor cells developed by the tumor emboli injection did not take any of the tracers, even under high pressure injection to exclude the presence of occluded or collapsed blunt end lymphatic (Zeidman et al. 1955). However, this long held view was challenged recently, partially due to the development of new techniques to trace and identify lymphatic endothelial cells. These include molecules such as lymphatic vessel endothelial hyaluronan receptor-1, Prox-1, podoplanin, and vascular endothelial growth factor receptor (VEGFR)-3 (Wigle and Oliver 1999; Soker et al. 1998; Breiteneder-Geleff et al. 1999; Banerji et al. 1999). In addition, vascular endothelial growth factor (VEGF)-C and -D, which are members of VEGF family, have been reported as lymphatic-specific growth factors (Orlandini et al. 1996; Joukov et al. 1996).

Despite the success of the current research strategies in identifying the presence of specific tumor induced lymphatic endothelial cell growth, the functionality of these lymphatic vessels remains essentially unproved in most experimental animal studies as well as in human tumor tissues specimens (Pepper 2001; Padera et al. 2002).

4.5.3 Vascular Endothelial Growth Factor (VEGF)

VEGF was first named vascular permeability factor by Dvorak because of its potent stimulatory effect on the permeability of the tumor microvasculature (Senger et al. 1983). Leung et al. (1989) coined the term VEGF to describe a mitogenic factor that selectively stimulated endothelial cell proliferation and angiogenesis. This was confirmed by Connolly et al. (1989), who reported that vascular permeability factor was mitogenic for endothelial cells and had the capacity to stimulate vascular proliferation. Recently, the VEGF family of growth factors has expanded with the addition of four new molecules: placenta growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D (Yamada et al. 1997; Olofsson et al. 1996; Maglione et al. 1991; Kukk et al. 1996). VEGF-C and VEGF-D have a specific lymphatic endothelial cells growth stimulatory role as described earlier (Orlandini et al. 1996; Joukov et al. 1996). VEGF comprises four main isoforms produced by alternative splicing of mRNA: VEGF-121, VEGF-165, VEGF-189, and VEGF-206 (Wei et al. 1996; Ferrara and Davis-Smyth 1997). VEGF is the major player in neoangiogenesis, both in physiological wound healing or in support of tumor growth.

VEGF stimulates the migration and proliferation of arterial, venous, and microvascular endothelial cells as well as angiogenesis in vivo and in vitro (Plouet et al. 1989; Leung et al. 1989; Ferrara and Davis-Smyth 1997; Connolly et al. 1989). In addition, VEGF promotes the balanced degradation of the extracellular matrix around the sprouting endothelium by inducing the expression of urokinase-type plasminogen activator, tissue type plasminogen activator (Pepper et al. 1991;

Folkman 1990) and interstitial collagenase (Unemori et al. 1992). By enhancing the permeability of venules to circulating proteins including fibrinogen, VEGF is believed to facilitate the perivascular deposition of fibrin, which further potentiates angiogenesis (Dvorak et al. 1995). Through its capacity to induce nitric oxide, VEGF may also mediate the vasodilation and increased blood flow that precede angiogenesis.

Though VEGF function is indispensable to wound healing to support new tissue formation after discontinuation of various tissue layers due to injury, tumor cells have exploited this mechanism for its own growth and spread. Masood et al. measured the VEGF level of expression in 10 different human cell lines in comparison to the nil level of expression expected in stable cells. They found that most tumor cells overexpress VEGF with the range of 419–1,476 pg/10^6 cells. Further they demonstrated that VEGF can function as tumor growth factor, where its inhibition can result in tumor cell growth inhibition (Masood et al. 2001). In addition, VEGF increases the barrier permeability of endothelial cells through destabilization of the intermingled adherens junction and tight junction, characteristic of endothelial cells. VEGF triggers endocytosis of VE-cadherin present in adherens junctions (Gavard and Gutkind 2006), as well as occludin in tight junctions through phosphorylation by activation of protein kinase C (PKC) (Erickson et al. 2007; Harhaj et al. 2006) and ubiquitination (Murakami et al. 2009). VEGF as such can be considered as the most prominent player in neoangiognesis development as well as its enhanced *leakiness*. Currently there are over 60 angiogenesis inhibitors in clinical trials for various cancer treatments (Lenz 2005; van Moorselaar and Voest 2002).

4.5.3.1 Other Mediators Involved in Enhanced Tumor Permeability

Besides VEGF, the EPR effect is further amplified by numerous other vascular mediators which include angiotensin II (AT II), bradykinin (BK), nitric oxide (NO), peroxynitrite ($ONOO^-$), matrix metalloproteinase (MMP) (or collagenase), prostaglandins (PGs), among others (Wu et al. 1998, 2001; Suzuki et al. 1981; Okamoto et al. 2001; Matsumura et al. 1988; Maeda et al. 1996). As discussed earlier, tumor blood vessels are deranged and as such they lack smooth muscle layer resulting in its lack of response to vasoconstrictors. Therefore, administration of a vasoconstrictor such as AT II that affects normal vessels and increases blood pressure would be expected to have no effect on tumor vessels. However, hypertension, induced by AT II, would have mechanical effects and cause dilation of tumor vasculature in a passive manner. Hori et al. (1991) showed clearly in a window model of solid tumor that some tumor vessels cannot be seen under normotensive conditions but can be visualized when an AT II induced hypertensive state is generated. They showed that apparently avascular tumor tissue actually does have vessels but that they are visible or functional sporadically, e.g. once every 15 or 25 min. However, the absence of smooth muscle in tumor vessels accounted for a three- to fivefold increase in blood flow volume under conditions of induced hypertension, when systolic pressure increased from 100 to 160 mmHg by infusion of AT II, which consequently enhanced

macromolecular drug delivery. In contrast to macromolecular drugs, when [^{14}C] methylglucose, a representative low-molecular-weight drug mimic, was studied under hypertensive condition, accumulation of this agent in tumor was much less than that of polymeric drugs and lasted no longer than 10 min (Li et al. 1993). Such low-molecular weight drugs seem to be washed out rapidly into the general circulation and are excreted via the urine.

In a converse approach, vasodilators, such as the NO-releasing agent isosorbide dinitrate (ISDN; Nitrol), were utilized to enhance the EPR effect via widening the tumor-feeding artery. This result was accomplished by local arterial infusion of ISDN by catheter (Greish et al. 2003; Greish 2007).

Another vascular mediator that greatly influences the EPR effect is bradykinin, which induces intense pain as well as increases vascular permeability. The increase of vascular permeability is associated with a down-regulation of the expression of the tight junction proteins, claudin-5, ZO-1 and occludin and a rearrangement of F-actin in a blood tumor barrier model (Liu et al. 2008). Furthermore, bradykinin cross-talks with prostaglandin and NO, resulting in greater vasodilatation as well as angiogenesis. A significant activation of the bradykinin generating cascade in the tumor compartment was reported, as well as [^{3}hydroxyprolyl] bradykinin. Further, BK was found to be involved in the accumulation of malignant ascetic and pleural fluid (Matsumura et al. 1988, 1991; Maeda et al. 1988). Angiotensin-converting enzyme (ACE) inhibitors such as enalapril and other similar agents can inhibit degradation of bradykinin in vivo and lead to higher bradykinin concentrations at sites of tumor and infection, because of an amino acid sequence homology to that of bradykinin near the C-termini. Consequently, ACE inhibitors did enhance the EPR effect (Matsumura et al. 1988, 1991; Hori et al. 2000) mediated by either bradykinin or NO. Therefore, increasing the local concentration of bradykinin by means of ACE inhibitors, and thereby improving tumor-selective delivery of macromolecular drugs would be possible. Interestingly, ACE inhibitors were found to beneficial on treatment of hepatocellular carcinoma (Noguchi et al. 2003) and prostate cancer (Uemura et al. 2005).

Another important mediators for EPR effect are prostaglandins (PGs) particularly PG_{E2}, generated via cyclooxygenase isozymes (COX 2), which is markedly elevated in inflammatory and cancer tissues. These increased levels of PGs can also enhance vascular permeability in solid tumor, as evidenced by significant suppression of vascular permeability in sarcoma 180 and other solid tumor models by the COX inhibitors indomethacin and salicylic acid (Wu et al. 1998; Maeda et al. 1996). It was found that a PG_{I2} analogue (beraprost sodium) with a much longer in vivo half-life (about 30 min vs. 3 s for PG_{I2}) was useful for the delivery of macromolecules (Tanaka et al. 2003); although a therapeutic advantage of beraprost sodium needs to be demonstrated. Prostaglandin E2 reverses the effect of the epidermal growth factor in epithelial cells and increases the permeability (Flores-Benitez et al. 2009).

Another potential modulator of the EPR effect is hydrogen peroxide. The role of hydrogen peroxide in regulating vascular permeability is currently attracting the interest of many researchers. Several studies have shown that H_2O_2 is involved

in the increase of the vascular permeability in various types of cells (Meyer et al. 2001; Jepson 2003). Hydrogen peroxide increases paracellular permeability by affecting the expression and localization of occludin and ZO-1 (Lee et al. 2004). Drummond et al. (2000) have discovered a role of hydrogen peroxide in transcriptional and posttranscriptional regulation of endothelial NO synthases expression by endothelial cells. Direct addition of 100 and 150 mmol/l H_2O_2 caused increases in bovine aortic endothelial cell eNOS mRNA that were time and concentration dependent (i.e. 3.1- and 5.2-fold increases), and elevated eNOS protein expression and enzyme activity, accordingly. In other studies, it had been found that elevated levels of H_2O_2 cause calcium dependent release of NO from the endothelium and potassium channel-dependent relaxation of vascular smooth muscles (Weir and Archer 1995; Yang et al. 1999). In addition, H_2O_2 was reported to stimulate multiple forms of vascular phospholipases and directly modify lipids to species that are vasoactive (Rao et al. 1995; Natarajan et al. 1998). Cseko et al. (2004) proposed that H_2O_2 in a concentration dependent manner activates several endothelial and smooth muscle pathways, resulting in biphasic changes on the diameter and myogenic tone of isolated skeletal muscle arterioles. The constrictions induced by H_2O_2 are mediated by endothelial PGH2/TxA2, whereas the dilations are caused primarily by the activation of both endothelial NO synthase and various Kþ channels in vascular smooth muscle cells. It seems plausible that exogenous administration of H_2O_2 upstream into a tumor feeding artery could enhance the anticancer drug delivery, similar to the effect produced by ISDN, however this needs to be verified. Maeda's group have demonstrated the role of H_2O_2 in enhancing the EPR effect utilizing polyethylene glycol conjugated D-amino acid oxidase, which can selectively produce H_2O_2 in tumor tissues upon injection of D-proline (Fang et al. 2002).

Photodynamic therapy, in which a photosensitizer is administrated systemically or locally and subsequently activated by illumination with visible light, leading to the generation of cytotoxic reactive oxygen species in the presence of oxygen, has been identified to have an active role in enhancing tumor vascular permeability (Fingar 1996; Dougherty et al. 1998). Chen et al. (2006) found that the concentration of 2,000-kDa FITC-dextran were fivefold higher in orthotropic MatLyLu rat prostate tumors treated with vascular-targeting photodynamic therapy verteporfin, at 15 min following light irradiation, compared to non-irradiated control group. When they studied the effects of verteporfin photosensitization on endothelial cell morphology, and cytoskeleton, they found that photosensitization causes endothelial cell microtubule depolymerization and induces the formation of actin stress fibers. Thus, endothelial cells were found to retract, disrupting the tight junctions and leading to the formation of intercellular gaps, which result in enhanced vascular permeability. In addition, endothelial cell damage leads to the establishment of thrombogenic sites within the vessel lumen and this initiates a physiological cascade of responses including platelet aggregation, the release of vasoactive molecules, leukocyte adhesion and increases in vascular permeability (Fingar 1996).

4.6 Capillary Fluid Transport at Tumor Vasculature

Physiologists have studied fluid dynamics in normal tissue for centuries. Markwalder and Starling (1914) referred to the constant blood volume between the arterial end and the venous end of a capillary under normal conditions. In both normal and tumor vessels, the difference between the hydrostatic and colloid osmotic pressures is known to affect the movement of fluid and solutes through the capillary vessel wall (Jain 1994; Guyton 2000). In normal human tissue blood vessels, the arterial end of a capillary has an average hydrostatic positive pressure of about 25 mmHg. This value drops to about 10 mmHg at the venous end of the capillary, while the interstitial colloid osmotic pressures remain constant at both arterial and venous ends of the capillary. This pressure difference facilitates leakage of fluid and nutrients into the interstitial space at the arterial end of the capillary, and then reabsorption at the venous end. This continuous translocation of fluid from the arterial end to the venous end of capillary through the interstitial space ensures a continuous supply of oxygen and nutrients for cells, as well as an efficient removal of metabolic waste products (Guyton 2000). Tumor vessels, however, demonstrate two major differences compared with normal vessels. First, significantly enhanced or almost unrestricted leakage of plasma proteins, from the luminal side of vessels into interstitial space, occurs because of the wide endothelial gap openings with large pore sizes estimated to be 0.2–0.4 mm (Yuan et al. 1995; Davies et al. 2002). Second, the lack of functional lymphatics in tumor tissue as described in an earlier section. This would lead to higher interstitial accumulation of macromolecules or Nanoparticles in tumor tissues than in normal tissue. In doing so, interstitial hydrostatic as well as colloidal pressures are expected to rise. As can readily be perceived, the raised interstitial colloid osmotic pressure would facilitate transfer of low-molecular-weight components as well as macromolecules from tumor vessels into the interstitial space of the tumor tissues, due to the higher solute level there. While the increased interstitial hydrostatic pressure at the arterial end of the capillaries will drive low-molecular-weight components in fluid from the interstitial space into the venous end of the capillaries and back to the luminal circulation. Whereas small molecules can move into and out of blood vessels in both normal and tumor tissues freely, transfer of macromolecules to the luminal side of blood capillaries does not occur effectively (Maeda 2001; Hori et al. 1991). Only the lymphatic system can clear macromolecules, Nanoparticles and lipids (Courtice 1968), as discussed previously functional lymphatics are lacking in tumor tissues. In effect this results in pronounced accumulation and retention of anticancer Nanomedicines into tumor tissues.

Furthermore, the venous return in tumor tissues was found to be an order of magnitude lower than that of normal tissues (Jain 1988). This condition is ideal for the accumulation of free drug liberated from drug carrier in the case of polymer conjugates or polymeric micelles delivering their cargo into the tumor interstitial space, in response to low pH environment or as a result of the equilibration dynamics between the polymer form and free form of drug. Thus, the slow venous return can also account, in part, for the EPR.

4.7 EPR Effect in Clinical Anticancer Management

Many different types of Nanomedicines have been designed and evaluated for drug targeting to tumors. Tumor–targeted Nanomedicines currently approved or tested in clinical trials are shown in Table 4.2. Nanomedicines carriers are classified into liposomes, micelles, polymers and Nanoparticles. Among these compounds, liposomal drugs and polymer-drug conjugates are two dominant classes, accounting for the majority of the products developed. These drugs could be used for either passive or active targeting. Active targeting is based on the molecular recognition of a specific biomarkers found to be overexpressed at the surface of the tumor cells by a ligand combined to a molecule, a protein or nucleic acids attached to a delivery platform (Allen 2002; Shi et al. 2011). The ligand moiety is constituted by antibodies, antibody fragments or peptides and can facilitate the retention and cellular uptake via receptor mediated endocytosis (Table 4.2). Since the first description of targeted liposomes, only few targeted liposomal systems have made it to clinical trial such as MCC-465(Matsumura et al. 2004a), MBP-426 (Sankhala et al. 2009), SGT53-01 (Heath and Davis 2008). More recently, self-assembly polymers Nanoparticles such as BIND-014 and CALAA-01 (Davis 2009) have been developed and are currently evaluated in phase I clinical trial (Table 4.2).

The exploitation of this EPR effect have led to the development of a few molecules approved for clinical use such as Myocet (Swenson et al. 2003), DaunoXome (FDA 1996), Doxil (Gordon et al. 2001; James et al. 1994), Depocyt (Chhikara and Parang 2010), Genexol-PM (Oerlemans et al. 2010), oncaspar (Dinndorf et al. 2007) and abraxane (Gradishar 2006). The liposomes were first described in 1965 (Bangham et al. 1965) and represent the majority of the Nanomedicine systems developed (Table 4.2) (Lammers et al. 2008). Liposomes are self-assembling colloid structure containing a single or multiple bilayered membranes composed of natural or synthetic lipids (Torchilin 2005). The size of particle can range from few nanometers to micrometers. The liposome platforms significantly improved the pharmacokinetics and biodistribution of several drugs such as doxorubicin for Myocet and Doxil (Lammers et al. 2008). Polymeric Nanoparticles used are either natural (albumin, chitosan, and heparin) or synthetic (for example polyethylene glycol (PEG) and polyglutamic acid (PGA)) polymers (Riggio et al. 2011). These Nanoparticles are generally biodegradable and are formed by a self-assembly process. The size of the particles is ranging from 10 to 1,000 nm in diameter (Riggio et al. 2011). These polymeric Nanoparticles rely essentially on the EPR effect and have shown a drastic improvement of the conjugated drugs to the tumor site (Duncan 2006). They are generally more stable than the liposomes which usually end up to a large extent in reticuloendothelial system.

The polymeric micelles are composed of amphiphilic molecules that self-assemble due to the energy minimization. These particles are generally biodegradable with a size between 10 and 200 nm. The hydrophobic core can be used to carry lipophilic drugs, making the particle highly suitable for *i.v.* injection. The stability of the micelles and the release of the drug is also conditioned by temperature and the

Table 4.2 Examples of clinically used tumor–targeted Nanoparticles

Type of Nanomedicine	Name	Therapeutic agent	Status	Cancer type	Reference-(Clinical trial number)[a]
Liposomes	Doxil/Caelyx	Doxorubicin	Approved	Ovarian cancer	Gordon et al. (2001)
			Approved	HIV-associated Kaposi's sarcoma	James et al. (1994)
			Phase IV	Breast cancer	Green et al. (2011)
	DaunoXome	Doxorubicin	Approved	HIV-associated Kaposi's sarcoma	FDA (1996)
			Phase III	Acute myeloid leukemia	Latagliata et al. (2008), Fassas and Anagnostopoulos (2005)
	Myocet	Doxorubicin	Approved-(Europe-Canada)	Metastatic breast cancer	Swenson et al. (2003)
	Depocyt	Cytarabine	Approved	Malignant lymphomatous meningitis	Chhikara and Parang (2010)
	MEPACT	Muramyl tripeptide phosphatidyl ethanolamine	Approved (Europe)	Osteosarcoma	Ando et al. (2011)
	ThermoDox	Doxorubicin	Phase III	Hepatocellular Carcinoma	(NCT00617981)[a]
			Phase II	Breast cancer	(NCT00826085)[a]
	Allovectin-7	HLA-B7 and beta2-microglobulin complex	Phase III	Melanoma	(NCT00395070)[a]
			Phase III	Head and Neck cancer	(NCT00050388) [a]
			Phase II	Melanoma	Stopeck et al. (2001)
	CPX-1	Irinotecan, Floxuridine	Phase II	Colorectal neoplasms	(NCT00361842)[a]
	SPI-077	Cisplatin	Phase II	Non-small-cell lung cancer	Kim et al. (2001), White et al. 2006)
			Phase II	Ovarian cancer	Seetharamu et al. (2010)
	Oncolipin	Interleukin-2	Phase II	Non-small-cell lung cancer	Neville et al. (2000), Wang et al. (2008b)
	L-Annamycin	Annamycin	Phase II	Breast cancer	Booser et al. (2002)
			Phase I	Leukemia	Apostolidou et al. (2007)

Aroplatin	Oxaliplatin	Phase II	Advanced colorectal cancer	Dragovich et al. (2006)
LE-SN38	SN38	Phase II	Colorectal cancer	Bayes and Rabasseda (2004) (NCT00311610)[a]
		Phase I	Neoplasm	(NCT00046540)[a]
OSI-7904L	Thymidylate synthase inhibitor	Phase I	Advanced biliary cancers	Ciuleanu et al. (2007)
		Phase I	Advanced solid tumor	(Beutel et al. 2005; Ricart et al. 2008)
LEP ETU	Paclitaxel	Phase II	Metastatic breast cancer	(NCT01190982)[a]
		Phase I	Advanced cancer	(NCT00100139)[a], (NCT00080418)[a]
Onco-TCS	Vincristine	Phase I/II	Non-Hodgkin's lymphoma	Boehlke and Winter (2006)
OSI-211	Lurtotecan	Phase II	Ovarian cancer	Seiden et al. (2004), Dark et al. (2005)
		Phase II	Head and neck carcinoma	Duffaud et al. (2004)
		Phase II	Small-cell lung cancer	(NCT00046787)[a]
		Phase I	Advanced leukemia	Giles et al. (2004)
		Phase I	Solid tumors	Gelmon et al. (2004)
INGN-401	FUS-1	Phase I	Lung cancer	(NCT 00059605)[a]
MCC-465	Doxorubicin	Phase I	Metastatic stomach cancer	Matsumura et al. (2004a)
SGT-53	p53 gene	Phase I	Solid tumors	Heath and Davis (2008)
LErafAON	c-Raf antisens oligo-nucleotides (AON), cationic	Phase I	Advanced solid tumors	Rudin et al. (2004)
PLD-E1A	Cationic-E1A pDNA	Phase I	Breast and ovarian cancer	Wang and Hung (2000)
CALAA-01	Small interfering DNA	Phase I	Solid tumors	Davis et al. (2010)
MBP-426	Oxaliplatin	Phase I	Metastatic solid tumors	(NCT00355888)[a]
		Phase I/II	Gastric and esophageal adenocarcinoma	(NCT00964080)[a]

(continued)

Table 4.2 (continued)

Type of Nanomedicine	Name	Therapeutic agent	Status	Cancer type	Reference-(Clinical trial number)[a]
Polymeric micelles	Genexol-PM	Paclitaxel	Approved (South Korea)	Metastatic breast cancer	Oerlemans et al. (2010)
			Phase II	Urothelial cancer	Lee et al. (2011)
			Phase II	Advanced non-small-cell lung cancer	Kim et al. (2007)
			Phase II	Advanced ovarian cancer	(NCT00886717)[a]
			Phase II	Pancreatic cancer	Saif et al. (2010)
	Oncaspar	PEG-L-asparaginase	Approved	Acute lymphoblastic leukemia	Dinndorf et al. (2007)
	PegAsys/PegIntron	IFNα2a/- IFNα2b	Phase III	Myelogenous leukemia	Michallet et al. (2004)
			Phase I-II	Solid tumors	Bukowski et al. (2002)
	SP1049C	Doxorubicin	Phase II	Adenocarcinoma of esophagus and gastroesophageal junction	Valle et al. (2011)
	NK-012	SN-38	Phase II	Small-cell lung cancer	(NCT00951613)[a]
			Phase II	Metastatic triple negative breast cancer	(NCT00951054)[a]
			Phase I	Solid tumors	Hamaguchi et al. (2010)
	NK105	Paclitaxel	Phase II	Gastric cancer	Kato et al. (2011)
	NC-6004	Cisplatin	Phase I-II	Pancreatic cancer	(NCT00910741)[a]
			Phase I	Solid tumors	Plummer et al. (2011)
	NK911	Doxorubicin	Phase I	Solid tumors	Matsumura et al. (2004b)
Polymer-drug conjugates	Smancs	Neocarzinostatin	Approved (Japan)	Hepatocellular carcinoma	Maeda et al. (2009)

Xyotax/CT-2103/ paclitaxel poliglumex/ Opaxio	Paclitaxel	Phase III	Non-small-cell lung cancer	Paz-Ares et al. (2008)
		Phase II	Esophageal cancer	Dipetrillo et al. (2011)
		Phase II	Ovarian cancer	Sabbatini et al. (2004), Sabbatini et al. (2008)
		Phase II	Metastatic breast cancer	Lin et al. (2007)
		Phase II	Prostate cancer	Beer et al. (2010)
PK1, FCE28068	Doxorubicin	Phase II	Breast, lung and colorectal cancer	Seymour et al. (2009)
IT-101	Campothecin	Phase II	Ovarian cancer	(NCT00753740)[a]
		Phase I/II	Advanced solid tumor	(NCT00333502)[a]
NKTR-102	Irinotecan	Phase II	Ovarian cancer	(NCT00806156)[a]
		Phase II	Breast cancer	(NCT00802945)[a]
		Phase II	Colorectal cancer	(NCT00598975)[a]
CDP-791	Anti-VEGFR2 Fab	Phase II	Non-small-cell lung cancer	(NCT00152477)[a]
EZN-2208	SN-38	Phase II	Metastatic breast cancer	(NCT01036113)[a]
		Phase II	Colorectal carcinoma (NCT00931840)[a]	(NCT00931840)[a]
		Phase I	Solid tumors and lymphoma	(NCT00520637)[a]
Prothecan	Campothecin	Phase II	Metastatic gastric and gastro-oesophageal junction adenocarcinoma	Scott et al. (2009)
		Phase I	Solid tumors and lymphomas	Posey et al. (2005)
CT2106	Camptothecin	Phase II	Colorectal cancer	(NCT00291785)[a]
		Phase II	Ovarian cancer	(NCT00291837)[a]
		Phase I	Advanced cancer	(NCT00059917)[a] Homsi et al. (2007)

(continued)

Table 4.2 (continued)

Type of Nanomedicine	Name	Therapeutic agent	Status	Cancer type	Reference-(Clinical trial number)[a]
	Hepacid/ADI-PEG	Arginine deaminase	Phase II	Hepatocellular carcinoma	(NCT00056992)[a] Shen and Shen (2006)
			Phase I	Melanoma	(NCT00029900)[a] Shen and Shen (2006)
	PK2, FCE28069	Doxorubicin	Phase I	Hepatocellular carcinoma	Seymour et al. (2002)
	PNU166945	Paclitaxel	Phase I	Breast cancer	Meerum Terwogt et al. (2001)
	MAG-CPT	Campothecin	Phase I	Solid tumor	Wachters et al. (2004), Bissett et al. (2004)
	AP5280	Platinum	Phase I	Solid tumor	Rademaker-Lakhai et al. (2004)
	AP5346	Platinum	Phase I	Solid tumor	Campone et al. (2007)
	DOX-OXD/AD-70	Doxorubicin	Phase I	Various cancers	Danhauser-Riedl et al. (1993)
	DE-310	Topoisomerase-I-inhibitor, exatecan mesylate	Phase I	Solid tumors	Soepenberg et al. (2005)
	BIND-014	Doxetaxel	Phase I	Solid tumors	(NCT01300533)[a]
Albumin based Nanomedicine	Abraxane (ABI-007)	Paclitaxel	Approved	Metastatic breast cancer	Gradishar (2006)
			Phase II	Non-small-cell lung cancer	Reynolds et al. (2009)
			Phase II	Melanoma	Kottschade et al. (2011), Hersh et al. (2010)
			Phase II	Ovarian cancer	Coleman et al. (2011), Teneriello et al. (2009)
			Phase I	Pancreatic cancer	Stinchcombe et al. (2007)
			Phase I	Bladder cancer	McKiernan et al. (2011)
	MTX-HSA	Methotrexate	Phase II	Kidney carcinoma	Vis et al. (2002)
	ABI-008	Docetaxel	Phase I/II	Metastatic breast cancer	(NCT00531271)[a]
			Phase I/II	Prostate cancer	(NCT00477529)[a]
	ABI-009	Rapamycin	Phase I	Solid tumors	(NCT00635284)[a]
	ABI-010	Tanespimycin (17-AAG)	Phase I	Solid tumors	(NCT00820768)[a]
	ABI-011	Microtubule and topoisomerase inhibitor	Phase I	Advanced solid tumors and lymphomas	(NCT01163071)[a]

[a] ClinicalTrials.gov Identifier number

external pH (Riggio et al. 2011). Genexol-PM is a polymeric micelle of paclitaxel approved for the treatment of metastatic breast cancer in South Korea (Oerlemans et al. 2010).

The dendrimers are a new class of synthetic macromolecules with a well-defined tree-like structure organized in a series of radially homocentric layers (Cheng et al. 2011). Physiochemical properties of these dendrimers make them suitable for anticancer drug delivery (Cheng et al. 2011). Encapsulation of cisplatin within the dendrimers demonstrated a higher accumulation within the solid tumor compare to the free drug (Malik et al. 1999). Other Nanoparticle drug delivery platforms that have been extensively include metal Nanoparticle and molecular targeted Nanoparticles. Their efficacy of drug delivery systems to enhance the pharmacokinetic properties of drugs has been confirmed in preclinical trials (Rippel and Seifalian 2011; Wang et al. 2008a).

4.8 Challenges to the Enhanced Permeability Strategy to Target Nanosize Drug Carriers

Recognition of the EPR effect among researchers in drug delivery field resulted in a considerable momentum to the emerging field of Nanomedicine. The expectation for realization of a selective anticancer drug as a result of adapting the EPR effect in Nanotechnology was high, however as shown in Table 4.2, only few Nanomedicine have found its way to the clinic (Duncan 2003). Following we discuss some of the factors that might interfere with the full exploitation of EPR effect for anticancer specific targeting.

4.8.1 EPR Effect in Animal Models

The EPR effect has been repeatedly proved in animal models through the use of Evans blue dye (EBD). EBD binds instantly to plasma albumin which results in large molecular weight complex about 7 nm in diameter that can simulate the effect of a Nanomedicine. After 6 h, usually there is a profound accumulation in tumor lesion compared to surrounding tissues. Similarly, many Nanomedicines have been proven to accumulate in tumor tissues compare to other organs, such as 11-fold higher concentration of SMA-doxorubicin micelle compare to free drug (Greish et al. 2004). The question whether the results of EPR-based drug targeting results in animal models can be translated clinically remained unanswered. A great difference between the tumor models in animal and human is the progression rate; animals usually develop a large clinically relevant tumor (>5 mm) 1 week after tumor cell inoculation subcutaneously, while such a tumor volume can take few years to develop in a human. This fast progression rate carries many confounding factors towards more effective EPR effect in animal models. Animal tumors developing

quickly produce large quantity of VEGF and vascular mediators to support the rapid tumor growth. In addition a 1 g tumor mass in a 30 g mouse is about 3% of its total weight. In human that would be 2–5 kg tumor, which is a rather an advanced tumor stage that do not represent an ideal condition for utilization of anticancer Nanomedicine. Further, it is a common practice to utilize immuncompromised mice to allow for the take of human tumor xenographs. Such condition in fact rarely represents a human cancer patient. Finally tumor is usually implanted in the dorsal skin of mice, which allow the developing tumors to take advantage of the extensive cutaneous vascular network for extending its blood supply. Weather this is a confounding factor towards more pronounced EPR effect in these models, is unanswered question.

4.8.2 Tumor Biology and Neoangiogenesis

EPR effect is a phenomenon that is completely related to tumor vasculature. Even though EPR effect has been repeatedly proven in animal models, to our knowledge, there are no studies that compare tumor vascular permeability in relation to tumors of different origins, at different stages, or in different organs. It is becoming clearer that only a subset of human tumors can exhibit the enhanced permeability. Human malignant tumors vary with malignant grades; the higher the grade is the further the tumor cells from its cell of origin. As tumor cells become poorly differentiated they tend to be more aggressive with higher metastatic potentiality. Usually this subset of cells can exhibit accelerated growth with active formation of neoangiogenesis and extensive production of growth factors and permeability enhancing mediators. On the contrary, slowly growing tumors characteristic of low grade tumors, usually grow slowly, with less production of VEGF and permeability factors, and as such the EPR effect will not be true in these tumors (Berger et al. 1995). In addition to the growth rate of various tumors, the nature of tumor tissue stroma can have an important role as well. For example, in pancreatic tumors, a dense connective tissue stroma composes the majority of the malignant lesions and impeded the vascular supplies to malignant cells, diffusion from neighboring tissue becoming the main life supply for the intervening malignant cells. In such cases the delivery of drugs based on the EPR effect will be challenging (Bachem et al. 2005). Similar to the above example is the tumor cells in the center of a lesion. Those cells have always and remain the greatest challenge in tumor management. This subset of cells usually survive the most unfavorable survival conditions in terms of poor oxygen and glucose supply, in addition to the highest acidic environment in the tumor mass (West et al. 1980). Central necrosis is a phenomenon known for tumors that overgrow their blood supply where the central tumor cells die from lack of proper oxygenation and nutrients. Lack of oxygen supply has long been the factor contributing to the radioresistance of these cells as oxygen is needed to mediate the ionizing toxic effect of radiation. Similarly, no anticancer drug can penetrate deep into the center of the tumor due to the poor blood supply at this central region (West et al. 1980).

This problem is valid to a greater extent for the case of large size molecules (Nanomedicine) that cannot transport through convection or diffuse freely due to their relative large size. One hypothesis is that impedance of drug diffusion can result from raising interstitial tumour pressure in tumour tissue. However, raised interstitial pressure can logically operate to force fluid and solutes towards the side of lower pressure, which is the venous end of the capillaries, rather than back towards the arterial end where the hydrostatic pressure derived for cardiac output and vascular resistance is much higher than that at the venous end. More in depth research of the controversial role of tumour interstitial pressure in relation to Nanomedicine drug targeting is essential for this drug delivery technology.

4.8.2.1 Biodistribution

The EPR effect utilizes the unique characteristic of large gaps between endothelial cells that make up the tumor vessels. Usually these gaps can be from few nanometers to up to 400 nm. With this large size Nanomedicine can penetrate through tumor tissues preferentially. However, tumors are not the only organs with this large fenestration size. Two major organs with such quality are the spleen and liver. Liver sinusoids can have fenestration around 100 nm in humans (Wisse et al. 2008). Spleen, on the other hand, has large sinusoid lumina of 20–40 μm that can support the extravasations of aged red blood cells. With this large fenestration size, a great amount of Nanosize drugs are filtered into the liver and spleen. While the spleen functions can be compensated for by other lymphatic organs as well as the liver, liver damage due to the concentration of cytotoxic Nanomedicine remains a potential challenge to successful anticancer drug targeting. It is not surprising that Nanoconstruct of Cis-platinum which has dose-limiting renal toxicity resulted in less toxicity of the kidney, but in exchange for liver dose limiting toxicity(Uchino et al. 2005).

4.8.3 Release Rate

In order for the successful Nanocarrier to provide selective antitiumor targeting, the carrier should have a stable chemical bond with the cargo drug while in circulation to prevent the rapid release of free drug that can otherwise result in a similar biodistribution and toxicity profile comparable to the free drug. However, with a stable linkage, the release rate of the drug at the site of action (tumor tissue) would be slow; here there is a dynamic that involves tumor doubling time. While x amount of drug can kill a certain amount of tumor cells, double the amount of the same drug will be needed to kill the same subset of tumor after one doubling time. Thus while a strong chemical bond between the Nanocarrier and the drug is beneficial during the circulatory phase of the drug, it becomes a disadvantage to encounter the progressive number of tumor cells. Multiple techniques have been utilized to address

this problem with different level of success such as devising specific bonds between the Nanocarrier and the drug that will only cleave upon exposure to specific enzymes in tumor cells or merely the high acidic condition in tumor tissue (Greish et al. 2003; Greish 2007).

4.8.4 Biocompatibility

After intravenous administration of a specific dose of a drug carried by Nanocarrier, a relatively high concentration is targeted to the tumor by the EPR effect. However this portion of the drug is usually less than 10% of the total administered dose. The remaining 90% still find its way to different organs and tissue (Bae and Park 2011). Unless the Nanocarrier is biodegradable this amount of Nanocarrier can remain in the body after releasing its anticancer drug cargo. The Nanosize particles used to carry the drug load are frequently recognized and dealt with as a foreign body. The innate elements of the immune system are non-specifically stimulated by many Nanocarrier through toll like receptor (TLR's), i.e. TLR-4 (Kedmi et al. 2010). Following activation by Nanocarrier, immune cells can produce cytokines which trigger inflammation and the Nanocarrier is phagocytized by monocyte/macrophage. At that point the phagocytic cells will try to degrade the Nanocarrier in the lysosomal compartment through lysosomal enzymes. Failure of this process can lead to (frustrated macrophage) or the formation of a giant foreign body cells that closely resemble the formation of granuloma. This will result in a pathological capsule with dense fibrous capsule replacing the original functional tissue, which can compromise organ function, especially in the liver (Kao and Lee 2001). Another concern of the Nanocarrier not being biodegradable is malignancy induction, where the prolonged unresolved inflammation may result in malignant transformation. A successful Nanocarrier thus must be biodegradable.

4.8.5 Intracellular Internalization

While the EPR effect will result in a high drug concentration in the tumor tissues in a specific subset of tumors, it cannot guarantee the internalization of these drugs through the tumor cell membrane into the cytoplasm or the nucleus. Usually Nanosize anticancer drugs are internalized into tumor cells through endocytosis with the final localization in the endosomes, then the lysosomal compartment (Zaki and Tirelli 2010). A major limitation in tumor cells is their phagocytic capability. It was found that macrophages are much more susceptible to toxicity associated with Nanosized silica Nanoparticles compared to different tumor cells in vitro, and this deferential cytotoxicity activity in macrophage-derived cell lines was clearly correlated with the higher intercellular uptake by the professional phagocytic macrophage (Yu et al. 2011). Based on this observation, it is clear that

the relatively large carriers (macromolecules and Nanocarriers) that adhere to cell surfaces without intracellular trans-localization may not give any additional benefit by the *retention* effect. Many Nanosize particles may locate juxtaposition to the leaky sites due to limited permeability or mobility in the extracellular space and non-specific interactions with the extracellular matrix. Thus, cell internalization is essential for Nanocarriers for effective drug delivery besides the enhanced permeation and retention (EPR) effect.

4.9 Conclusion

The EPR effect can be considered a hallmark concept that exploits the anatomical and pathophysiological defects in the tumor vasculature. It plays a critical role in selective delivery of Nanomedicine-based-anticancer agents to tumor tissues. EPR effect outcome can be influenced by variables such as tumor diversity, animal models, biodistribution, intracellular interaction, and release rate of active cytotoxic cargo from its Nanosize carrier. Understanding and manipulating the different variables contributing to the EPR effect, can further improve the selective targeting of high-molecular-weight biocompatible or anticancer Nanomedicine to tumor, thus ensuring bright future for EPR based anticancer Nanomedicine.

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