Challenges of Drug Delivery Systems That Contribute to Cancer Chemotherapy

Nanoparticle-Based Passive Drug Targeting to Tumors: Considerations and Implications for Optimization

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There are many potential barriers to the effective delivery of small-molecule drugs to solid tumors. Most small-molecule chemotherapeutic drugs have a large volume of distribution upon intravenous administration, which is often associated with a narrow therapeutic index due to their high levels of toxicity in healthy tissues. Nanoparticle-based therapeutics for tumor targeting have emerged as one of the promising approaches to overcome the lack of tissue specificity of conventional chemotherapeutic drugs. Various different concepts have been envisioned for nanoparticle-mediated drug targeting. Among them, the passive drug targeting strategy has been the most widely investigated, and numerous preclinical studies have provided insights into the validity of the strategy. This review article briefly introduces our recent findings related to the passive drug targeting strategy including its application in anti-angiogenic therapy, along with considerations to be taken into account and implications for the rational design of a passive drug targeting strategy.

Key words passive targeting; nanoparticle; anti-cancer drug; angiogenesis; endothelial cell

1. INTRODUCTION

Cancer is a leading cause of death around the world. The World Health Organization estimates that 84 million people will die of cancer between 2005 and 2015. For effective cancer therapy, it is necessary to improve our knowledge of cancer pathophysiology, discover new anti-cancer drugs, and develop novel biomedical technologies. Currently, cancer therapy is a multidisciplinary challenge requiring close collaboration among clinicians, scientists in the field of biology and pharmaceutics, and biomedical engineers. There are many potential barriers to the effective delivery of a drug in its active form to solid tumors. Most small-molecule chemotherapeutic drugs have a large volume of distribution upon intravenous administration, which is often associated with a narrow therapeutic index due to their high levels of toxicity in healthy tissues.1,2) Given the potency of modern anticancer drugs, therefore, their tissue selectivity is of great importance to achieve efficient and safe cancer chemotherapy. Hence, cancer therapeutics are expected to increase the survival time and quality of life of cancer patients by improving the in vivo disposition characteristics of anticancer drugs and by reducing the systemic toxicity of current chemotherapy regimens.

In this context, nanoparticle-based therapeutics for tumor targeting have emerged as one of the promising approaches to overcome the lack of tissue specificity of conventional chemotherapeutic drugs. Nanoparticles are submicron-sized drug carriers that are expected to improve the biodistribution of systemically administered chemotherapeutic drugs. By delivering pharmacologically active agents more selectively to pathological sites (tumor tissues) and/or by guiding them away from healthy tissues, nanoparticle-based formulations aim to improve the balance between the efficacy and toxicity of systemic chemotherapeutic interventions.

Various different concepts have been envisioned for nanoparticle-mediated drug targeting. Among them, the passive drug targeting strategy has been most widely investigated, and a huge number of preclinical studies have provided insights into the validity of the strategy.3–5) As described below, this strategy is based on the abnormalities of tumor vasculatures, allowing nanoparticles access to tumors while avoiding distribution into normal healthy tissues. In this review article, we briefly introduce our recent findings related to the passive drug targeting strategy including its applications in anti-angiogenic therapy.

2. ENHANCED PERMEABILITY AND RETENTION (EPR) EFFECT

Most solid tumors possess unique pathophysiological characteristics that are not observed in normal tissues/organs, such as extensive angiogenesis, defective vascular architecture, and impaired lymphatic drainage/recovery system.6,7) Generally, the capillary permeability of the endothelium of newly vascularized tumors is significantly greater than that of normal organs. Passive drug targeting approaches to solid tumors take advantage of these unique pathophysiological properties of tumor vasculature. Due to the long circulation time of polyethylene glycol (PEG)-modified liposomes (PEG liposomes) and the leakiness of the microcirculation in solid tumors, PEG liposomes containing anticancer drugs have been shown to accumulate preferentially in tumors (Fig. 1). This phenomenon
known as the EPR effect, has been generally observed in many types of solid tumor and provides a good opportunity for passive targeting of liposomal anticancer drugs to tumor tissues. However, the extent of vascularity and permeability of the vasculature within tumors may differ from one type of tumor to another. Therefore, differences in the pathophysiological characteristics of tumors may result in different therapeutic effects in EPR effect-based therapy.

3. DETERMINANTS OF EPR EFFECT-DRIVEN ANTI-TUMOR EFFECTS OF PEG LIPOSOMAL DRUGS

To elucidate the important determinants of the EPR effect-driven in vivo anti-tumor efficacy of PEG liposomal doxorubicin (DOX), we examined its anti-tumor effects against three different tumor cell lines with various phenotypes [Lewis lung cancer (LLC), Colon-26 (C26), and B16BL6 melanoma (B16)] under in vitro and in vivo experimental setups. In the in vitro evaluation, LLC was the most sensitive to DOX and liposomal DOX based on the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. However, the most potent in vivo anti-tumor effects were observed in C26 tumor-bearing mice. From these results, it was revealed that the in vivo anti-tumor effects of PEG liposomal DOX did not directly reflect the sensitivity of tumor cells to DOX. On the other hand, the in vivo accumulation of PEG liposomes in C26 tumors 48 h after intravenous injection was significantly greater than in other tumors, suggesting that the efficient tumor disposition of PEG liposomes in C26 tumor tissues would be one of the main reasons behind the prominent in vivo anti-tumor effects in C26 tumor-bearing mice. Furthermore, it was found that the extent of vascularity assessed by immunohistochemical staining of CD31 was not directly related to the tumor accumulation of PEG liposomes. On the other hand, Evans-blue extravasation in C26 tumors was higher than in other tumors, clearly demonstrating that the vascular permeability was higher within C26 tumors. These results indicated that the vascular permeability within the tumor substantially affects the tumor accumulation of PEG liposomes and would be one of the important determinants in the EPR effect-driven anti-tumor efficacy of PEG liposomal DOX.

4. STRATEGIES TO OVERCOME MULTIDRUG RESISTANCE: HITTING TUMORS WHERE IT HURTS

Generally, chemotherapeutic drugs are administered to cancer patients over a long term at low dosage to prevent severe side effects, which often causes the cancer cells to acquire resistance against the chemotherapeutic drug and the effectiveness of the drug gradually decreases. The acquisition of resistance by cancer cells during long-term exposure to chemotherapeutic agents, called multidrug resistance, has been considered as a major obstacle in current clinical cancer chemotherapy.

Various mechanisms of multidrug resistance have been proposed, including the induced expression of multidrug efflux transporters [P-glycoprotein (P-gp) or multidrug resistance-associated proteins]. Accumulating knowledge on the mechanism behind multidrug resistance shows that two or more mechanisms simultaneously contribute to the acquisition of resistance, but the over-expression of P-gp on tumor cells has been considered to be the main factor associated with it.

To develop a strategy for overcoming multidrug resistance, we established the DOX-resistant Colon-26 cancer cells (C26/DOX) in which P-gp is over-expressed. To confirm the acquisition of resistance of C26/DOX to DOX, the in vitro sensitivity of C26/DOX to DOX was evaluated. The IC50 value of DOX in C26/DOX was found to be about 20-fold higher than that in the C26/control, demonstrating that the C26/DOX is much more resistant to DOX than the C26/control. Then, the EPR effect-based anti-tumor activities of PEG liposomal DOX were evaluated in both C26/control- and C26/DOX-bearing mice. It was found that PEG liposomal DOX significantly inhibited tumor growth in not only C26/control-bearing mice but also in C26/DOX-bearing mice in a dose-dependent manner. These results clearly demonstrate that there is a marked difference in the efficacy of DOX to C26/DOX between the in vitro and in vivo studies.

To elucidate the reasons for this discrepancy between the in vitro and in vivo results, various evaluations and analyses were conducted. Based on the outcomes of those studies, we propose the following mechanisms underlying the in vivo anti-tumor effects of PEG liposomal DOX recognized in C26/DOX-bearing mice (Fig. 2). First, PEG liposomes in the blood circulation gradually extravasate into the interstitial space of the tumor tissue due to the EPR effect. DOX encapsulated in

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**Fig. 1. Schematic Representation of EPR Effect-Driven Tumor Targeting of Drugs**

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Liposomes must first be released into the interstitial space of the tumor to be taken up by the tumor cells via passive diffusion. In the case of C26/control tumors, DOX passively taken up by C26/control would exert its anti-tumor activity against the tumor cells directly, leading to the apoptosis of cells throughout the tumor. On the other hand, in the case of C26/dOX tumors, due to the over-expressed P-gp on the surface of tumor cells, DOX passively taken up by C26/dOX would be subjected to efflux out of the cells, which would lead to much less apoptotic cell death in the tumor. Subsequently, the resultant larger amount of DOX accumulated in the interstitial space of the C26/dOX tumor tissue would penetrate into neighboring vascular endothelial cells. Finally, the apoptotic cell death of vascular endothelial cells would lead to the suppression of angiogenesis necessary for tumor growth, resulting in the in vivo anti-tumor effects of PEG liposomal DOX in C26/DOX-bearing mice. These findings indicate the potent efficacy of targeting anti-angiogenic drugs to vascular endothelial cells for cancer chemotherapy to overcome multidrug resistance.

5. ANTI-ANGIOGENIC THERAPY

Angiogenesis, the sprouting of capillaries from pre-existing blood vessels, is essential for the sustained growth of solid tumors. In 1971, Folkman suggested that tumor growth might be inhibited by preventing tumors from recruiting new blood vessels. In this strategy, ligand-targeted nanocarriers encapsulating cytotoxic drugs are expected to bind to and kill angiogenic blood vessels, leading to the death of tumor cells surrounding these vessels. These mechanisms of action differ from those of conventional chemotherapy in the following ways: 1) there is no need for the extravasation of nanocarriers to arrive at their target site; 2) direct binding to their receptors is possible after intravenous injection; 3) the potential risk of emerging resistance is decreased because of the better genetic stability of endothelial cells compared with tumor cells; and 4) most target molecules expressed on endothelial cells in tumor tissues are expressed irrespective of the type of tumor, providing a ubiquitous approach and eventual broad-spectrum application. Due to these potential advantages of anti-angiogenic therapy, the strategy has attracted much attention and many preclinical studies have been conducted to attack tumor tissues by inhibiting the angiogenesis.

The vascular endothelial growth factor (VEGF) family and its receptors constitute the most important signaling pathways in tumor angiogenesis and have been well characterized by many researchers over the last two decades. In a variety of solid tumors, tumor cells secrete VEGF and the secreted VEGF then binds to VEGF receptor-2 (VEGF-R2) exclusively expressed on the surface of endothelial cells. The binding of VEGF to VEGF-R2 is the main trigger to initiate angiogenesis. SU5416 is a potent and specific inhibitor of VEGF-R2 tyrosine kinase which efficiently shuts down VEGF signal transduction. However, since SU5416 has poor water solubility, its appropriate formulation has been expected. We tried to formulate SU5416 into an O/W PEGylated emulsion (PE-SU5416) and evaluated its anti-angiogenic potency in the in vitro and in vivo experiments.

The MTT assay revealed that SU5416 inhibited the proliferation of human umbilical vein endothelial cells in a concentration-dependent manner but did not show such an inhibitory effect in all types of tumor cells used, demonstrating the specificity of SU5416 for endothelial cells. Furthermore, multiple injections of PE-SU5416 into tumor-bearing mice significantly suppressed the growth of C26 and B16 tumors, but had no effects on the growth of LLC tumors. Since the secretion of VEGF from tumor cells is the first step in angiogenesis, we evaluated the VEGF level in each type of tumor tissues using ELISA. The VEGF levels within C26 and B16 tumors were about 10-fold and 20-fold higher than the level in LLC tumors, respectively. This result suggests that VEGF plays a major role in angiogenesis in C26 and B16 tumors, while other pro-angiogenic factors excluding VEGF should trigger angiogenesis in LLC tumors, since no in vivo anti-tumor effects of PE-SU5416 were observed in LLC tumor-bearing mice. From these results, it was suggested that intravenously injected PE-SU5416 would inhibit angiogenesis in certain types of tumor tissue such as C26 and B16 where VEGF plays a major role in initiating angiogenesis, leading to the suppression of in vivo tumor growth. In this regard, VEGF could be an adequate biomarker for selecting patients who should be enrolled.
in anti-angiogenic therapy with compounds such as SU5416.

6. VESSEL NORMALIZATION STRATEGY: AN EMERGING CONCEPT IN ANTI-ANGIOGENIC THERAPY

As described above, EPR effect-driven passive targeting of drugs is based on the abnormalities of tumor vasculature such as extensive angiogenesis, defective vascular architecture, and impaired lymphatic drainage/recovery system, allowing access of nanoparticles to tumors. On the other hand, it is known that a variety of pathophysiological features of solid tumors compromise the efficacy of conventional nonsurgical therapies. Due to the imbalance between pro- and anti-angiogenic factors secreted in tumor tissues, the resulting tumor vasculature is structurally and functionally abnormal.\(^{18,19}\) For example, vessel coverage by pericytes is generally poor in solid tumors, and the impaired pericyte-coverge contributes to chaotic blood flow in tumor tissues. Furthermore, regions of severe oxygen deprivation (hypoxia) arise within solid tumors due to rapid division of tumor cells and aberrant blood vessel formation. These structural and functional abnormalities of the tumor vasculature cause spatial and temporal heterogeneous tumor blood flow. In addition, proliferating cancer cells within tumor tissues generate high internal pressures, resulting in the impairment of blood flow due to the compression of intratumoral blood vessels. These abnormalities in the tumor vasculature lead to a unique tumor microenvironment characterized by hypoxia, low pH, and elevated interstitial fluid pressure (IFP). Specifically, from the therapeutic point of view, impaired blood supply and elevated IFP pose a barrier against delivering therapeutics to solid tumors.\(^{20}\)

Jain proposed that judicious attenuation of pro-angiogenic (VEGF) signaling, within a dose- and time-dependent schedule, might selectively prune immature blood vessels and remodel others.\(^{21}\) The resultant vasculature is less chaotic with greater pericyte-coverage and reduced hyper-permeability, resembling that of normal tissue (vessel normalization).\(^{21}\) These structural and functional transformations are further thought to be accompanied by physiological normalization of parameters such as decreased IFP and improved blood flow, leading to tumor oxygenation. These changes are considered to make the overall vascular network more stabilized and better suited to drug delivery. To date, a number of VEGF inhibitors have become available, including neutralizing anti-VEGF antibodies and small molecular-weight compounds inhibiting VEGF receptor tyrosine kinase activity. The concept of “vessel normalization” has already been confirmed using various VEGF inhibitors such as bevacizumab, sorafenib, TSU68 (SU6668), pazopanib, DC101 (VEGF-R2 antibody), and SU5416 in terms of the structural and functional transition of vessel vasculature.\(^{22,23}\) However, there are only a few reports addressing the anti-tumor effect of subsequently injected anticancer drugs after pretreatment with these VEGF inhibitors.

In our study, we selected SU5416, a hydrophobic molecule with potent tyrosine kinase inhibitory activity toward VEGF-R2. Other groups have already demonstrated that structural and functional transition of the tumor vasculature could be achieved by treatment with this compound. However, in those studies, due to its poor water solubility and poor tumor disposition, SU5416 was dissolved in DMSO or Cremophor EL, which is known to induce undesirable side effects such as anaphylactic shock or hemolysis and was administered as a peritoneal injection frequently at high doses. To prepare safer dosage forms with better tumor-targeting properties, we evaluated the effects of pre-treatment with PE-SU5416 on tumor disposition and in vivo anti-tumor efficacy of subsequently administered PEG liposomal paclitaxel (PL-PTX) that was developed by our group\(^{24}\) in C26 solid tumor-bearing mice.\(^{25}\)

Pre-treatment with PE-SU5416 significantly enhanced the in vivo anti-tumor effects of PL-PTX, although PE-SU5416 administration alone did not show any anti-tumor effect. Immunostaining for endothelial cells and pericytes demonstrated that pre-treatment with PE-SU5416 enhanced the pericyte coverage of the tumor vasculature. In addition, tumors treated with PE-SU5416 contained significantly smaller hypoxic regions compared with untreated control group, demonstrating that structural normalization of the tumor vasculature resulted in an improvement in tumor vessel functions, including oxygen supply. Furthermore, the pre-treatment with PE-SU5416 increased the distribution of PEG liposomes and included PTX in the core region of the tumor, as well as conversely decreasing the ratio of their peripheral distribution. These results suggest that the structural and functional normalization of the tumor vasculature by pre-treatment with PE-SU5416 enabled liposomes to reach deeper regions within tumor tissues, leading to more potent anti-tumor activity of PL-PTX.\(^{25}\) A follow-up study using tumor cells with different phenotypes is underway and will provide further information that will be of great importance to appreciate the potential of the vessel normalization strategy for future therapeutic applications.

7. CONCLUSIONS AND FUTURE PERSPECTIVES

Despite extensive researches and developments in nanotechnology, only a few nanoparticle-based drug delivery systems have been approved and are available for cancer treatment. This is partly due to the fact that (patho)physiological features of the tumor microenvironment and molecular mechanisms underlying tumor angiogenesis are quite different and are dependent on the type of tumors. Therefore, a deeper and more precise understanding of these characteristics of tumor tissues will be necessary to further ensure a successful future for EPR effect-based cancer chemotherapy. In this regard, tumor biopsy samples from cancer patients would be valuable sources of information on their own tumor tissues which can be retrieved by adequate measurements of several biomarkers. We hope that such biomarker-based diagnoses will lead to the rational design of more beneficial and more effective cancer treatments using the passive drug-targeting strategy.

REFERENCES


