EPR Effect for Cancer Treatment

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The EPR effect was first discovered by Maeda and colleagues in solid murine tumors. The polymer-drug conjugates were i.v. administered, and 10-to-100-fold higher concentrations were achieved relative to free drug administration. The concentration of nanodrugs builds up in tumors due to the EPR effect, reaching several times higher than that of plasma due to the lack of lymphatic drainage.

EPR-based therapy | passive targeting | heterogeneity | solid-tumor | EPR-imaging techniques

1. Introduction

The EPR effect was first discovered by Maeda and colleagues in solid murine tumors [1][2]. The polymer-drug conjugates were i.v. administered, and 10-to-100-fold higher concentrations were achieved relative to free drug administration [2][3][4]. Passively targeted cancer drugs at first reached the clinic about 30 years ago with the approval of an EPR-based drug, a PEGylated liposomal drug, DOXIL. Nanocarriers preferentially accumulate in the tumor through passive targeting due to a leaky vasculature and defective lymphatic drainage in solid tumors. The permeability of a chaotic vasculature and tumor microenvironment (TME) and retention can lead to the accumulation of macromolecules in TME by 70-fold. The leaky and defective vasculature created due to the rapid vascularization vital to the support of malignant tumors, coupled with imperfect lymphatic drainage, allows the EPR effect. The tumor vasculature is leaky and also irregular in diameter, shape, and density with discontinuous vessels. This results in several conditions, including heterogenous perfusion in the tumor, elevated interstitial fluid pressure from the extravasation of fluid, hypoxia, and an acidic environment [5]. EPR-based drug delivery depends on various factors, including circulation time, targeting, and the ability to overcome barriers, which are dependent on size, shape, and surface properties of the drug particles. Passive-targeting is mainly based on a diffusion mechanism. As a result, size is a crucial factor in the EPR-dependent delivery process. Studies have indicated that a nanoparticle size range of approximately 40 to 400 nm is suitable to ensure long circulation time, and enhanced accumulation in tumors with reduced renal clearance [6]. Shape and morphology also play important roles in passive targeting. Generally, rigid, spherical particles of size ranging from 50 to 200 nm have the highest tendency to long circulation, to avoid uptake by liver and spleen, but large enough to avoid kidney clearance [7][8]. Surface properties play a critical role in determining the internalization process of the drug particles into the target cell. To avoid opsonization and subsequent clearance by the RES, surface modification of polymers using PEG can be effective to a certain extent. Thus, the EPR-based drug delivery can be modulated by modifying the size, shape and sometimes by surface alteration of the nanoparticles.

Currently, a number of passively targeted nanoparticles are in clinical use including, Abraxene, Doxil, Marqibo, Myocet, and DuanoXome. Many other nanoparticles have shown promising therapeutic efficacy in clinical trials.

Major drawbacks of passive targeting include the inability to distinguish between healthy and diseased tissues, inadequate tumor accumulation, intra- and inter tumoral as well as inter-individual tumor heterogeneity. Different vascular and TME parameters contribute to the heterogeneity in EPR-mediated nanoparticle accumulation. These include vascular permeability, endothelial cell receptor expression, and vascular maturation at the vessel level. Several stromal parameters, including extracellular matrix (ECM), tumor cell density, hypoxia, and interstitial fluid pressure, contribute to heterogeneity in EPR-based tumor targeting responses. All of these pathophysiological parameters are factors necessary to be taken into consideration for the development of personalized and improved nanodrug treatments using the EPR effect. The extent of tumor accumulation always varies between tumor types, and in patients, making it necessary to determine the EPR effect. Thus, the application of direct and indirect imaging and other technologies is necessary to evaluate the degree of the EPR-effect. The presence of an EPR and non-EPR tumor in the EPR and non-EPR patients may help improve the EPR-based drug delivery systems for success in the clinic.

Dense cancer stroma is a critical component of the TME, where it has crucial roles in tumor initiation, progression, and metastasis. Most anticancer therapies target cancer cells specifically. However, tumor stromal factors can promote resistance of cancer cells to such therapies, ultimately resulting in deadly diseases such as PDAC (pancreatic ductal carcinoma). Therefore, novel anticancer therapies should be a combination of anticancer and anti-stroma therapeutic agents [9]. Approaches have been made to enhance the EPR-targeted drug accumulation to the tumor while considering cancer stromal barriers. For instance, in the use of the ADC (antibody-drug conjugate) drugs with a scaffold for cancer (CA) stromal (S) targeting (T) (CAST) [10]. In CAST therapy, stroma-targeting immunoconjugates bound to the stroma generate a scaffold, from which controlled release of cytotoxic drugs occurred and afterward diffused throughout the tumor tissue to damage both tumor cells and tumor vessels. The CAST therapy was thus reported as a new mode of cancer therapy, especially for refractory, stromal-rich cancers. Since the first CAST therapy was reported over 10 years ago, there have been several appreciated experimental studies and review works supporting and promoting CAST therapy [11][12][13][14][15][16][17][18].

Several strategies to overcome heterogeneity in EPR-based tumor accumulation can be employed to improve nanoparticle-based cancer treatments, including enhancing, combining, bypassing, and imaging. Enhancing pharmacological and physical means such as radiotherapy, hyperthermia, and sonoporation can be used to enhance the EPR effect. Combining active targeting with a pharmaceutically active ligand and the drug molecules encapsulated within a nanoparticle formulation can improve the EPR effect in a targeted tumor. Bypassing the EPR effect in cases of tumors with low or non-EPR, vascular targeting, or the use of triggerable nanocarriers to release the payload intra-vascularly can be used to enhance dr accumulation in spite of a low or non-EPR effect. To address the heterogeneity in EPR-mediated tumor targeting, direct or indirect imaging techniques, employing either nanotheranostics or companion nano-diagnostics to monitor the biodistribution and tumor accumulation or using standard imaging probes and protocols to visualize tumor blood vessels and the TME are required. Further, EPR-based tumor targeting can help to pre-select a patient for individualized therapies [19][20][21][22].

Thus, complementary active targeting with passive targeting, enhancing circulation, tumor accumulation, drug penetration in the target cell and finally release into the cytoplasm for action through circulation, accumulation, penetration, internalization, and release (CAPIR) cascade to improve the EPR effect is necessary for the development of effective cancer therapy and its translation to the clinic [23].

2. Passive Versus Active Tumor Targeting

Active targeting was at first employed to enhance the EPR-based drug delivery as a complementary approach with passively targeted drugs to improve tumor accumulation by nanoparticles to increase targeting efficiency and enhance their retention at targeted tumors [21]. Passively targeted drugs, which are dependent on the EPR effect, may not be sufficient to achieve effective targeting at target sites. However, a meta-analysis of preclinical data indicated that a median of only about 0.7% of injected dose (ID) of nanoparticles actually reaches the target tumor [21]. Several pre-clinical studies have also shown that only 0.1 to 0.2% of the ID are effective against cancer cells and show anticancer therapy with significant patient benefit [20][21].

Active targeting approaches are necessarily much more complex than a passive one. Several challenges associated with these active targeting strategies include physiological barriers and tumor heterogeneity and complex design and engineering needed for these drug delivery systems. The latter may pose major challenges and complicate pharmaceutical development and scale-up under GMP production and, significantly, to the overall cost of the therapy. In spite of several difficulties, one major advantage of active targeting is the ability to target sites disseminated throughout the body, including hematological malignancies and metastatic cancers in which the EPR is not effective [21].

Both passive and active targeting have their own limitations. To ensure clinical success of active targeting, pre-clinical tumor models need to be significantly improved to ensure effectiveness against diseases including solid tumors, hematological malignancies, and metastasis. There are significant barriers to passive targeting resulting in very low tumor accumulation leading to reduced therapeutic efficacy. Passive targeting may not distinguish between normal and diseased tissues. On the other hand, in cases of active targeting, increasing accumulation into tumor cells cannot guarantee the delivery of desired therapeutic agents to the target cells, as drug release may be hindered by the components within the cells. Moreover, endosomal escape of the drug and initiation of drug activating mechanisms is always challenging for targeted delivery. Conjugated nanoparticles may compromise the stealth capacity of the polymer because PEGylating may not be at a sufficient level. Encountering the tumor cell over-expressing receptors proteins without hurdles is a major limitation in targeted delivery. If the stealth properties of the nanoparticles are compromised, then the carriers may be rapidly uptaken/absorbed by the liver, spleen, and other RES organs, resulting in a very low accumulation of drugs in the target tumor.

For both passive and active targeting approaches, the development of companion diagnostic imaging technologies to evaluate the targeting efficiencies is very important. Selection of suitable patients and modifying treatments for specific patients may improve tumor accumulation, efficacy, and therapeutic outcome reducing the adverse effects,

unnecessary treatments, and overall health expenses. Finally, active targeting can be used to complement passive targeting for better treatment results.

3. Factors Affecting the EPR Effect

The EPR effect has been observed by researchers working in cancer therapeutics for a long time. The preferential accumulation of these nanoparticles in the tumor region is a much more complex aspect than initially envisioned. This process includes several biological processes, including angiogenesis, hemodynamic regulation, vascular permeability, lymphangiogenesis, and heterogeneity of the tumor microenvironment. There is a lot of subject-to-subject variabilities related to these above-mentioned factors. The accumulation of the nanoparticles also depends on various factors, such as the physicochemical properties of each material. A rapidly growing tumor needs an enhanced blood supply as the blood vessels surrounding the tumor are enough to provide the oxygen required for cell growth. New blood vessels are formed to meet the nutritional demands of the tumor cells [24]. The process of angiogenesis surrounding the tumor is rapid, and due to this rapid growth, the blood vessels are irregular with discontinuous epithelium and lack of a basal membrane, constituting a leaky vasculature with fenestrations of 200 to 2000 nm [25][26][27]. This allows enhanced permeation of the blood components as it reduces resistance to extravasation into the tumor interstitium.

Unlike normal tissue, tumors have defective lymphatic drainage resulting in minimal uptake of their interstitial fluid [28]. Molecules smaller than 4 nm can be reabsorbed and diffuse back into the circulation, whereas the diffusion of larger nanoparticles is hindered by their hydrodynamic radii, which results in the accumulation of these nanoparticles in the tumor interstitium [29][30][31].

4. Heterogeneity of EPR: A Clinically Relevant Phenomenon

In the last couple of years, research reports citing nanocarriers and EPR effect-based therapies have been increased markedly. The basic rationale for tumor targeting via EPR effect has been presented in thousands of research papers that claim improved therapeutic potentials and consider this phenomenon a royal gateway. However, at present, scientists and oncology specialists are of a view that these therapies are failing in the clinic and that the EPR effect is misinterpreted and overrated. This approach, “one size fits all,” worked in lab animal tumor models but not in humans, possibly because they were transient in nature, thus limiting the bench to bedside translation of most targeted tumor therapies. The heterogenous outcomes of clinical trials have led to a new understanding that the EPR effect varies greatly between lab animals and humans as well as among different tumor types and metastases within the same individual. To address the complex nature of the EPR effect, the research is now moving towards a custom-fit approach to personalize the patient therapy for better outcomes and to identify the most responsive patients from clinical trials [20][32][33][34][35].

Human tumors differ greatly from animal tumors with respect to the rate of growth, size of the tumor, tumor-to-body weight ratio, and heterogeneity of the tumor microenvironment that collectively alters the pharmacokinetics of most

drugs. The degree of tumor heterogeneity varies in different types of tumors as well as with the same types of tumors in different patients. Thus, complete control and performance monitoring throughout therapy might help develop successful clinical trials [36][37].

5. Strategies to Overcome Heterogeneity

Various treatment modalities based upon specific pathophysiology of tumor and EPR effect have been proposed with more than 7350 citations over the first report of EPR (as of June 2021 from Google Scholar). The CAST therapy received considerable attention from researchers after the successful development of new strategies to achieve highly localized concentration of topoisomerase-1 inhibitor, SN-38, conjugated with monoclonal antibody (mAb) targeted against collagen-4. This newly developed immunoconjugate was optimized to bound with stromal collagen creating a scaffold with sustained release of anticancer agent [13][14]. Gebleux and coworkers proposed non-internalizing antibody drug conjugates, ADC, that rely on extracellular release of drug thus preventing antigen barriers [15]. ADC might overcome heterogeneity of tumors by utilizing TME to facilitate cleavage of linkers and payload release [16]. Tumor endothelial marker-8 (TEM-8) is overexpressed in perivascular stromal cells and can be used as a useful stromal target for locally triggered drug release from anti TEM-8 ADC [17]. The heterogenous antigen distribution in malignant cells and the difference in targeted gene copy number among patients are serious challenges for researchers, and a single mAb may not be effective for all patients [14].

Many approaches have been proposed for mAb-based tumor targeting and mechanisms to overcome therapeutic resistance that is caused by the heterogeneity of tumor antigen and also the resistance executed by TME, including inefficient delivery to the tumor, alteration of effector functions in the TME, and Fc-γ receptor expression diversity and polymorphism. mAbs-based therapies are potential approaches to overcome these barriers using several diagnostic and prognostic biomarkers for envisaging response to mAb-based therapies [18].

EPR-effect has been proved by many preclinical animal models. However, results obtained from animal models are usually conflicting with clinical observations. Unlike hematological malignancies, in solid tumors, administered anticancer agents (ACA) must diffuse through the tumor mass, overcoming cancer stromal barriers and tumor mass itself. It has been demonstrated that hypercoagulability caused by cancer stroma, and the more aggressive cancer, the greater the deposition of insoluble fibrin (IF) in cancer tissue [14]. An ant-IF mAb was developed and conjugated with an ACA using V-L-K linker. The resultant ADC drug linker is degradable by plasmin. The plasmin is activated during the IF formation only. ACA is released from the ADC drug particularly when the conjugate binds to the IF. This novel approach was beneficial to deliver ACA to tumor cells through the stromal barrier due to the small size of the drug [14].

Numerous strategies have been used to modify the abnormal tumor microenvironment in humans by combination with nanomedicines. The direct permeability enhancement by various methods has been explored that take advantage of the EPR effect and facilitate the delivery of drugs/macromolecules inside tumors. Examples include the selective inhibition of angiotensin-converting enzymes [38][39], generation of NO or CO within tumors [40][39][41],

blockage of VEGF and other angiogenic signaling factors [42][43][44], inhibition of pericyte recruitment and BM activation [45][46], and image guiding systems [47].

The recent advancements and technological innovations have allowed novel insights into the drastic differences between murine and human cancers that can hamper the clinical translation of tumor-targeted nanotherapeutics. The laboratory-established models are not true representatives of human cancer in many respects and require modifications to explain the heterogenous events responsible for compromised EPR effects in humans. To maximize the clinical outcomes of investigational cancer therapeutics, new strategies to mimic the individual tumors are required that closely recapitulate the patients' responses to preclinical drug testing [48][49]. This approach provides the potential for guided clinical decision-making in translational cancer research by individual performance metric calculation. Tailoring the cancer therapy to patient groups that are more prone to respond and benefit from the investigational treatment offer a potential solution to overcome the heterogeneity of the EPR effect. Patient-derived tumor xenografts (PDX) involve the engraftment of specific tumor tissues in immunocompromised mice. Izumchenko et al. integrated PDX models via implantation of 92 different solid cancers from a 237 cohort of patients into immunodeficient mice. They analyzed and compared the patient responses and PDX models after whole exome sequencing. Their findings suggested that these models accurately replicated the patient outcomes over a repetitive course of therapy, enabling an oncologist to assess the patient-specific cellular events [50]. The mouse models offered numerous benefits, such as their small size, ease of reproduction, transgenicity, and closely mimicked physiology. However, various limitations involving mice such as high cost, complex genetic manipulations, and prolonged duration of experimentation have forced researchers to utilize alternatives. Numerous current publications reported the use of chick chorioallantoic membrane (CAM) and Zebrafish for implantation as alternatives to mice. Hu and coworkers demonstrated that CAM is an efficient system to analyze pilot drug responses in patients with bladder tumors, accelerating the discovery of critical molecular mechanisms [51]. Mercatali et al. studied the metastatic potential of breast cancer after injecting primary culture of bone metastasis derived from a 67-year-old patient into zebrafish embryos. Their findings suggested zebrafish are a suitable substitute for mouse models and provide for a better understanding of chemotherapeutic sensitivity and prognostic marker identification [52].

6. Targeting Tumor Tissues via an EPR Effect

Since the discovery of the EPR concept, it has been utilized widely for many applications (Figure 1), especially for the delivery of anticancer drugs. The EPR effect helps promote a favorable biodistribution of nanoparticles in blood and a high level of nanoparticle accumulation in solid tumors. However, for the optimal development of nanoparticles for enhanced drug delivery by EPR effect, multiple factors should be considered, including blood half-life of nanoparticles, minimal nonspecific delivery, and effective elimination from the body [53].

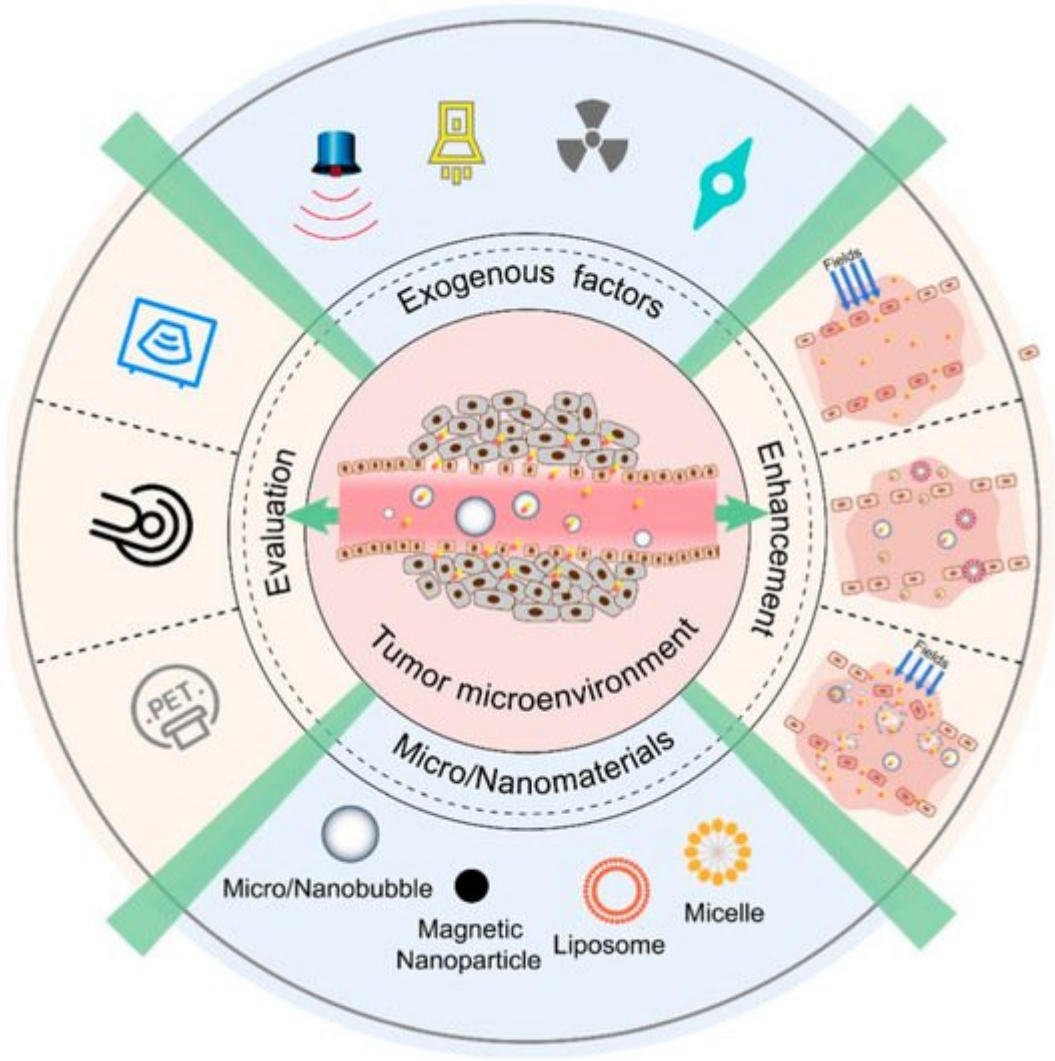

Figure 1. Common strategies utilizing the EPR effect [54].

The EPR effect discovery was a milestone in drug delivery systems, and expectations for utilizing this effect in a selective anticancer drug delivery were high. However, the transition of nano-drug delivery medicine from benchtop to clinic has been very difficult. An EPR effect-mediated drug accumulation has been proved with various natural and synthetic molecules with molecular sizes greater than 40 kDa or 7 to 8 nm in diameter. Encapsulation of small molecules inside macromolecular vehicles, including liposomes, nanospheres, or polymeric micelles, led to full utilization of the EPR effect and made it a universal method for targeting the tumor side known as passive targeting. The characteristics of the EPR effect are at disposal for this method of targeting, including (i) defective architecture of blood vessels, known as a “leaky vasculature,” with large gaps (around 400 nm) between capillary endothelial cell linings; (ii) overproduction of vascular mediators including bradykinin and nitric oxide [NO]); and (iii) improved retention of the macromolecules in tumor tissue due to impaired lymphatic recovery [55][56].

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