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Perspective

Enhanced transcytosis and retention (ETR) effect

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The enhanced permeability and retention (EPR) effect, first proposed by Maeda and co-workers [1] in 1986, has been considered as a classic principle for decades in the field of nano drug delivery for oncology. This theory, though recently controversial, has been frequently used to interpret the accumulation of nanoparticles within various experimental tumor tissues [2–4]. EPR effect is primarily attributed to the structural abnormalities of tumor vasculature (*a.k.a.*, leakiness), which result in increased blood vessel permeability. However, emerging evidence indicates that not all tumor vasculature exhibits this leakiness, particularly in human cancers that develop over several years, unlike the more rapidly growing murine experimental tumor models. Even within animal tumor models, variability in vascular permeability exists. Our observations have highlighted certain tumor models (e.g., human KB-31 subcutaneous xenograft) often demonstrate pronounced vascular leakiness [5]. This is characterized by compromised vessel integrity, low pericyte coverage and round-like vascular structures, thereby facilitating substantial nanoparticle accumulation within the tumor tissue likely through EPR. Conversely, tumors such as pancreatic ductal adenocarcinoma (PDAC), triple-negative breast cancer (TNBC), and certain types of lung cancer frequently exhibit collapsed vasculatures with relatively high vessel integrity, often accompanied by high pericyte coverage [6]. These characteristics significantly impede nanoparticle delivery due to diminished vascular leakiness, presenting a considerable challenge in the effective application of the EPR effect for these cancer types.

Recent studies including ours underscore the pivotal role of transcytosis in augmenting nanoparticle delivery within stroma-rich tumors such as PDAC and TNBC (Table S1 online). The dense stromal and composition in the tumor microenvironment significantly diminishes the efficacy of the conventional EPR effect. Under physiological condition, transcytosis plays a crucial role in various processes by enabling the transport of macromolecules across cell interiors. This process facilitates nutrient and antibody absorption in the intestines [7], regulates the selective permeability of the blood–brain barrier [8], and supports immune defense by transporting antibodies across epithelial barriers [9]. Additionally, transcytosis is essential during pregnancy for transporting maternal antibodies and nutrients to the fetus [10]. In endothelial cells, it helps maintain vascular homeostasis by transporting proteins, lipids, and signaling molecules, etc. For example, albumin binds to specific receptors such as the albumin-binding protein gp60, triggering vesicle formation and facilitating its transendothelial passage [11]. Vascular endothelial growth factor (VEGF) is similarly transported via receptor-mediated transcytosis, binding to VEGFR-1 and VEGFR-2 on the endothelial surface, which leads to its internalization and transport. Transferrin, essential for iron transport, binds to the transferrin receptor on endothelial cells, enabling its transcytosis and subsequent iron delivery to tissues including passing through blood–brain barrier [12].

We propose considering pharmacological, nutritional, and biochemical approaches to enhance the robustness of transcytosis as a novel strategy for advancing (nano) drug delivery in stroma-rich solid tumors (Fig. 1a–c). The targeted cancer indications are those with limited vasculature leakiness and consequently poor EPR effect. To distinguish this mechanism from the traditional EPR effect, we introduce the term "Enhanced Transcytosis and Retention" (ETR) effect. We prefer this abbreviation also because it evokes the English word "enter" (Fig. 1a). Representative transmission electron microscopy (TEM) pictures were provided to show the morphological feature of nanoparticle transcytosis at solid tumor site (Fig. 1b).

To fully understand and utilize ETR, several experimental requirements are needed. It is crucial to introduce stringent tumor models, such as orthotopic models, spontaneous tumor, and patient-derived xenograft model, to accurately mimic the tumor microenvironment observed in patients. Methodologically, it is essential to employ a comprehensive approach to study nanoparticle transcytosis. TEM provides detailed morphological insights, including filopodia formation at lumen side, intracellular nanoparticle transport within endothelial cells (ECs), and particle exocytosis. Complementing TEM, flow cytometry coupled with sorting technologies allows for the precise isolation of nanoparticle-containing ECs, facilitating the detection of intracellular (transcytosing) nanoparticles including related singling cascade. Moreover, introducing experimental conditions such as the "zombie" model (disabling ATP-mediated active transport through ECs) and pharmacological inhibitors (blocking transcytosis pathways) can indirectly validate nanoparticle transcytosis. These integrated methodologies offer a robust framework for advancing our understanding of nanoparticle transport mechanisms.

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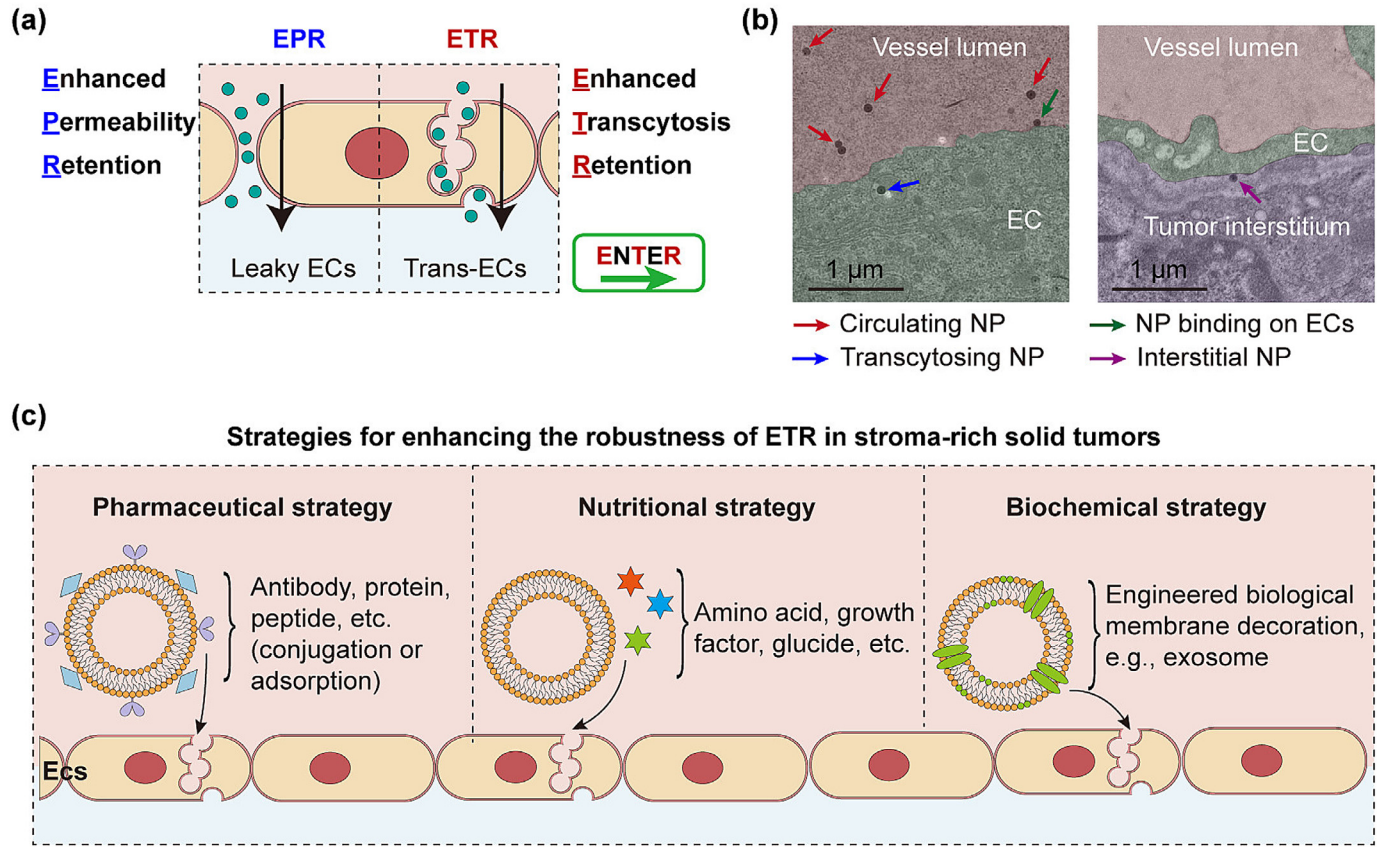

Fig. 1. Schematic illustration of the EPR effect versus the ETR effect. (a) The EPR effect is characterized by passive diffusion through leaky endothelial cells (ECs), whereas the ETR effect involves transcellular transport that does not depend on vascular leakiness. (b) Representative TEM picture for nanoparticle (NP, lipid coated silica model nanoparticles) transcytosis at KPC cancer site. (c) We advocate the use of pharmacological, nutritional, and biochemical strategies to achieve efficient ETR outcome. This would provide a novel and effective approach for drug delivery in tumor types with poor EPR effect.

Identifying endogenous or external stimuli at the nano/bio interface, including sensor mechanisms that can trigger ETR, is essential. ECs express a variety of receptors, including integrins, that play a pivotal role in nanoparticle recognition and internalization processes. Integrins, known for their involvement in cell adhesion and signaling, can mediate the binding of nanoparticles to EC surfaces and facilitate subsequent cellular uptake pathways. The specificity of integrin-nanoparticle interactions, which are largely unknown, is influenced by nanoparticle surface properties and the activation state of ECs, impacting the efficiency and specificity of nanoparticle delivery across EC barriers. Therefore, we believe that elucidating the interplay between integrins and nanoparticle recognition mechanisms in ECs is essential for optimizing nanoparticle-based drug delivery systems. This could yield profound impact in various disease contexts, such as cancer and more. For example, our previous work [6] demonstrated that the iRGD peptide enhances transcytosis by targeting integrin/NRP-1 pathways and activating transcytotic transports. Surface modifications of nanoparticles, such as using albumin coating, can promote interaction with endothelial receptors and enhance transcytosis. However, further research is still required to identify such tumor-specific targets that can improve drug access therapeutically without affecting normal organs.

Importantly, our aim is not to replace the EPR effect with ETR effect, as both mechanisms can coexist within the same cancer type or even within the same patient. Even within the EPR effect, additional barriers, such as the basement membrane barrier, may prevent particle extravasation [13]. We propose leveraging both effects synergistically to optimize therapeutic outcomes. Identifying patient populations with specific tumor microenvironment characteristics that predict favorable drug delivery outcomes could significantly improve the precision of cancer treatments. This is particularly important in the highly fatal and aggressive disease such as PDAC. The advocated approach would move beyond the empirical testing currently prevalent in oncology clinics, towards a more personalized and predictive model. By incorporating advanced diagnostics to assess the likelihood of EPR and/or ETR effects in individual patients, one can tailor treatment strategies that maximize drug delivery efficiency and therapeutic efficacy.

We also want to briefly comment on precision medicine in cancer (nano) treatment, which tailors medical care to the individual characteristics of each patient, thereby enhancing the effectiveness of therapies and minimizing side effects. Key technological contributors include genomic profiling to identify genetic mutations and select targeted therapies, as well as the use of biomarker analysis and pharmacogenomics to guide treatment decisions and personalize drug selection and dosing based on a patient's genetic information. Liquid biopsies offer a non-invasive method to monitor tumor dynamics, while multimodality imaging, which combines techniques such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET), provides comprehensive tumor characterization. Additionally, AI leverage big data and machine learning to predict treatment outcomes and guide personalized treatment plans. However, in this chain reaction, a fairly weak piece is the accurate prediction of drug accessibility and delivery. We envision that

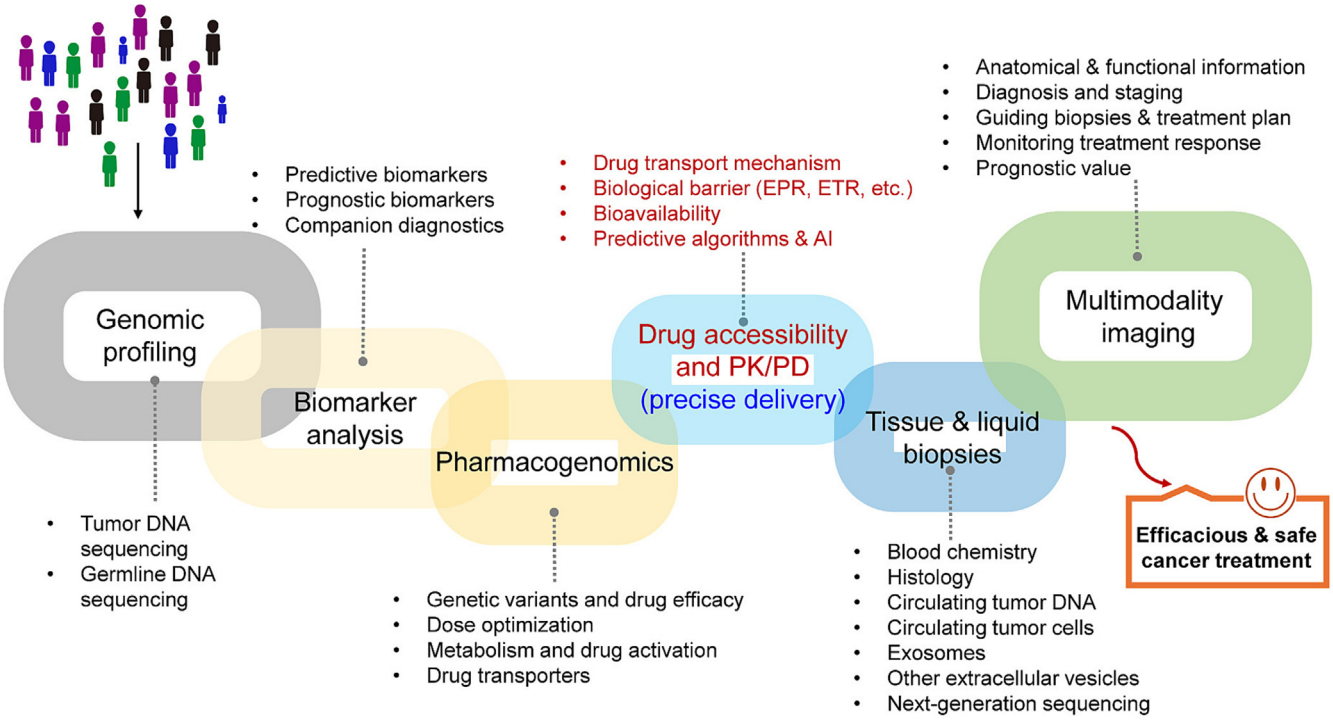

Fig. 2. Ideal workflow for precise cancer treatment, which requires consideration of the drug delivery mechanism and its ability to overcome biological barriers such as tumor endothelial cells.

selecting a rational delivery mechanism, in this case, a nanocarrier, can enhance pharmacokinetics and biodistribution at the tumor site. As shown in Fig. 2, the ideal precision workflow should include the experimental selection of active pharmaceutical ingredients, the rational choice of delivery mechanisms capable of overcoming various biological barriers, and the use of complementary imaging and biochemical techniques for monitoring before, during, and after treatment. This integrated strategy forms the foundation for achieving high overall treatment efficacy while minimizing adverse effects, thus advancing the field of oncology by providing more personalized and effective therapeutic options.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary materials

Supplementary materials to this perspective can be found online at https://doi.org/10.1016/j.scib.2024.10.003.

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