BIO Integration

The Entry Mechanism of Nanoparticles Determines the Future Direction of Nanomedicine Development

Phei Er Saw

Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China

Medical Research Center, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China

*Correspondence to: Phei Er Saw
E-mail:

Received xxxx xx, xxxx
Accepted xxxx xx, xxxx

Available at:
http://bio-integration.org/

According to the classic theory of enhanced permeability and retention (EPR) proposed by Maeda in 1986, nanoparticles passively enter targeted tumor cells via gaps between endothelial cells (inter-endothelial gaps) in tumor blood vessels. This theory implies that enhanced localization of nanoparticles at the tumor site are due to the endothelial pores created through angiogenesis. At that point, it was known that tumor cells possess high angiogenesis to create neo-blood vessels to aid their survival and growth. The exponential growth of blood vessels is created at the expense of their structural integrity, resulting in nonhierarchical, messy, and incomplete vessels. Since these pores, ranging in size from nanometers to micrometers, only occur near tumors, this became the manipulation point for scientists to specifically deliver nanoparticles into the tumor vicinity. The study of Maeda et al. focused on the question of how nanoparticles enter tumors by carrying out detailed visualization and data quantification on specific areas, such as blood vessel gaps in tumors, accumulation and localization of nanoparticles in tumors, and on the comparison of the transendothelially transported rate of nanoparticles to the rate of diffusion across blood vessel gaps [1]. Through this study, they claimed that the main mechanism of nanoparticle tumor entry, represented by gold nanoparticles (GNPs), is through the transendothelial openings. They analyzed 313 blood vessels across several tumor models, such as U87-MG glioma, 4T1 breast cancer, MMTV-PyMT breast cancer, PDX breast cancer, and so on, and found only 26 gaps, which implied that gaps occurred at a very low frequency in all the tumor types mentioned above, which is consistent with the knowledge that gaps are also rare in tumor vessels from cancer patients. Furthermore, they demonstrated that only 7 (26.9%) out of 26 openings were intercellular gaps, whereas the rest were transendothelial pores. In fact, in 1997, researchers at Harvard University proposed that the endothelial cell gaps that were induced by vasoactive mediators were mostly transcellular pores [2]. Dvorak et al. [3] defined a novel endothelial cell organelle in the endothelial cells of both humans and animals, termed the vesiculo-vacuolar organelle. It participates in the regulation of intracell transportation through the endothelial cells of soluble macromolecules. In this regard, there is structure basis for nanoparticles to enter tumors through trans-endothelial pathways. According to this article, the extravasation of nanoparticles shows no significant relevance with tight junction, fenestra, vesicle, endothelial gaps, or transcellular pathways, which infers that nanoparticles primarily enter tumors by active transportation. Based on the above, there are two possible ends when nanoparticles enter the circulation. The first one is that nanoparticles are also capable of entering other organs by crossing the endothelial cells. Feng et al. [4] showed that colloidal carbon traversed endothelial cells via the development of pores that did not communicate with or disrupt intercellular junctions by gap formation in permeability factor-exposed normal vessels. If the hypothesis that "nanoparticles enter the normal vessels" is proven to be true, this may help explain why the efficiency rate of nanoparticle delivery to tumors was lower than 1% in clinical experiments [5]. However, this theory seems unable to explain why nanoparticles show different accumulations in major organs, especially when the accumulation is not positively correlated with the number of blood vessels. It suggests that the blood vessels in different organs might show different active transportation abilities, leading to the other possibility, that is, the

BIOI xxxx 2020, Vol 1, No. 1, 0001– 0003

endothelial cells in tumor regions develop stronger active transportation ability than normal blood vessels.

Hida et al. [6] reported that endothelial cells in tumors exhibit characteristics different from those of normal vascular endothelial cells in terms of cell proliferation, migration ability, gene expression, and response to growth factors and drugs. Akino et al. [7] proposed that, in human clear cell renal cell carcinoma, 22% to 58% (median, 33%) of tumor-associated endothelial cells are aneuploid, whereas normal endothelial cells are diploid. In contrast with normal vascular endothelial cells, tumor endothelial cells express epidermal growth factor receptor, which activates the mitogen-activated protein kinase signaling pathway and accelerates the proliferation rate of tumor cells. Meanwhile, tumor endothelial cells do not express *ErbB3*, a gene that encodes for a protein that inhibits endothelial cell growth by coupling with neuregulin ligands. Therefore, tumor endothelial cells proliferate faster than normal vascular endothelial cells [8]. In this regard, it could be inferred that the endothelial cells in tumors might have stronger active transportation ability due to their faster proliferation. Further experimental investigations are needed to make this conclusion, which reveals one problem in current nanomedicine research: the lack of mechanism research. In December 4, 2019, an editorial, titled "The Two Directions of Cancer Nanomedicine," was published in *Nature Nanotechnology* [9]. This editorial discussed the two future directions of nanomedicine development, which are acceleration of the clinical transformation of nanomedicine and increasing research on the mechanism of action of nanomedicine. One reason that scientists are advocating for the latter is that the efficiency of nanomedicine delivery *in vivo* remains very low. According to the theories suggested in the article, if the active transportation of nanoparticles occurs in both normal and tumoral blood vessels, the advantages of the proposed nanomedicine, such as lower systemic toxicity, will either be negligible or not so ideal anymore. Therefore, research and development in nanomedicine should focus on the mechanism of action first, in order to build a stronger understanding of the interaction between drugs and the body, and then followed by pursuing the acceleration of clinical transformation, for example, studying the physical differences between normal and tumor-associated endothelial cells in order to improve the active transportation ability of tumor blood vessels, therefore increasing the accumulation of nanoparticles in tumor. In addition, endothelial cellular transportation needs to be taken into consideration in the research and development of responsive-type nanomedicine. At present, many different types of responsive nanocarriers have been developed according to specific tumor microenvironments, including pH-, hypoxia-, and reactive oxygen species (ROS)-responsive nanocarriers. The characteristics of tumor microenvironments are highly related to the rapid proliferation of tumor cells. Hence, the rapid proliferative behavior of tumor endothelial cells may trigger prompt response from the nanocarriers, which in turn affects the ultimate tumor accumulation. Hojo et al. [10] showed that ROS accumulated in the blood vessels of human tumor tissues including hepatocellular, colorectal, and renal cell carcinoma, compared with normal counterparts. There reports showed that contact with cancer cells induced a rapid increase of ROS in human umbilical vein endothelial cells [11, 12]. However, a few reports have shown that pH, glutathione, and other substances can trigger the release of nanoparticles. Overall, it is necessary for us to explore the dynamic of nanoparticles in tumor and normal endothelial cells.

This article is of great significance. It provides a profound horizon for nanomedicine, leading us to focus research on the mechanism of nanoparticle activity instead of clinical transformation itself. It reminds us that only when we have a deep understanding of the mechanism can we design better nanoparticles to achieve efficient clinical transformation. Surely, some questions remain. For example, although it is known GNPs enter tumors transendothelially, the understanding of the entry of other nanocarriers into tumors is insufficient. Nanocarriers come in many forms including high-molecular-weight polymeric nanoparticles, liposomes, virus-like particles, iron oxide nanoparticles, and water-based nanogels, to name a few. Besides GNPs, do other nanoparticles also enter the tumor via transendothelial openings? Why were the nanoparticles released polarly from the opposite end of endothelial cells after entering from one end? Why were they not released adjacently or retained in the endothelial cells? All these unanswered questions demand further in-depth research on the mechanism of nanomedicine.

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