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Nanoparticles squeezing across the blood–endothelial barrier via caveolae

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Nanomedicines have a potential to shift the paradigm of medicine. Therapeutics coupled with nanoparticles can be tailored for personalized medicine because they can precisely deliver multiple therapeutics to the targeted cells in diseased tissue. Thus far, nanotechnology has made progress in design and manufacture of therapeutic nanoparticles in drug delivery [1]:

- Improved efficiency of delivery of poorly water-soluble drugs;
- Potential for targeted delivery of drugs to cells based on binding to unique receptors or *de novo*-expressed receptors;
- Co-delivery of multiple therapeutics in a single nanoparticle;
- Visualization of sites of drug delivery using imaging techniques;
- Real-time readouts of therapeutic efficacy *in vivo*.

Toxicity of administering nanotherapeutics, however, presents a fundamental challenge for translating nanomedicines to reality. Most nanoparticles are taken up by the reticulo-endothelial system of the liver rather than diseased organs even though nanoparticles may be designed for targeting of the diseased tissues [2]. A primary reason for this is that we have

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little knowledge of transvascular trafficking of nanotherapeutics *in vivo*: that is, at the molecular level, the fundamental question is how nanotherapeutics interact with endothelial cells lining the lumen of blood vessels in order to avoid the liver and only distribute in diseased tissue. To target a specific organ, the nanoparticles must breech the endothelial monolayer lining the vessels of the organ. The endothelial monolayer presents a real barrier for the transport of nanoparticles because openings of inter-endothelial junctions (the gaps between contiguous endothelial cells) have an average size of 3 nm [3]. The other less well known is the antigen profile of each organ vascular bed. Having a specific antigen address (e.g., the lung endothelium) would allow the nanoparticles to deliver drugs specifically to that organ [4].

Targeting of anticancer drugs using nanoparticles has been proposed based on the hypothesis that nanoparticles can be transported readily across the leaky vasculatures of tumors because of increased endothelial permeability of tumor blood vessels [1,2]. While the tumor vascular endothelial barrier has been considered to be leaky [5], recent studies using defined size of dendrimers [6] showed that the endothelium deep in solid tumors was, in fact, restrictive with the gaps of less than 12 nm, and, hence, it represents a barrier for the transport of protein-based drugs and nanotherapeutics. Thus, endothelial permeability of different organ vasculatures, including solid tumor vessels, likely depends on both size and concentration of nanoparticles due to the inherent restrictiveness of the endothelial junction barrier [3]. This restrictiveness depends on the organ (e.g., the brain endothelial monolayer, which is impermeable to protein), whether the endothelium is continuous or noncontinuous, and whether it is fenestrated or not [7]. The blood–brain barrier presents the most restrictive, based on its highly developed tight junctions consisting of claudins [8]. We have recently described the transport of nanoparticles not through inter-endothelial junctions, but rather across endothelial cells via the transcellular caveolar pathway. This caveola-based pathway is present in endothelial cells of different organs and cancer tissue, and its function is to transport proteins and other macromolecules into tissues [9].

What are caveolae? Caveolae are flask-shaped invaginations of the plasma membrane, which, interestingly, occupy approximately 70% of the total endothelial membrane in blood capillaries [10]. They ‘bud’ or ‘pinch’ from the luminal endothelial cell plasma membrane and transport cargo to the basal side of the monolayer [11]. They have no protein coat unlike clathrin-coated pits [11]. The main protein lining the caveolar membrane is CAV1 [12], which helps to give caveolae their characteristic structure and serves as a scaffold for a variety of signaling molecules [13]. Approximately 144 molecules of CAV1 are present in a caveola [12]. Recent studies showed that CAV1, a protein, binds to CAV1, but the function is not yet defined [14]. Cholesterol, glycosphingolipids (e.g., monosialotetrahexosyl-gangliosides) and sphingomylin are enriched in caveolae compared with the plasma membrane [11]. Caveolae, thus, represent specialized, morphological entities that are stabilized by CAV1, and do not disassemble during the trafficking in cells [11–13].

Since the caveola size of 50–100 nm is in the range of nanoparticles that can be used to deliver drugs, we determined whether caveolae efficiently transport nanoparticles across the endothelial monolayer without disrupting the endothelial cell monolayer itself. For this, we used a high-resolution optical method to visualize a caveola in living endothelial cells interacting with nanoparticles. Using this approach we measured size and dynamics of caveolae. Albumin-conjugated fluorescence polystyrene nanoparticles were shown to be internalized by caveloae [15,16]. Thereafter, the caveolae loaded with nanoparticles pinched-off to form intra-cellular vesicles, which migrated to the basal membrane [15]. We also found that uptake of albumin-conjugated fluorescent polystyrene nanoparticles in cells was dependent on the particle size (20–100 nm nanoparticles used in the experiment) and required coating of particles with albumin [15]. Interestingly, albumin-coated nanoparticles

20 nm in diameter were preferentially transported versus particles 100 nm in diameter, since more of the smaller particles fitted into each caveola [15]. The albumin-coated nanoparticle size-selective uptake is consistent with the physical size of caveolae and albumin-binding proteins located in the caveolar membrane [17]. These results provide a rationale for using caveolae to deliver drugs conjugated to albumin-coated nanoparticles to underlying tissue. If specific organ antigens located in caveolae are determined, the antigen's ligands can be coupled to nanotherapeutics and guide the nanotherapeutics to the correct 'address' or 'zip code' (antigens) on endothelial cells of a specific organ.

The signaling mechanisms regulating caveola-mediated transcytosis are not well understood. Albumin appears to bind in a saturable manner to a 60-kDa glycoprotein (gp60) albumin-binding protein on the endothelial cell surface [17,18]. Binding to gp60 activated Src kinase resulting in phosphorylation of CAV1, gp60 and DNM2 (a 'pinchase' associated with the neck of the caveolae invagination) that initiated budding and release of caveolae [18]. The mechanisms of trafficking of caveolae to the opposite side of the vascular endothelial barrier and how caveolae avoid lysosomes are not known. When caveolar vesciles arrived at the basal membrane, caveolae fused to the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors on the basal membrane, where caveolae discharged their contents [19].

In summary, albumin nanoparticles of 20 nm in diameter are effectively internalized by caveolae and activate caveola-mediated transcytosis. While this pathway may be exploited for delivery of drugs and therapeutic proteins, there are important questions that need to be addressed. Among them is whether this approach would be effective in delivering nanotherapeutics, whether a correct address can be attached to the nanoparticles, and whether pharmacokinetics of drugs and proteins are enhanced by this mechanism. Further studies are needed to identify organ-specific caveola proteins that might provide the organ-specific address. Proteomics and electron microscopy are needed to determine the organ specific receptors located in the cavoelar membrane and identify these 'zip codes'. Future studies should also address the role of caveolae in mediating transport of therapeutic nanoparticles in the intact micro-circulation of animals. To address this question, it is required to establish advanced optically microscopic methods [20] combined with careful design of multifunctional nanoparticles (nanoparticle size, shape and bioconjugation). The studies will enable the rational design and manufacture of therapeutic nanoparticles targeted to caveolae for treating a wide range of cardiovascular and cancer diseases.

References

1. Faokhzad OC, Langer R. Impact of nanotechnology on drug delivery. ACS Nano. 2009; 3:16–20. [PubMed: 19206243]
2. Wang X, Yang L, Chen Z, Shin DM. Application of nanotechnology in cancer therapy and imaging. CA Cancer J Clin. 2008; 58:97–110. [PubMed: 18227410]
3. Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. Physiol Rev. 2006; 86:279–367. [PubMed: 16371600]
4. Giordano RJ, Edwards JK, Tuder RM, Arap W, Renata Pasqualini R. Combinatorial ligand-directed lung targeting. Proc Am Thorac Soc. 2009; 6(5):411–415. [PubMed: 19687212]
5. Hashizume H, Baluk P, Morikawa S, et al. Openings between defective endothelial cells explain tumor vessel leakiness. Am J Pathol. 2000; 156:1363–1380. [PubMed: 10751361]
6. Sarin H, Kanevsky AS, Wu H, et al. Physiologic upper limit of pore size in the blood–tumor barrier of malignant solid tumors. J Trans Med. 2009; 7:51–63.
7. Zetter, BR. Endothelial heterogeneity: influence of vessel size, organ localization and species specificity on the properties of cultured endothelial cells. In: Ryan, US., editor. Endothelial Cells. Vol. 2. CRC Press; Boca Raton, FL, USA: 1989. p. 63-79.

8. Sandoval KE, Witt KA. Blood–brain barrier tight junction permeability and ischemic stroke. Neurobiol Dis. 2008; 32:200–219. [PubMed: 18790057]

9. Komarova Y, Malik AB. Regulation of endothelial permeability via paracellular and transcellular transport pathways. Annu Rev Physiol. 2010; 72:463–493. [PubMed: 20148685]

10. Johansson BR. Size and distribution of endothelial plasmalemmal vesicles in consecutive segments of the microvasculature in cat skeletal muscle. Microvasc Res. 1979; 17:107–117. [PubMed: 449718]

11. Parton RG, Simons K. The multiple faces of caveolae. Nat Rev Mol Cell Biol. 2007; 8:185–194. [PubMed: 17318224]

12. Pelkmans L, Zerial M. Kinase-regulated quantal assemblies and kiss-and-run recycling of caveolae. Nature. 2005; 436:128–133. [PubMed: 16001074]

13. Pelkmans L, Kartenbeck J, Helenius A. Caveolar endocytosis of simian virus 40 reveals a new two-step vescular-transport pathway to the ER. Nat Cell Biol. 2001; 3:573–483. [PubMed: 11389442]

14. Hill MM, Bastiani M, Luetterforst R, et al. PTRF-cavin, a conserved cytoplasmic protein required for caveola formation and function. Cell. 2008; 132:113–124. [PubMed: 18191225]

15. Wang Z, Tiruppathi T, Minshall R, Malik AB. Size and dynamics of caveolae using nanoparticles in living endothelial cells. ACS Nano. 2009; 3:4110–4116. [PubMed: 19919048]

16. Wang Z, Tiruppathi C, Cho J, Minshall RD, Malik AB. Delivery of nanoparticle-complexed drugs across vascular endothelial barrier via caveolae. IUBMB Life. 2011; 63:659–667. [PubMed: 21766412]

17. Tiruppathi C, Song W, Bergenfeldt M, Sass P, Malik AB. Gp60 activation mediates albumin transcytosis in endothelial cells by tyrosine kinase-dependent pathway. J Biol Chem. 1997; 272:25968–25975. [PubMed: 9325331]

18. Minshall RD, Sessa WC, Stan RV, Anderson RG, Malik AB. Caveolin regulation of endothelial function. Am J Physiol Lung Cell Mol Physiol. 2003; 285:L1179–L1183. [PubMed: 14604847]

19. Predescu SA, Predescu DN, Malik AB. Molecular determinants of endothelial transcytosis and their role in endothelial permeability. Am J Physiol Lung Cell Mol Physiol. 2007; 293:L823–L842. [PubMed: 17644753]

20. Hidalgo A, Chang J, Jang JE, Peired AJ, Chiang EY, Frenett PS. Heterotypic interactions enabled by polarized neutrophils micro-domains mediate thromboinflammatory injury. Nat Med. 2009; 15:384–391. [PubMed: 19305412]