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AUTHOR'S VIEW

Major effect of transcytosis on nano drug delivery to pancreatic cancer

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ABSTRACT

We demonstrated that activated transcytosis is a major mechanism to complement the classic enhanced permeability and retention effect in pancreatic cancer. This was achieved by using an iRGD peptide that triggers transcytosis pathway at the tumor site. Co-administration of unconjugated iRGD substantially improved the effect of the chemotherapeutics delivering nanocarrier, and resulted in survival improvement in mice. Since the iRGD effect is commensurate with neuropilin-1 expression on tumor vasculature, it is necessary to contemplate a personalized approach to implement this technology.

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While nanocarrier mediated drug delivery is an exciting technology that promises a fundamental change in cancer treatment, most nanocarriers that enter into clinic rely on passive targeting, *a.k.a.* enhanced permeability and retention (EPR) effect, based on the big fenestration in tumor blood vessels.[1] Evidence shows that the effectiveness of EPR effect can vary case by case.[2] It is possible that either the carrier does not effectively reach the tumor or the nanocarrier reaches the tumor site but the drug-laden particle cannot extravasate from the tumor fenestration. Both effects result in insufficient tumor targeting. What's more, there are increasing concerns that the heterogeneity and presence of thick tumor stroma among different tumor types could lead to variation in the magnitude of the putative EPR effect due to differences in vascularity, vasculature structure or lymphatic drainage. Moreover, considerable amount of nanocarriers are particulates that can be effectively removed by reticuloendothelial organs. With this in mind, it is not a surprise that passive tumor targeting usually achieves approximately half to a few percentage of the total administered dose, with occasional exception that leads to greater than 10% administered nanoparticle dose delivered to a solid tumor.

Generally, the effectiveness of EPR effect is fairly low in pancreatic ductal adenocarcinoma (PDAC) when compared with other cancer types. This is partially because of the restricted vascular access to therapy (including nanomedicine) due to vascular abnormalities and the presence of desmoplastic stroma.[3] Though 2 nanocarriers, *i.e.* Abraxane® (paclitaxel/albumin complex) and Onivyde® (irinotecan liposome), were recently approved by FDA, these early-stage nano formulations (that rely on EPR for tumor targeting) only lead to ~2 months overall survival in PDAC patients.[4,5] PDAC develops a range of disease-specific barriers, including high pericyte coverage as well as high interstitial fluid pressure within tumors. This either physically blocks particle extravasation or promotes therapeutics outflow from the tumor, collectively resulting in a reduced bioavailability to tumor cells. In our recent study, we demonstrated a major mechanism to complement the classic EPR effect.[6] Unlike particle egression through tumor fenestration, we experimentally demonstrated the use of activated transcytosis to bypass vasculature barriers in PDAC, leading to an enhanced drug access at tumor site that is potent enough to show survival improvement in stringent PDAC animal models. This transcytosis mechanism has also been described by Dr. Erkki Ruoslahti.[7,8] We showed that the efficacy of an irinotecan-laden silicasome nanocarrier could be significantly improved by the co-administration of an unconjugated iRGD peptide that does not need to be attached to the carrier to enhance tumor uptake. Co-administration of the free iRGD peptide increased silicasome uptake at orthotopic PDAC tumors sites 3–4-fold, leading to enhanced killing of the primary tumor, metastasis inhibition and survival improvement.[6] The iRGD effect is mediated by interaction with tumor-associated integrins initially, followed by peptide cleavage and the release of the C-terminal end that engages neuropilin-1 (NRP-1) (Fig. 1).[7,8] Although the physiologic role of NRP-1 is to control transcytosis for nutritional purposes, the vesicular system can also be used for the transport of nanoparticles. Our effort on TEM visualization provided the 1st time ultrastructural evidence that iRGD could induce the appearance of grouped vesicles in endothelial cells, with the ability to carry Au-labeled silicasomes from the blood vessel lumen to the tumor matrix. Since the iRGD effect is commensurate with NRP-1 expression on tumor blood vessels in patient-derived xenografts, it is necessary to contemplate a personalized approach to implement this technology.

EPR effect is not prominent in PDAC for several reasons.[3] One of the reasons is the presence of pericyte that tightly adheres to vascular endothelial cells. To address the restricted vascular

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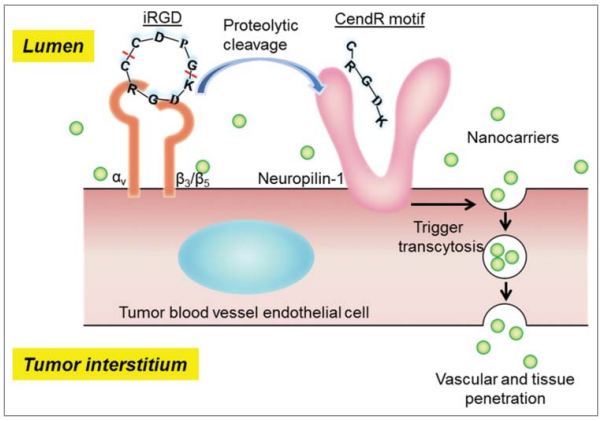

Figure 1. Principles of iRGD-mediated transcytosis at tumor sites. The iRGD effect is mediated by binding of the cyclic peptide with tumor-associated $\alpha_v\beta_3$ or $\alpha_v\beta_5$ integrins initially, followed by peptide cleavage and the release of the C-terminal end (CendR motif) that engages neuropilin-1 (NRP-1). The NRP-1 binding triggers a system of vesicles that can assist nanocarriers transport through tumor vasculature.

access in PDAC, it is also possible to use vasculature manipulation by targeting the transforming growth factor β (TGF-β) pathway, which recruits pericyte and facilitates its coverage on vascular fenestrations.[9] Moreover, several agents have been introduced to improve the tumor permeability that could be applied to enhance the tumor access of nanocarriers.[2,9] To name a few, it includes the use of 1) a hedgehog pathway inhibitor to provide a temporary increase in PDAC vascular density, 2) a PEGylated PH20 hyaluronidase to induce the re-expansion of PDAC blood vessels, and 3) pharmacological vascular permeability enhancer such as nitric oxide and angiotensin-converting enzyme inhibitors to increase particle penetration.

It is also necessary to comment on the co-administration *versus* conjugation approach when combining an iRGD peptide with a nanocarrier. This is advantageous for pharmaceutical activity and nano formulation manufacture. The co-administration approach addresses a major limitation of peptide-conjugated nanocarriers, which rely on the available number of NRP-1 receptors that transport particle in a "one-by-one" fashion because of the limited number of target receptors on the vasculature. This contrasts with unconjugated peptide co-injection that triggers bulk transfer of bystander nanoparticle (silicasomes in our case) at PDAC.[6] Moreover, introducing covalent conjugated peptide may add complexity on nano surface that may lead to profound impact in the complicated *in vivo* system.[10] From the perspective of translational feasibility, the use of the free peptide (for which the good manufacturing practice has been well-established by pharma industry) is more practical and less expensive for clinical use in contrast to a bio-conjugation process that inevitably enhances the cost and complexity of the nanocarrier.

Moreover, to the best of our knowledge, the iRGD-mediated transcytosis pathway has never been directly visualized at ultrastructural resolution in a tumor biopsy receiving nanocarrier. Observing a low electron density nano objective (*e.g.* silica based nanoparticle) in a complex PDAC microenvironment is a very challenging task. In response, we combined the use of optimal time/dose in the animal experiment and introduced a high electron density gold core in our nanocarrier.[6] This allowed us to successfully demonstrate that iRGD co-administration induces a novel vesicular transport pathway, an ultrastructural feature that has not previously been accomplished.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

1. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Controlled Release 2000; 65(1–2):271-84; PMID:10699287; https://doi.org/10.1016/S0168-3659(99)00248-5
2. Kobayashi H, Watanabe R, Choyke PL. Improving conventional enhanced permeability and retention (EPR) effects; what is the appropriate target? Theranostics 2014; 4(1):81-9; PMID:24396516; https://doi.org/10.7150/thno.7193
3. Feig C, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. Clin Cancer Res 2012; 18 (16):4266-76; PMID:22896693; https://doi.org/10.1158/1078-0432.CCR-11-3114
4. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N. Engl J Med 2013; 369(18):1691-703; PMID:24131140; https://doi.org/10.1056/NEJMoa1304369
5. Wang-Gillam A, Li CP, Bodoky G, Dean A, Shan YS, Jameson G, Macarulla T, Lee KH, Cunningham D, Blanc JF, et al. Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. Lancet 2016; 387(10018):545-57; PMID:26615328; https://doi.org/10.1016/S0140-6736(15)00986-1
6. Liu X, Lin P, Perrett I, Lin J, Liao YP, Chang CH, Jiang J, Wu N, Donahue T, Wainberg Z, et al. Tumor-penetrating peptide enhances transcytosis of silicasome-based chemotherapy for pancreatic cancer. J Clin Invest 2017; 127(5):2007-18; PMID:28414297; https://doi.org/10.1172/JCI92284
7. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, Hanahan D, Mattrey RF, Ruoslahti E. Tissue-penetrating delivery of compounds and nanoparticles into tumors. Cancer Cell 2009; 16(6):510-20; PMID:19962669; https://doi.org/10.1016/j.ccr.2009.10.013
8. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Greenwald DR, Ruoslahti E. Coadministration of a tumor-penetrating peptide enhances the efficacy of cancer drugs. Science 2010; 328(5981):1031-5; PMID:20378772; https://doi.org/10.1126/science.1183057
9. Meng H, Zhao Y, Dong J, Xue M, Lin YS, Ji Z, Mai WX, Zhang H, Chang CH, Brinker CJ, et al. Two-wave nanotherapy to target the stroma and optimize gemcitabine delivery to a human pancreatic cancer model in mice. ACS Nano 2013; 7(11):10048-65; PMID:24143858; https://doi.org/10.1021/nn404083m
10. Zhao F, Meng H, Yan L, Wang B, Zhao Y. Nanosurface chemistry and dose govern the bioaccumulation and toxicity of carbon nanotubes, metal nanomaterials and quantum dots in vivo. Sci Bull 2015; 60(1):3-20; https://doi.org/10.1007/s11434-014-0700-0