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RESEARCH HIGHLIGHT

Organotropic metastasis: role of tumor exosomes

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A recent paper in *Nature* shows that tumor exosomes expressing unique integrins can determine organotropic metastasis by preparing pre-metastatic niche through their integrins-mediated fusion with and fertilization of organ-specific resident cells.

Tumor metastasis is a critical step in malignant progression of tumors and has been implicated in the failure of most cancer therapeutics. One salient feature of metastasis is that some types of cancer cells preferentially colonize and metastasize to specific organs, under the control of a range of cellular and molecular programs [1]. Many studies focus largely on identifying cell-intrinsic determinants of such organotropic metastasis, including genes and chemokine receptors expressed on cancer cells [2]. The adhesion and extracellular matrixc molecules, such as integrins, tenascin and periostin, have been also shown to promote colonization of metastatic cancer cells [3]. In 2005, Dr Lyden and colleagues proposed a term of pre-metastatic niche to describe the phenomenon that primary tumor could promote its own metastasis by recruiting bone marrow-derived cells to the distant organ and establish supportive metastatic environments [4]. Besides, tumor-derived soluble factors such as lysyl oxidase [5] have been also reported to induce organ-specific metastasis by formation of pre-metastatic niche in certain sites. However, the exact mechanism for metastatic organotropism is still unclear. In recent years, tumor-derived exosomes have been demonstrated to promote cancer progression [6]. Exosomes are small membrane-bound vesicles (30-100 nm) containing functional biomolecules (including proteins, RNA, DNA and lipids) that can be horizontally transferred to recipient cells [7]. For example, brain astrocyte-derived exosomes can promote the outgrowth of brain metastatic cancer cells by transferring PTEN-targeting microRNA-19a to these cancer cells [8]. Nevertheless, whether molecules present on tumor exosomes can determine organ-specific metastasis is an unresolved question.

Lyden and colleagues in their recent *Nature* paper gave the answer that tumor exosome integrins can determine organotropic metastasis by fusing with organ-specific resident cells to establish pre-metastatic niche through activating Src phosphorylation and pro-inflammatory S100 expression [9] (Figure 1). The authors isolated exosomes from organotropic human and mouse breast and pancreatic cancer cell lines known to primarily metastasize to the lung, liver, or both [9]. Then, they retro-orbitally injected these near infrared (NIR) or red fluorescently labeled exosomes into nude mice and quantified exosome biodistribution and uptake in distant organs after 24 h by NIR whole-lung imaging and confocal microscopy. Using this approach, they observed that exosomes from different cancer models selectively interact with the same future metastatic organs as their cell of origin. Thus, the authors proposed that exosomes could promote organ-specific metastasis. To test exosome-mediated education of target organs functionally, they injected luciferase-expressing 4175-LuT (LuT, Lung-tropic) or 1833-BoT (BoT, bone-tropic) cells into 4175-LuT or 1833-BoT exosome-educated mice, and found that education with 4175-LuT-derived exosomes increased the lung metastatic capacity of 4175-LuT tumors, and interestingly, even significantly redirected the bone-tropic 1833-BoT cells to disseminate in the lung. These observations suggested that certain type of tumor exosomes can prepare pre-metastatic niches to facilitate organ-specific metastasis and redirect metastatic distribution even for cancer cells poorly capable of metastasizing to these sites.

To further dissect the molecular mechanisms involved in organ-specific metastasis, through quantitative mass spectrometry of lung-, liver- and brain-tropic exosomes, they identified integrins (ITGs) — the most highly represented cell adhesion receptor proteins in exosomes — as determinants of metastatic organotropism. Subsequent analysis showed that lung-tropic exosomes expressing ITG$\alpha_6\beta_4$ and ITG$\alpha_6\beta_1$ could interact with S100A4-positive fibroblasts and surfactant protein C-positive epithelial cells in laminin-rich lung microenvironments, ITG$\alpha_v\beta_5$-expressing pancreatic exosomes co-localized with F4/80$^+$ macrophages and fused with Kupffer cells in fibronectin-rich liver niches, and brain-tropic 831-BrT exosomes interacted mainly with CD31$^+$ brain endothelial cells. Furthermore, inhibiting the exosomal integrins ITGβ_4 and ITGβ_5 expression via short hairpin RNAs or blocking their binding by HYD-1/RGD peptides markedly reduced exosome uptake as well as lung and liver metastasis, respectively. Thus, the authors demonstrated that certain exosomal integrins govern organ-specific metastasis by fusing with

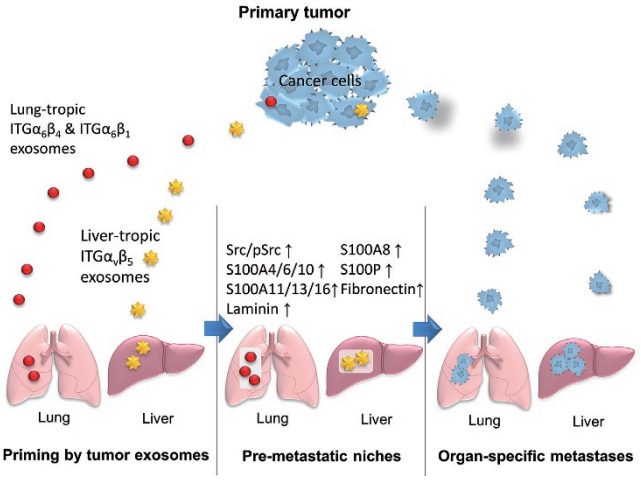

Figure 1 Tumor exosomes direct organ-specific metastasis via integrins. Tumor-derived exosomes transport proteins, nucleic acids and lipids to specific organ and fuse with resident cells, which can prepare distant organ site as pre-metastatic niche. Lyden and colleagues [9] report that exosomes derived from different type of cancer cells can display different integrin proteins on their surface; ITG$\alpha_6\beta_4$- and ITG$\alpha_6\beta_1$-expressing exosomes preferentially interact with fibroblasts and epithelial cells in lung, and ITG$\alpha_v\beta_5$-expressing exosomes preferentially fuse with Kupffer cells in liver. Once uptaken, tumor exosomes induce cellular changes (Src activation and pro-inflammatory S100 gene expression) in the target organ, thus promoting cancer cell colonization and organ-specific metastasis.

target cells in an organ-specific manner. When using RNA sequencing to analyze the gene expression in distinct cells targeted by exosomes, they found that exosomal ITGs could prominently upregulate pro-inflammatory S100 gene expression (i.e., several S100 genes (S100A4, -A6, -A10, -A11, -A13 and -A16) were upregulated in 4175-LuT exosome-educated WI-38 fibroblasts; S100A8 and S100P were upregulated in BxPC-3-LiT exosome-educated Kupffer cells). Moreover, exosomal ITGβ_4 uptake could increase Src or phosphorylated Src (pSrc) levels in resident cells. S100 proteins promote metastasis [10], and ITG$\alpha_6\beta_4$ can active Src and S100A4 expression [11]. Taken together, the mechanisms for exosomal integrins in promoting tumor organotropic metastasis in this *Nature* paper [9] may be summarized as follows: (1) promotion of adhesion by fusing with specific resident cells; (2) activation of Src signaling pathways and inflammatory responses (pro-inflammatory S100 gene expression) in these target cells, thus preparing favorable pre-metastatic niche for further metastasis (Figure 1).

Finally, their clinical data showed that ITGβ_4 level is higher in exosomes from breast cancer patients with lung metastasis and increased exosomal ITGα_v in pancreatic cancer patients who developed liver metastasis than in those without metastasis. These results indicated that exosomal integrins isolated from circulating plasma may be used as organotropism biomarkers to predict organ-specific metastasis in cancer patients.

The study by Lyden and colleagues [9] expands our understanding of the organ-specific metastasis mechanisms and also highlights a crucial role of exosomes in promoting tumor metastasis. Indeed, another recent work in *Nature Cell Biology* shows that pancreatic cancer exosomes can increase liver metastatic burden by transferring macrophage migration inhibitory factor (MIF) to Kupffer cells and by recruiting immune cells to initiate pre-metastatic niche formation in the liver [12]. Thus, strategies targeting these particular exosomal integrin molecules in this *Nature* paper [9] may not be enough to prevent organ-specific metastasis. Besides, the limited integrin repertoire of bone-tropic exosomes identified by the proteome analysis calls for further investigation. This study also raises several intriguing questions: Do exosomal integrins have any effect on the recruitment of bone marrow-derived cells or immunosuppressive cells in priming pre-metastatic niche? What is the function of other exosome components such as RNA and DNA in determining organ-specific metastasis? Are these results universal to other type of cancer metastasis? Most importantly, how to translate this finding into clinical applications? Addressing these questions will help us unveil the mystery of organotropic metastasis.

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