

Special delivery: microRNA-200-containing extracellular vesicles provide metastatic message to distal tumor cells

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An emerging view is that breast cancer is a systemic disease that utilizes intrinsic and extrinsic tumor cell processes to support both primary tumor growth and metastatic dissemination into distal tissue. Delineation of factors involved in these processes should facilitate a better understanding for both assessing and preventing disease relapse. In this issue of the *JCI*, Le et al. investigate whether intrinsic properties of metastatic breast cancer cell growth can be regulated through an extrinsic process – contact with tumor cell-derived extracellular vesicles containing microRNAs of the miR-200 family. The authors provide compelling evidence that miR-200s within extracellular vesicles secreted from highly metastatic tumor cells can be internalized by weakly metastatic cells. Thus, internalization and delivery of this metastatic “donor” cell-derived message provide plausible mechanisms by which oncogenic and regulatory factors confer the capability of tumor growth at metastatic lesions. This study provides a strong rationale for detailed assessment of the prognostic and predictive value of circulating extracellular vesicle-bound miR-200s in breast cancer progression and treatment.

Promoting metastasis: microRNA-200 family members and extracellular vesicles

Considering the large number of tumor cells that are estimated to be released daily into the circulation of cancer patients, metastasis and distal colonization is a relatively rare event (1). In this issue, Le and colleagues (2) provide new insight into potential mechanisms that regulate multiple steps of the metastatic process via the intercellular transmission of nucleic acids and proteins packaged within tumor cell-secreted extracellular vesicles (EVs). In an elegant series of experiments, Le and colleagues expand on initial observations that correlate metastatic properties of isogenic mouse breast cancer cell lines (4T1 and 4T07) to the intrinsic expression of miR-200 family microRNAs (3). Lieber-

man and colleagues previously demonstrated that the strongly metastatic cells could confer metastasis-associated properties upon otherwise nonmetastatic cells through ectopic expression of miR-200 microRNAs (3). This observation furthered the general understanding that miR-200 microRNAs control both the epithelial phenotype (via epithelial-mesenchymal transition) and metastatic properties of tumor cells (4–6). In their current report, Le and colleagues analyze a series of physiologically relevant studies demonstrating that EVs secreted from metastatic breast tumor cells contain a blueprint of prometastatic regulatory cargo that is apparently distinct from that within EVs of nonmetastatic cells. Quite simply, the results of this study indicate that the metastatic potential of an otherwise nonmetastatic tumor cell

can be increased through exposure to and uptake of cargo encapsulated in EVs from a metastatic soul mate.

Le and colleagues compared EVs derived from a metastatic murine cancer cell line (4T1E) and a nonmetastatic murine cancer cell line (4T07). 4T1E cells are an epithelial variant with high E-cadherin expression that uniformly metastasizes to the lungs, while 4T07 cells are nonmetastatic in murine models. Both cell lines produced EVs of similar size and distribution (50–310 nm), indicating exosome and ectosome secretion. Particles that were enriched for the EV markers TSG101 and ALIX also contained AGO2, a protein associated with mature miRNAs. Importantly, members of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-429, miR-141, collectively referred to as miR-200s) were elevated in metastatic 4T1E cell-derived EVs compared with EVs derived from nonmetastatic 4T07 cells. Extracellular miRNAs were encapsulated within an intact vesicle, as miR-200s in culture supernatants were only sensitive to RNase treatment in the presence of membrane-solubilizing agents. Furthermore, the level of miR-200s in EVs paralleled their relative abundance in the host cell, suggesting that these microRNAs are not specifically selected for packaging during EV biogenesis: therefore, the EV-associated miR-200 fingerprint is the same in both the vesicle and the host tumor cell. This observation has important implications for the development of therapeutics and biomarkers for breast cancer (7). Future work should expand our understanding of whether specific miRNAs are targeted to EVs or whether the miRNAs within EVs are simply an inherent product of the host tumor cell miRNA transcriptome.

The observation by Le et al. that tumor cell-derived EVs carry the miRNA fingerprint and prometastatic code of the donor cell conjoins earlier observations that EV-

► Related Article: p. 5109

Conflict of interest: The author has declared that no conflict of interest exists.

Reference information: *J Clin Invest*. 2014;124(12):5107–5108. doi:10.1172/JCI179191.

mediated transfer of RNA and oncogenes (8, 9) from donor tumor cells to recipient endothelial, hematopoietic, and stromal cells promotes tumor progression (10, 11). However, the observations that nonmetastatic tumor cells can serve as EV recipient cells, both in vitro and in vivo, and that the metastatic message can be conveyed without direct cell-to-cell contact distinguish the study by Le and colleagues. The authors used several methods to demonstrate that the miR-200-regulated metastatic program can be transferred from a metastatic donor to a nonmetastatic recipient via EVs. EV-dependent transfer of metastatic capacity was demonstrated both by direct cell-contact studies, in which metastatic donor cells and nonmetastatic recipient cells were cocultured, and by Transwell assays, in which metastatic donor cells were separated by a semipermeable membrane from the nonmetastatic recipient cells. In both systems, miR-200 target genes were downregulated in the nonmetastatic recipient cells in a dose-dependent manner, indicating that transfer of miR-200 cargo between donor and recipient tumor cells and subsequent abrogation of gene expression associated with a tumor reepithelialization (MET) program do not require direct cell-to-cell contact. Importantly, metastatic human breast cancer cell lines produced miR-200-containing EVs that were transferred to nonmetastatic human breast cancer cells and altered gene expression in these cells.

EV and miR-200 function in vivo

Circulating EVs and miRNAs have been identified in the serum of cancer patients (9, 12), yet the physiological relevance of tumor-derived EVs in disease progression has remained unresolved. Le and colleagues provide compelling experimental support for the idea that miR-200-expressing donor cells promote the growth of neighboring nonmetastatic tumor cells in murine cancer and human xenograft models. Specifically, mice bearing mixed tumors comprising differentially labeled metastatic and nonmetastatic cells exhibited metastasis of both populations, while animals implanted with only

nonmetastatic populations had no apparent metastases. In a series of elegant experiments, Le et al. isolated circulating EVs carrying miR-200 miRNAs from the serum of mice bearing human tumor xenografts derived from strongly metastatic cells and demonstrated that EV transfer could be accomplished through both direct and indirect means with similar consequences on miR-200 target gene expression. Exposure of MB-231 cells, nonmetastatic human breast cancer cells, to EVs from metastatic donor cells prior to i.v. injection resulted in miR-200 transfer and promoted tumor colonization in the lungs of injected mice. In contrast, MB-231 cells not exposed to miR-200-containing EVs did not form tumors in the lung. Importantly, Le and colleagues demonstrated that circulating tumor-secreted EVs bearing miR-200 cargo could promote metastasis to the lung by recipient MD-231 cells.

Conclusions and future direction

The current study by Le and colleagues provides compelling evidence that the heterogeneous tumor environment may contain “donor” metastatic cell populations that confer metastatic capacity to weakly metastatic “recipient” tumor cells. The concept that tumor cell-derived EVs provide a means of intercellular communication is highly intriguing, as these EVs may modify primary tumor growth as well as growth and colonization of tumor cells in tissue at distal metastatic sites. The results from Le and colleagues suggest miR-200 cargo within EVs secreted from donor cells provides one version of the prometastatic code. If tumor cell-derived EVs truly serve as intercellular messengers, then what other programs/instructions are they capable of delivering? The role of EV-dependent miR-200 in cancer metastasis is an evolving story. The results of Le et al. support the need for in-depth longitudinal analysis of circulating miR-200 levels in cancer patient-derived EVs before, during, and after treatment. Moreover, the potential of miR-200-containing EVs to serve as predictive and prognostic biomarkers in breast cancer (13, 14) and other tumor

types should be further evaluated. Additional work is needed to determine whether EVs and their contents are indeed sentinels of cancer growth and development.

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