CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor***Expanding on Exosomes and Ectosomes in Cancer**

Lorraine O'Driscoll, Ph.D.

Exosomes are tiny vesicles that are enriched in nucleic acids and proteins and released from cells. Originally considered to have no biologic significance, these nano-sized blebs are now considered to be mini-maps of their cells of origin, with physiological and pathologic relevance. In cancer, they have been implicated in the muddling of cell-to-cell communication and in the transfer of “undesirable” information from one cell to another. Consequences include stimulating the proliferation, motility, and invasive properties of the recipient cell, transferring drug resistance, inducing the formation of endothelial tubules (e.g., in angiogenesis), and attracting cancer cells to secondary sites within living organisms. That said, our understanding of exosomes is rudimentary.

Several recent studies provide further support to show that exosomes derived from cancer cells are dynamic mini-factories that actively contribute to the progression of disease. These studies have focused on the microRNA (miRNA) content of exosomes. MiRNAs are short, double-stranded RNA fragments that are generated from precursor miRNAs (pre-miRNAs). They do not encode proteins but, rather, regulate the levels of expression of specific sets of messenger RNAs (mRNAs), and therefore their protein products, by mechanisms that include binding to these mRNAs and targeting them for degradation. This binding-and-degradation process requires the pre-miRNA to be incorporated into a multi-protein complex called the RNA-induced silencing complex-loading complex. Within this complex, pre-miRNAs mature into miRNAs by means of their interaction with two proteins: an enzyme called Dicer and the transactivating response RNA binding protein (TRBP). Finally, a protein called argonaute 2 (AGO2) binds to miRNA and guides it to its target complementary mRNA.

Starting with exosomes from breast-cancer

cell lines and those from nontumorigenic breast-cell lines (human, MCF-10A; mouse, NMuMG), Melo et al.¹ found that the exosomes derived from the cancer cell lines but not those from the nontumorigenic breast cells were enriched in miRNAs and that the exosomes from cancer cells could convert pre-miRNAs into mature miRNAs. They cultured exosomes from both types of cells separately for 3 days and monitored the conversion of six pre-miRNAs into miRNAs (including two specific miRNAs that are known to be relevant to breast-cancer biology, miR-10b and miR-21). In exosomes derived from the breast-cancer cell lines, the ratio of miRNA to pre-miRNA increased with time, indicating active miRNA formation. The investigators did not detect a change in this ratio in the exosomes derived from nontumorigenic breast cells. Next, the group introduced synthetic pre-miRNAs into exosomes from the cancer cells; these were converted to miRNAs over the same time frame. Consistent with these findings was the detection of Dicer, TRBP, and AGO2 in exosomes that were derived from breast-cancer cells only.

To test the effect of exosomal contents on normal cells, the authors exposed MCF-10A cells to exosomes that were derived from the breast-cancer cell line MDA-MB-231. Exposure of these normal cells to the exosomes that were derived from the cancer-cell line and cultured over a period of 3 days increased cell survival and proliferation. This effect was accompanied by decreased expression of the tumor-suppressor protein PTEN and the transcription factor HOXD10, which suppresses the expression of genes that promote invasion, migration, and tumor progression. The investigators then found that the nontumorigenic cells, when coinjected with exosomes from the cancer cells into mice, formed tumors (Fig. 1) — unless Dicer activity was blocked, which suggests that Dicer is critical to

Figure 1 (facing page). The Exacting Toll of Exosomes and Ectosomes.

Exosomes are released into the surrounding medium (conditioned medium) by cells in culture and are also found in serum specimens. Melo et al.¹ found that exosomes in the serum specimens from healthy persons carry immature precursor microRNAs (pre-miRNAs), which remain as pre-miRNAs even when the exosomes are cultured under laboratory conditions for 3 days. When such exosomes were cocultured with nontumorigenic cells or coinjected with these cells into mice, Melo et al. observed no notable effects (Panel A). Serum specimens from patients with cancer contain substantially more exosomes than do serum specimens from healthy controls. These exosomes are enriched in pre-miRNAs, as compared with the exosomes from normal cells, and, unlike exosomes from normal cells, they also have molecular machinery (in the form of the Dicer enzyme, the transactivating response RNA-binding protein [TRBP], and the argonaute 2 [AGO2] protein) that can convert pre-miRNAs to mature miRNAs while in culture. After coculture of these exosomes with nontumorigenic cells for 3 days, the cells showed increased survival and growth rate, with an associated reduction in the proteins PTEN (encoded by a tumor-suppressor gene) and HOXD10 (a transcription factor), which are known targets of the miRNAs miR-10b and miR-21. When these exosomes were coinjected with nontumorigenic cells into mice, the cells formed tumors, unless the activity of Dicer in the exosomes was blocked. Exosomes and ectosomes, collectively termed extracellular vesicles, from highly metastatic cells transfer this trait to poorly metastatic cells, causing a substantial increase in metastasis to the lungs, except when the activity of miR-200 is blocked in the extracellular vesicles, in which case fewer and small metastases result (Panel B).

the transformation of normal cells into tumor cells on exposure to exosomes.

Melo and colleagues also found that serum specimens from patients with cancer had more exosomes than did those from healthy controls. They also observed that the same six pre-miRNAs, when cultured, matured to miRNAs in the exosomes from patients but not in those from healthy donors. The exosomes from 5 of 11 of these patients, when injected with the nontumorigenic breast epithelial (MCF-10A) cells, induced tumor formation in mice; those from 8 healthy donors did not.

Metastasis to secondary organs is the major cause of death from breast cancer and often involves an epithelial cell-to-mesenchymal cell transformation and subsequent reversion to epithelium, a process that is regulated by the miR-200 family of miRNAs. Le and colleagues found that exosomes and larger vesicles (termed ectosomes)

can transfer miR-200s from highly metastatic cells to poorly metastatic cells and thereby increase the metastatic potential of the poorly metastatic cells.² To begin, they used mouse triple-negative breast-cancer cells. Mouse triple-negative breast-cancer cells that were poorly metastatic were exposed for 3 days to either their own extracellular vesicles or to those derived from the highly metastatic mouse triple-negative breast-cancer cell line, 4TE1. When injected into the tail vein of mice, the cells that were preincubated with the extracellular vesicles from highly metastatic cells formed substantially more lung metastases than did the cells that were preincubated with their own extracellular vesicles. This finding was maintained except when miR-200 family members were blocked, in which case there were fewer and smaller lung metastases. Le et al. then carried out similar experiments, and obtained similar results, with human breast-cancer cell lines.

It appears that exosomes in the context of the stromal microenvironment also exert influence on tumor behavior. Boelens and colleagues³ found that exposure to stromal exosomes expanded a subpopulation of breast-cancer cells that are resistant to therapy and can initiate tumor formation. Shimoda and colleagues⁴ found that tissue inhibitors of metalloproteinases (TIMPs) guard against the release of tumor-promoting exosomes by the stroma: a depletion of TIMPs resulted in cancer-associated fibroblast-like cells. Squamous-cell carcinomas of the head and neck are a source of TIMP-less fibroblasts; exosomes derived from such fibroblasts enhance the motility of breast-cancer cells.

Although much remains to be learned about exosomes and ectosomes in vivo, these studies improve our understanding of extracellular vesicles by showing that they carry — and use — the necessary machinery to render mature miRNAs, that exosomes derived from cancer cells are qualitatively different from those derived from noncancer cells, and that extracellular vesicles seem capable of mediating the transfer of molecules (specifically, miRNA machinery and miRNAs themselves) that influence metastatic potential. Stromal exosomes also seem to be important influences on cancer-cell biology. Additional studies are warranted to determine the separate and collective influences of exosomes and ectosomes on cancer.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

From the School of Pharmacy and Pharmaceutical Sciences and Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin.

1. Melo SA, Sugimoto H, O'Connell JT, et al. Cancer exosomes perform cell-independent microRNA biogenesis and promote tumorigenesis. *Cancer Cell* 2014;26:707-21.
2. Le MT, Hamar P, Guo C, et al. miR-200-containing extracel-

lular vesicles promote breast cancer cell metastasis. *J Clin Invest* 2014;124:5109-28.

3. Boelens MC, Wu TJ, Nabet BY, et al. Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways. *Cell* 2014;159:499-513.

4. Shimoda M, Principe S, Jackson HW, et al. Loss of the Timp gene family is sufficient for the acquisition of the CAF-like cell state. *Nat Cell Biol* 2014;16:889-901.

DOI: 10.1056/NEJMcibr1503100

Copyright © 2015 Massachusetts Medical Society.