cofactor in hematologic malignancies containing mixed-lineage leukemia (MLL) gene fusions (*10*). In both disorders, menin acts by regulating MLL-mediated histone methylation (*11*, *12*), which may explain why inhibitors of menin counteract the oncogenic effects of K27M mutations. Although the role of menin in DIPG is unclear, these studies suggest it may be an important therapeutic target.

Hashizume *et al.* took a different approach to identify therapies for K27M-mutant DIPG. They hypothesized that the global loss of histone methylation induced by the K27M mutation (and the resulting sequestration of PRC2) is critical for tumor maintenance. The authors used patient-derived DIPG cell lines (established from biopsies and passaged in vivo) to evaluate the effects of a K27 demethylase inhibitor on tumor cells. Treatment of H3.3K27M-mutant DIPG cells with this inhibitor increased H3K27 methylation and decreased cell growth. By contrast, treatment of cells harboring wild-type H3.3 or a different histone mutation (H3.3G34R/V) had little effect. This suggests that global loss of H3K27 methylation may be the primary mechanism of K27M-driven gliomagenesis and raises the possibility that demethylase inhibitors may be valuable therapeutic agents for the disease.

The discovery of K27M mutations was an important step forward in understanding DIPG and promises to yield new approaches to treating the disease. The studies of Funato *et al.* and Hashizume *et al.* take us closer to that goal, creating models that can be used to study DIPG biology and demonstrating that these models can be useful for identifying therapies. It will be interesting to see whether these therapies synergize with one another, or with focal radiation, the standard of care for children with DIPG. Given the dismal prognosis associated with this disease, there will be strong incentive to move them forward into clinical trials. ■

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ILLUSTRATION: C. BICKEL/*SCIENCE*

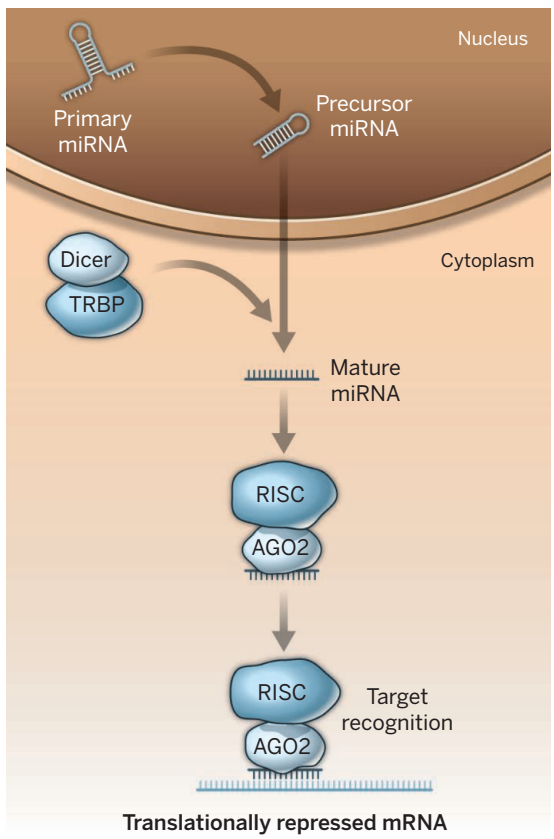
CANCER

Malicious exosomes

Nanovesicles derived from cells of cancer patients carry microRNAs that initiate tumor growth in normal cells

By **Eleni Anastasiadou** *and* **Frank J. Slack**

Nanovesicles known as exosomes are secreted from a variety of cell types and circulate in biological fluids such as urine and plasma. These exosomes "hijack" membrane components and cytoplasmic contents of these cells and play an important role in intercellular communication, often inducing physiological changes in recipient cells by transferring bioactive lipids, nucleic acids, and proteins (*1*). These tiny vesicles also have been implicated in a number of human diseases, including cancer, and are becoming an appreciated fundamental aspect of tumor progression and metastasis (*2*). Recently, Melo *et al.* (*3*) showed that exosomes from breast cancer cells transfer microRNAs (miRNAs) to normal cells and stimulate them to become cancerous. This potentially expands the mechanisms by which cancer spreads and may provide opportunities to develop exosome-based diagnostics and therapies.



MiRNA biogenesis. MiRNAs combine with AGO2 and other proteins in an RNA-induced silencing complex (RISC) to repress the translation of target mRNAs.

Many physiological processes involve exosomes, such as cell growth, neuronal communication, immune response activation, and cell migration, and in the case of cancer, may transfer angiogenic proteins or oncogenes from one cell to another (*4*–*7*). Thus, analyzing the macromolecules harbored by exosomes could have important diagnostic and therapeutic implications. Experimental evidence shows that exosomes mediate interactions between cancer and normal cells. For example, exosomes secreted by breast cancer cells inhibit exosome release from the normal counterparts. These cancer exosomes may trigger extracellular acidity in which cancer cells (but not healthy cells) can survive and which activates hypoxia-dependent angiogenesis during tumor development (*1*). Exosomes can also induce drug resistance of cancer cells by sequestering chemotherapeutic agents (*8*); and can stimulate metastasis (*2*).

Interestingly, exosomes contain messenger RNA (mRNA) and miRNA that can be transferred to other cells and regulate gene expression of the target cell (*9*). Likewise, miRNAs are present in apoptotic bodies (small membrane vesicles that are produced by cells undergoing programmed cell death) (*10*), or they are in the plasma, associated with Argonaute2 (AG02), the key effector protein of a miRNA-mediated gene silencing mechanism (*11*). However, miRNAs detected in human serum and saliva are mostly concentrated inside exosomes (*12*). Virally encoded miRNAs are also found in exosomes, indicating how oncogenic viruses could manipulate the tumor microenvironment (*13*).

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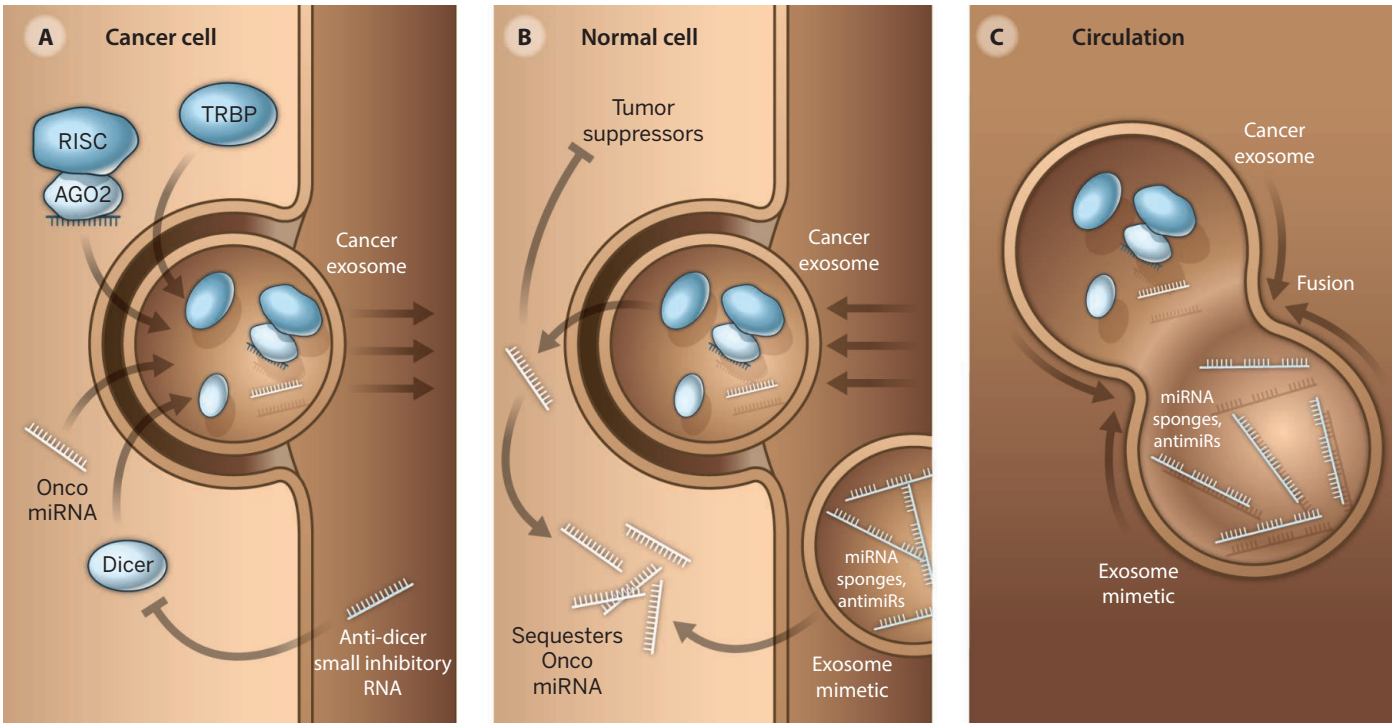

Targeting cancer exosomes. Three possible therapeutic scenarios are shown for targeting tumor-derived exosomes within a cancer cell (**A**), in a normal recipient cell (**B**), or in the circulation (**C**).

The dysregulation of certain miRNAs has been associated with cancer-forming (oncogenic) events and has been identified in numerous types of human cancers. Fabbri *et al.* (*14*) detected nine miRNAs, including the oncogenic miRNAs (oncomiRs) miR-21, miR-27b, and miR-29a, in nanovesicles derived from supernatants of lung cancer cell lines, but not from normal cells. This suggests a cancer-specific pattern of secreted miRNAs. In particular, Fabbri *et al.* found that tumor-secreted miR-21 and miR-29a can bind to toll-like receptors (TLRs), murine TLR7 and human TLR8, to induce protumoral inflammation that leads to tumor growth and metastasis.

Melo *et al.* (*3*) reveal a role of exosomes in cell-independent miRNA biogenesis that affects cancer progression. The authors show that only exosomes derived from cancer cells, but not those derived from normal cells, contain key enzymes involved in miRNA biogenesis such as Dicer, TAR (trans-activation response) RNA-binding protein (TRBP), and AGO2 (see the first figure). The exosomes also contain the membrane protein CD43, which plays a role in accumulating Dicer in cancer exosomes. The study also shows that Dicer-containing cancer exosomes process precursor miRNAs into mature miRNAs (including oncomiRs) over time, and upon encounter with normal human mammary epithelial, cells induces them to become cancerous. Healthy mammary human epithelial cells formed tumors when they were injected into mice that were treated with cancer exosomes. Moreover, miRNAs in the cancer exosomes inhibited the expression of their respective mRNA targets—phosphatase and tensin homolog [(PTEN), a tumor suppressor protein] and the transcription factor homeobox D10 (HOXD10)—in the recipient epithelial cells. The authors suggest a possible temporal oncogenic "field effect" induced by cancer exosomes that recruits surrounding normal cells to become tumorigenic. The tantalizing results of Fabbri *et al.* and Melo *et al.* merit further investigation in immunocompetent mouse models.

The findings of Fabbri *et al.* and Melo *et al.* could be harnessed to design exosome-based cancer diagnostics and therapeutics. For example, using exosomes as biomarkers in early cancer diagnosis and prognosis might involve detecting the presence of Dicer and mature miRNAs in bodily fluids, which could serve as an additional cancer biomarker without requiring an invasive tissue biopsy. Given the role of oncomiRs such as miR-21 in cancer initiation, progression, and maintenance (*15*), the findings suggest a new route for anticancer therapy in targeting the malicious exosomes (see the second figure). For example, Dicer could be silenced directly in cancer cells by small interfering RNA. Alternatively, Dicer concentration within the cancer exosomes could be reduced by blocking CD43 expression in tumor cells with a specific monoclonal antibody. This may reduce further processing of oncomiRs from taking place within the exosomes. Another possibility for therapeutic intervention could be exosome mimetics containing antimiRs or miRNA sponges that either capture the incoming oncomiRs in the recipient cells or fuse with the cancer exosomes and neutralize them while still in circulation.

Gone are the days of considering exosomes as mere "garbage cans" for the cell. We have entered the very early and exiciting stage of understanding how cancer cells might use exosomes as drones to assemble and transport a menacing cargo of oncomiRs. Hopefully, evaluation of this phenomenon will spur the development of cancer therapies and broaden our understanding of how cancer spreads. ■

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