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CORE CONCEPTS

Extracellular vesicles garner interest from academia and biotech

Karen Hopkin, *Science Writer*

Extracellular vesicles are the subject of conferences, have had two societies and two journals dedicated to their study, and the NIH has earmarked millions in financing to explore and exploit their biology. However, only 10 years ago, most cell biologists thought that extracellular vesicles were little more than membranous debris.

"It took me three years to publish our paper in *Leukemia* in 2006," says Mariusz Ratajczak of the University of Louisville. "We were rejected from all the major journals because they said we were describing an artifact" (1).

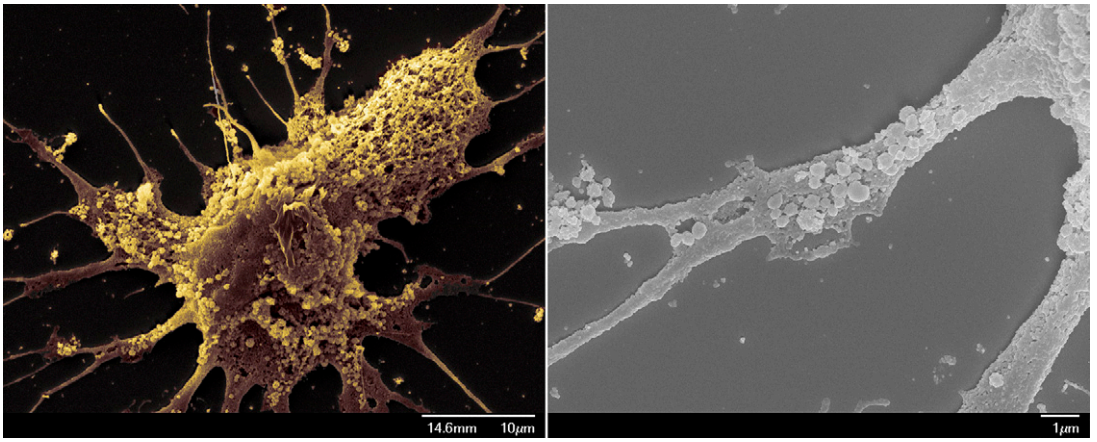
Now, extracellular vesicles—an umbrella term for a family of membrane-enclosed spheres that bud from the surface of nearly all living cells—are a hot topic. By carrying lipids, proteins, mRNAs, and small regulatory RNAs from one cell or tissue to another, these transport vehicles can influence the behavior and even the identity of cells. And because the same vesicles also convey molecules involved in pathological conditions, such as neurodegeneration and cancer, several biotech companies want to use extracellular vesicles for diagnosing or treating disease.

Overlooked and Underappreciated

With their ability to alter cell fate, extracellular vesicles can shape their microenvironment. "This suggests that cell biology may be even more fluid and less fixed than many people thought," says Peter Quesenberry of Brown University, a pioneer in the field.

Scientists have known since the 1980s that immature red blood cells shed bits of their membrane as they develop into concave oxygen-carrying discs. Platelets, too, jettison membrane fragments during clotting. But a decade or so ago, researchers started noticing these vesicles elsewhere. Ratajczak was looking at cell lines. "And I wondered, why is this debris present when the cells look perfectly healthy under the microscope?" he says. Johan Skog, then a postdoctorate at Massachusetts General Hospital in Boston, spotted similar vesicles springing from the surface of the stem cells from a glioblastoma (2).

Such observations were just the start. It's now clear that all cells secrete such vesicles: not only stem cells or cells in culture, but plant cells, parasites, and bacteria. "Even migrating amoebas leave behind a trail of vesicles that other cells can follow," notes Ratajczak, adding that their ability to ferry information across so many



Glioblastoma cells produce microvesicles containing RNA, as shown in this scanning electron microscopy image (*Left*). Higher magnification reveals microvesicles—ranging from 50 to 500 nm in diameter—on the cell surface (*Right*). Reproduced from ref. 2, with permission from Macmillan Publishers Ltd.: *Nature Cell Biology*, copyright (2008).

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phyla means that extracellular vesicles likely represent "the first form of cell-to-cell communication."

So how could such a universal agent of communication have been overlooked—or actively disregarded—for so long? Perhaps because they are far from homogeneous, says Alexander (Sasha) Vlassov of ThermoFisher Scientific. "Cells secrete a bunch of different vesicles of different sizes, with different compositions, different functions, and different mechanisms of formation," he says. "So it took a while to explain to reviewers what you were talking about."

Indeed, not everyone in the field even agrees on what they should be called. Some stick with the term extracellular vesicles, others prefer microvesicles. Many use the term "exosomes" to refer only to the smaller vesicles that work their way through the interconnected network of intracellular membranes before being shed from the cell surface. But there are those, including Vlassov, who consider "exosomes" the default terminology, because "it's catchy and NIH understands what it means."

Crucial Cargo

The lack of what Vlassov calls a "rock solid nomenclature" may have kept extracellular vesicles from receiving early recognition, but their cargo has piqued the interest of a rapidly expanding cadre of investigators in both industry and academia.

In the study Ratajczak published in 2006 (1), he isolated vesicles from cultured stem cells and found these vesicles contained RNA, and that this RNA could be delivered to other cell types, such as hematopoietic precursor cells. This transfer of molecular information drove the recipient cells to produce marker proteins that are typical of the pluripotent, developmentally supple stem cells from which the vesicles arose.

The finding has implications for regenerative medicine. Extracellular vesicles from stem cells—even induced pluripotent stem cells produced from an individual—could be used to direct repair of damaged tissue. Best of all, the approach would obviate the need to introduce potentially immunogenic cells into patients. "Using vesicles derived from stem cells, we could do stem-cell therapy without the cells," says the University of Torino's Giovanni Camussi, who, along with Quesenberry, is organizing the Gordon Conference on extracellular vesicles in Maine this August.

In his laboratory, Camussi has also found that extracellular vesicles carry RNAs with regenerative potential. Using vesicles derived from endothelial progenitor cells, he has triggered the sprouting of new capillary-like blood vessels (3). And Quesenberry and others have shown that extracellular vesicles' powers of phenotypic persuasion extend to bone marrow cells, which can be directed to behave like cells from the lung, brain, or liver by vesicles derived from those tissues (4).

Of course not all vesicular RNAs modulate cell behavior; a substantial portion have no biological activity at all, says Vlassov. However, he and others in the community remain excited about how the vesicular communication system can be exploited.

Extracellular Exploitation

Unfortunately, extracellular vesicles can also promote disease. Cancer cells, for example, use extracellular vesicles to prepare a microenvironment optimally suited for their survival. Vesicles derived from tumor cells harbor factors that can attract a nutrient-bearing blood supply, blunt the activation of surveilling T cells and macrophages, and even pave the way for their malignant spread to other tissues. In 2011, Camussi and colleagues published a study showing that in renal cancer, cancer stem cells of the kidney release vesicles that favor the formation of metastases (5).

Cancer cells are not the only ones exploiting the system. Quesenberry finds that in mouse models of pulmonary hypertension, vesicles from the damaged lung can convert bone marrow cells into cells that induce pulmonary hypertension in a healthy animal (6). Viruses, too, may hijack vesicles to assist their spread. Ratajczak has found that extracellular vesicles from platelets can pass the receptor protein CXCR4 to hematopoietic cells, rendering them susceptible to infection with HIV (7). And studies show that HIV itself could hide inside extracellular vesicles, which then sneak the virus into unsuspecting cells like a microscopic Trojan horse.

Exosomes are the perfect natural vessels for delivering antisense oligonucleotides, miRNA, or siRNA to different organs or tissues.

—Alexander Vlassov

But researchers, too, are taking advantage of extracellular vesicles to design therapies to battle some of the same diseases. Camussi, for example, is using vesicles derived from mesenchymal stem cells—multipotent cells that can give rise to cells of the bone, cartilage, muscle, or fat—to deliver a payload of drugs that hinder angiogenesis and disable the expansion of carcinomas (8).

On the biotech front, a start-up company called Codiak BioSciences has set its sights specifically on therapeutics, starting with cancer. Researchers there are loading purified extracellular vesicles with a small-interfering RNA molecule—an siRNA—designed to block the activity of a mutated gene that is known to drive a variety of human malignancies.

"Exosomes are the perfect natural vessels for delivering antisense oligonucleotides, miRNA, or siRNA to different organs or tissues," says Vlassov, who has been working on protocols for isolating and characterizing extracellular vesicles and their cargo. But some challenges remain, including producing vesicles on a large scale, loading them with the appropriate therapeutic agents, and targeting them to the desired tissue.

Promising Diagnoses

In the meantime, exploiting extracellular vesicles for diagnostic purposes may turn out to be a more straightforward endeavor. Because they bear the signature molecules of their cell of origin, and are secreted into the blood and other bodily fluids, extracellular vesicles provide an easy-to-access calling card for a variety of disorders. The vesicles Skog first

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spotted contained tumor-specific RNAs and a mutant form of EGF receptor commonly found in glioblastomas, a finding that prompted him to found a company called Exosome Diagnostics, based in Cambridge, Massachusetts.

In January, Exosome launched a test that can detect a mutation characteristic of nonsmall cell lung cancer—a gene fusion known as *EML4–ALK*—in exosomes purified from a patient's blood sample. And additional "liquid biopsies" are in the pipeline, including a new screen that Skog predicts will eliminate more invasive screens for prostate cancer. "The patient just pees in a cup," he says.

If the field of exosomal research was slow to start, its subsequent explosion was perhaps driven in large part by such medical applications. "Pharmaceutical companies started to see the potential for exploiting this mechanism of communication for diagnostics and therapy," says Camussi. "I think this is the main factor driving the enhanced interest."

"Six or seven years ago, when we started studying extracellular vesicles, the reaction of my colleagues was: 'Why are you looking at that junk?'," says Quesenberry, who currently serves as the American editor of the *Journal of Extracellular Vesicles*. "But now it's beginning to be respected as a field."

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