A New Diagnostic Tool With the Potential to Predict Tumor Metastasis

By Joanne Nicholas

A recent discovery that microscopic particles once considered cellular trash are actually oncogenic exosomes may hold the key to diagnosing an individual's type of cancer and determining its likelihood of future metastasis. A study published in the journal *Nature Medicine* in May 2012 was the first to show that measuring a melanoma patient's exosome activity can predict survival.

The findings grew out of earlier work by senior author David C. Lyden, M.D., Ph.D., of Weill Cornell Medical College and Memorial Sloan–Kettering Cancer in New York. "Using confocal and electron microscopy, I noticed flecks that pathologists said were debris were actually homogeneous small particles at future sites of metastasis known as premetastatic niches where the metastatic process is initiated," said Lyden.

With metastatic melanoma exosomes the focus of the new research, the team examined exosomes in patients with metastatic disease. Because exosomes are found in all biofluids, they used stage IV melanoma plasma samples as a reference for exosome profiling. "We can perform a blood test to determine the exosome number and protein content [and] then correlate that to the stage of disease progression. This has helped validate the use of tumor exosomes as prognostic biomarkers," explained Lyden.

"For the first time, we have correlated circulating exosome profiles to melanoma metastasis and people are excited about its new role," said Hector Peinado, Ph.D., instructor of molecular biology in the department of pediatrics at Weill Cornell Medical College and first author of the *Nature Medicine* study. "Like normal exosomes, tumor exosomes participate in cell signaling but with a more sinister result. They circulate to bone marrow progenitor cells and transfer information that promotes a prometastatic phenotype with a long-lasting memory. The cells are primed to promote cancer growth but can remain dormant only to metastasize years later."

Peinado says exosomal activity can now be measured and characterized. "Every cancer type has its own exosome signature. Our data show a five- to 10-fold increase in protein content in tumor exosomes derived from stage III and stage IV melanoma patients compared to healthy people. This is the first study suggesting that circulating exosome signatures may determine which patients are at risk for metastasis and which ones do not need to be monitored."

Another new finding was "the first demonstration of a transfer and functional role of oncogenic information between tumor cells and noncancerous cells," said Lyden. "Exosomes are one of the most important mediators for the crosstalk between tumor cells and cells in the microenvironment." Lyden sees exosome proteins as a potential target for drug development to reduce tumor growth and prevent metastasis.

Lauren Pecorino, Ph.D., a cancer biologist at the University of Greenwich in London and author of *Why Millions Survive Cancer*, characterized their "landmark paper" as "the most far-reaching discovery in recent cancer research history." In an e-mail, she wrote that it "elucidates a functional role of exosomes in metastasis, characterizes their use as a noninvasive prognostic indicator for melanoma patients, and demonstrates proof of principle for new molecular drug targets to inhibit metastasis." Pecorino adds, "Metastasis—the crux of treating cancer—may finally be within our grasp."

Co–corresponding author Jacqueline F. Bromberg, M.D., Ph.D., an oncologist and breast cancer scientist at Memorial Sloan–Kettering Cancer Center, is optimistic that continued research of cancer-derived exosomes will change aspects of cancer treatment, leading to personalized medicine.

"What I find interesting about these particles is their concentrated and stable protein/RNA composition, which represents the cell of origin," said Bromberg. "One could potentially follow the expression profile of exosomal molecules in response to therapies and monitoring exosomal activity throughout treatment to detect new mutations and protein expression patterns that alter the tumor's phenotype and help understand the mechanisms responsible for drug resistance."

David C. Lyden, M.D., Ph.D.

Further study may identify each tumor's specific exosomal protein profile and subtype (e.g., ER$^+$, HER2$^+$, and ER/PR/HER2 for breast cancer), which would be useful for prognosis in predicting which patients could be at risk for future metastatic disease. Bromberg feels their research has substantial promise, but "using exosomes for cancer diagnosis and treatment is not yet ready for prime time."

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Clinical Trials Begin

Clinical trials are just beginning. Glioblastoma is one disease with an early clinical trial. Xandra O. Breakefield, Ph.D., professor of neurology at Massachusetts General Hospital in Boston, together with Johan Skog, Ph.D., a postdoctoral fellow,

accidently discovered a new form of extracellular membrane vesicles. "Examining biopsy tissue from glioblastoma patients from the operating room, we saw their cells were covered with vesicles that contained tumor RNA that was protected from rapid degradation in the bloodstream. When these glioblastoma vesicles were added to human brain endothelial cells, they started forming tubules before our eyes within a day. We had found a very potent mechanism of promoting tumor growth that we hadn't even known existed," said Breakefield.

The Breakefield laboratory's findings, published in the December 2008 *Nature Cell Biology*, raised new questions. Could they pick out glioblastoma extracellular vesicles in blood samples? Examining blood serum from 25 glioblastoma patients confirmed tumor vesicles in all patients by virtue of a glioblastoma-specific activating mutation for the epidermal growth factor receptor.

"This biofluid-based diagnostic technology provides a liquid biopsy for glioblastoma," said Breakefield. "It can be used to monitor tumor progression and for disease risk stratification." She is participating in a 10-center glioblastoma biomarker project that is part of a clinical trial looking for therapeutics to suppress tumor activity.

"RNA and nucleic acid in exosomes can be used to noninvasively interrogate what is happening in the tumor in real time," said Skog.

Skog, now director of research at Exosome Diagnostics in New York, and colleagues are part of a multi-institution clinical trial led by the Prostate Cancer Foundation to develop a more effective diagnostic test for prostate cancer by evaluating RNA and nucleic acid transcripts released into the urine in microvesicles.

Although the prostate cancer trial is farthest along, another is looking for neurodegenerative biomarkers in cerebrospinal fluid for Alzheimer disease and Parkinson disease. They are also part of a multi-institution consortium looking at brain tumor mutations such as EGFRv3 in cerebrospinal fluid and plasma to diagnose glioma.

NIH Encourages Exosome Research With Grants

The new understanding of extracellular RNA (exRNA) and extracellular vesicles (exosomes) suggest potential uses in diagnosis, prognosis, and treatment of several diseases. "For the past decades, exosomes have been viewed as inconsequential," said Danilo A. Tagle, Ph.D., associate director for special initiatives at the National Center for Advancing Translational Sciences at the National Institutes of Health. "Through a convergence of advances in sequencing technology and cell biology research, it was recently learned that extracellular vesicles are actually filled with RNA, DNA, proteins, and lipids and are very important signaling molecules."

Tagle was one of the organizers of an NIH working group that identified exRNA as an area for multidisciplinary research that could have tremendous impact. After an extensive review by NIH leadership, the NIH Common Fund announced a 5-year, $133 million funding opportunity in May 2012 to stimulate and support multidisciplinary study in five areas of exRNA communication.

The grants are for the following purposes:

- Development and demonstrate the potential for clinical utility of exRNA as therapeutic agents.
- Creation of a data-management resource/repository.
- Development of reference profiles of exRNA from healthy human blood and body fluid samples.
- Determine the principles that guide the selection of regulatory RNA molecules for extracellular transport and determine their function.
- Identify and qualify exRNA-based biomarkers from human body fluids to diagnose and monitor disease progression and response to therapy.

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Palliative Care Programs Still Face Obstacles in Mainstream Cancer Care

By Steven Benowitz

By all accounts, the past several years has seen palliative care leap into the nation's health care headlines and perhaps its consciousness as never before. Many point to 2010 as a turning point, when Massachusetts General Hospital oncologist Jennifer Temel, M.D., reported a landmark study in the *New England Journal of Medicine* showing that terminal lung cancer patients given palliative care at the time of diagnosis, along with curative cancer treatment, not only had a better quality of life but also lived a median of 3 months longer. And in 2012, the American Society of Clinical Oncology (ASCO) released a report concluding that according to available evidence, all patients with metastatic cancer can receive palliative care—defined as symptom and pain management, and psychosocial and other supportive care aimed