Why Exosomes Will Power Companion Diagnostic Development in Applications for Neurodegenerative Disorders

By Jonah R. Riddell, PhD, Exosome Diagnostics

Abstract

Exosomes were discovered 40 years ago, and the ability to perform high-throughput analysis of their molecular contents has become a prominent discussion topic recently. Exosomes have established clinical utility as proven by commercialized tests like the ExoDx™ Prostate Test and others. Expanding potential is observed for exosomes to power companion diagnostics and clinical utility for neurological disorders. For scientists interested in *ex vivo* studies, exosomes present an especially appealing target because they contain high-content biomolecules and can be studied *in vivo* as well as with *in vitro* models. Since cultured cells continue to release their exosome messengers, scientists in pharmaceutical and biotech companies have readily available model systems to create a steady supply of new or engineered materials that generate data for discovering, developing, and validating biomarkers and drugs. Furthermore, exosomes may serve as the gateway for these biomarkers to become candidates for companion diagnostics development. Such studies are of particular interest for neurodegenerative disorders and follow-up confirmation in clinical trials.

Background

Recent clinical trials for candidate Alzheimer's therapies have led to disappointing results.

Those same therapies could possibly have produced better outcomes if their use had been restricted to patients with certain biomarker profiles, but that approach would not be feasible without extensive development and validation of the right biomarkers – this validation is key to diagnostic development.

Accelerating the discovery, development, and clinical validation of new treatments could be enabled through the use of liquid biopsies and, as we shall discuss, novel exosome-based biomarkers for patient stratification. We have learned from so many other areas of medicine that *targeted* treatments can be more successful in clinical trials and regulatory review than treatments aimed at *all* people with a certain disease or condition (so-called all-comers vs enrichment trials).

Unfortunately, the world of neurodegenerative disorders has been left behind for biomarker-driven precision diagnostics. No wonder, then, that more diagnostic developers are focusing on liquid biopsies in the quest for new, actionable biomarkers for neurodegenerative disorders research that may be useful for clinical diagnosis of patients with these disorders.

Introduction: First-generation liquid biopsies

Liquid biopsy approaches have transformed patient care in several therapeutic areas, especially oncology, but more recently in fields such as neurodegenerative disorders. By its very nature, the approach involves non-invasive tests that allow for insights without time-consuming and potentially painful tissue biopsies. Oncology insights from liquid biopsies rely on data culled from circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), or cell-free DNA (cfDNA) from biological fluid samples (see **Inset 1** for more details on types of liquid biopsy samples and **Inset 2** for an oncology case study). In some cases where tissue biopsies were logistically improbable and rarely performed, liquid biopsies may now give scientists and clinicians their first view of key biological data.

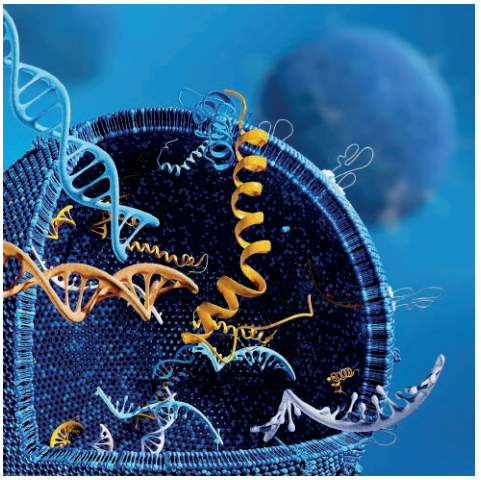

Figure 1: Exosomes are released from cells and contain DNA, RNA and protein.

These first-generation liquid biopsy approaches have yet to be fully realized in neurodegenerative disorders for brain-specific biomarker interrogation, largely because the blood-brain barrier usually prevents first-generation type biopsy material (e.g., demyelinated, sloughed, or damaged cells) from the body's circulatory system.

Next-generation liquid biopsies: Exosomes and the potential for biomarkers in diagnostic applications

A more recent entrant to the biomarker landscape may address issues that first generation biopsies cannot. Exosomes are a type of extracellular lipid vesicle routinely released by cells through membrane budding from the cell's lipid bilayer; bits of the extruded membrane break away to form protective bilayer vesicles for the exosomes' internal cargoes. As relatively compact packages (30 nm to 200 nm in diameter packed with DNA, RNA, and proteins; see **Figure 1**), these small vesicles are able to be shuttled in the circulatory, lymphatic, and cerebrospinal fluid (CSF) systems and the urinary tract to provide important information to other cells.[1-3] For example, analyses have revealed that exosomes play a key role in the crosstalk between various neural cell types, such as between neurons and glial cells.[4]

Exosomes are often present in greater abundance than other liquid biopsy targets (e.g., cfDNA or CTCs). Their cargo of DNA, RNA (including miRNA and mRNA), and proteins offers a wealth of information about the cell of origin, its state of health, dynamic changes, and much more.[5] Even a limited and tissue-specific population of these vesicles can shed light on the genome, transcriptome, metabolome, glycome, and proteome of the cells they came from. By looking at the total exosome population, scientists can get a diverse view of what's going on throughout the body by characterizing the many different exosome-wrapped messages produced by each cell.

INSET 1

First- and second-generation liquid biopsy targets

To understand where exosomes fit into the biomarker landscape, it's worth taking a moment to compare the various materials typically analyzed in liquid biopsies.

cfDNA. As its name suggests, cell-free DNA is released when cells die off or are lysed. cfDNA circulates throughout the body and can easily be collected from plasma. It is often used to characterize tumors as the cfDNA is shed from the tumor, or in prenatal testing to analyze fetal DNA circulating in the mother's blood. The fraction of cfDNA available to scientists or clinicians is limited since each split cell only offers two copies of the person's genome. Of course, once the cell has been lysed and released its DNA, there is no chance to go back and get more information from that cell or to get any dynamic information on a changing state of the specific cell.

CTCs. In patients with solid-tissue cancers, tumors slough off cells that can circulate in the body. This is a source of metastasis, but it's also an opportunity for scientists or doctors to intercept these CTCs in the bloodstream and learn more about the tumor they came from. CTCs are useful exclusively for oncology applications. CTCs can be cultured in the lab, so they can give scientists a continuous source of information so long as they are kept in the right conditions to remain viable. While CTC analysis has become fairly common for research and clinical applications associated with a range of cancers, it has not yet been widely used to investigate brain cancers.[10]

ctDNA. A specific type of cfDNA is ctDNA, which in many ways represents a mix of the CTC and cfDNA approach. ctDNA is the cell-free DNA released by tumor cells. Like CTCs, ctDNA is used solely for analysis of cancer. It is readily accessible in the bloodstream and offers a snapshot of the tumor's DNA profile. But it suffers two limitations: it can be difficult to find, since it only comes from tumor cells and releases just two copies of DNA; and it cannot be cultured in a lab since it does not come from a viable cell.

Exosomes. The fourth common target of liquid biopsies is the exosome – the second generation of biopsy targets. These vesicles hold the appeal of being a renewable resource, since they can be released by cells, collected in biofluids, and kept in frozen storage for years. Their abundance is also a significant advantage; thousands of exosomes may be shipped off by a single cell each day, and there can be billions of exosomes in a milliliter of plasma, urine, or other biofluids. Unlike cfDNA and ctDNA, they offer a glimpse of the genome, transcriptome, and proteome of their originating cell for a more comprehensive view of biology. The presence of various analytes gives the advantage of multi-analyte or multi-omic approaches using mixes of analyte information, such as RNA and protein. Because they are produced by almost every cell in the body regardless of disease state, they are useful in a wide range of applications, from cancer to neurological disorders to rare diseases and much more.

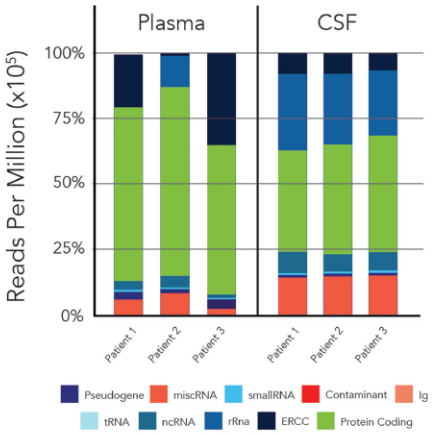

Figure 2: Exosomes are actively released from living tissues and can be captured from biofluids such as blood, urine, cerebrospinal fluid (CSF) and saliva. The exosome compartment stabilizes the RNA transcriptome and if isolated correctly, the entire transcriptome can be mapped (RNAseq). From as little as 0.5 ml of plasma we can see over 30,000 of the 56,000 RNA targets in gencode. A variety of RNA psudotypes can be found in exosomal RNA isolated from Plasma and CSF. Data from Exosome Diagnostics Research Laboratories, unpublished.

The information collected provides a unique and broad snapshot of cellular activity at any given time. In this regard, exosomes can be harvested to detect DNA and RNA elements (e.g., splice variants or components that manage gene rearrangements).[6]

Potential for Biomarker Discovery for Neurodegenerative Disorders

Obtaining a tissue or liquid biopsy of a person's brain comes with some degree of risk. Because of the relative impermeability of the blood-brain barrier, extracting molecular information produced by neurons and other brain cells is challenging. The presence of exosomes from brain cells in body fluids could offer a means to collect neuronal information as noninvasive samples, making them ideal candidates to discover high-content biomarkers. Exosomes are already showing real promise for providing such higher-content markers for neurodegenerative disorders.[1] A pressing need, however, exists to extend the benefits of precision diagnostics to patients with neurological diseases or disorders.

Alzheimer's disease, Huntington's disease, and Parkinson's disease are just a few of the well-known neurodegenerative disorders for which patients and physicians are desperate for better detection and treatment options. According to expert analysis, the global disease burden of neurological disorders and brain-related cancers is high – nearly 1 in 6 people are affected.[7] As five-year survival rates for other types of cancer have improved, the rates for people with neurological-related cancers have seen little change in the past several decades.[8]

"For neurodegenerative disorders, exosomes offer three distinct advantages: first, unlike cfDNA, exosomes keep their contents stable because the lipid bilayer protects the exosome's content from enzymes and subsequent degradation. Second, exosomes circulate throughout the body and, third, of perhaps the most critical significance, exosomes can freely cross the blood-brain barrier."

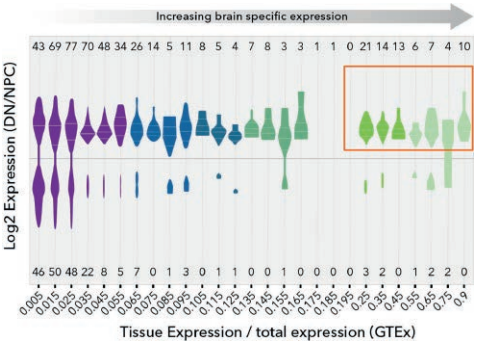

Figure 3: The violin plot displays gene expression patterns in fully differentiated neurons (DN) vs. neuronal progenitor cells (NPCs). Data from Exosome Diagnostics Research Laboratories, unpublished.

Recent exosome-based studies in these and other diseases have shown that exosomes contain relevant molecular cargo that could aid in the development of companion diagnostics. For example, exosomes contain proteins and microRNAs associated with Alzheimer's disease as well as biomarkers indicative of Parkinson's disease.[1] Already, many of these biomarkers have been moved into clinical trials to better gauge their potential for healthcare use.[9]

Moving from Biomarkers to Companion Diagnostics Development

With a better source of neurodegenerative biomarkers, it would be possible to match patients with drugs in development and give new diagnostics and therapies a better chance at reaching the market to make a difference in patients' lives. Exosomes represent an opportunity to accelerate this process as these biological messengers enable real-time, longitudinal monitoring of cellular processes to characterize dynamic changes in the brain over time, something that is not possible with standard tissue biopsy (see **Inset 2**). With their cargo of DNA, RNA, and proteins, exosomes can be analyzed with a number of approaches, including genome and transcriptome sequencing as well as proteomic analysis. In fact, exosomes have already been proven to contain actionable markers, including lncRNAs, microRNAs, proteins, and key genetic variants.[4] This versatility in target analytes can increase the chances of identifying a useful biomarker, thereby allowing researchers to leverage develop high-value diagnostics (**Figure 2**).

Often in neurology there are rare cell types contributing to disease; these cell types are sorely lacking in useful molecular diagnostics. Molecular biomarkers from rare cells may be candidates for diagnostics, but they are difficult

INSET 2

An example from neuro-oncology

Diagnostics derived from exosomes offer the opportunity to define and stratify patient populations. For example, in a study of patients with glioblastoma, scientists from Harvard Medical School and other institutions used RNA sequencing of exosomes to identify a biological signature that separated responders from non-responders to a tyrosine kinase inhibitor.11 In separate unpublished work, a single microRNA biomarker proved sufficient to distinguish between responders and non-responders to a glioblastoma treatment (Data from Exosome Diagnostics Research Laboratories, unpublished). These kinds of biomarkers can be developed into a robust, exosome-derived companion diagnostic. The approach is ideal for clinical trials, where testing with a noninvasive liquid biopsy makes it easier to collect more data from participants more often, for a comprehensive evaluation of therapeutic efficacy.

Since exosome analysis has the potential to offer in-depth views of gene activity in the brain, exosome-based biomarkers may also be valuable for noninvasive monitoring of disease progression. In one glioblastoma study based on exosomes that has not yet been published (Data from Exosome Diagnostics Research Laboratories, unpublished), gene expression analysis identified more than 450 differentially expressed genes. The top 25 genes linked to therapeutic response were associated with eight key biological pathways and revealed clear differences between healthy controls and glioblastoma samples. Another internal glioblastoma-related analysis showed the value of comparing exosomes from different sample types. Plasma and cerebrospinal fluid samples were collected from a patient; gene expression analysis was conducted, and clear differences were seen in the levels of thousands of genes, suggesting that biofluid-specific signatures could be possible.

to identify in low concentrations. An important technical consideration is that exosome analysis can allow for enriching targeted cell types of interest, thereby enriching certain targets or analytes. Enrichment and depletion assays can be used to boost the signal-to-noise ratio to ensure more targeted results and focus the analysis. This allows scientists to tease out rare disease signatures that might otherwise go undetected. The technique has already been used to enrich low-abundance proteins important for brain research, such as phosphorylated tau. Hence, new companion diagnostics might be made possible in a field otherwise wanting for the earliest possible signal of onset.

Partnering Strategies for Drug and Companion Diagnostics Development – the key role of communication, shared goals, and clear timelines

Clearly, exosomes have strong potential for use in the design and development of companion diagnostics for neurodegenerative disorders applications. But performing the deep exosome analysis needed to create those diagnostics may not fit easily into traditional pipelines at pharmaceutical and biotech companies. As with other types of companion diagnostics, pharma and biotech scientists have chosen to partner with diagnostic developers rather than designing the clinical assays themselves. This model is also a good approach for diagnostic tests based on exosomes.

The expertise required to collect and isolate exosomes, and then to enrich for desired targets and perform biomarker analysis, is still limited to niche groups that focus on extracellular vesicles. **Figure 3** demonstrates brain specific gene expression is observed as neuron differentiate from a progenitor cells. A partnership model allows drug discovery scientists to team up with exosome experts who have established technology workflows and deep familiarity with exosome biology.

This commonly-used divide-and-conquer approach also makes it possible to parallelize the development of a candidate therapy and its companion diagnostic. Drug discovery scientists can focus on what they know best – ushering new leads through the rigors of discovery and development processes – while exosome scientists are performing the studies needed to identify and validate biomarkers that will form the basis of a new diagnostic test. By running these projects simultaneously, candidate therapies can be sent to clinical trials and then to regulatory review months or even years sooner than would be possible if the same team of pharma or biotech scientists had to build their own diagnostic as they developed the new drug.

For neurodegenerative diseases and disorders, where so little is known about potential biomarkers and the ability to stratify patients, the partnership model would be most beneficial. Brain-related biomarkers may be more complex than most biomarkers – for instance, many neurological conditions are associated with repeat expansions, which are difficult to detect and quantify accurately with traditional methods. Ensuring that development of both the therapy and the companion diagnostic is overseen by scientists who specialize in those areas is critical to achieving a successful outcome. Ideally for pharma and biotech companies, diagnostics development partners will have global reach and provide not only expertise in exosome analysis and biomarker development, but also in test commercialization, regulatory review, and reimbursement best practices.

Summary

Exosome analysis has potential value in research and drug development for neurodegenerative disorders conditions, and in the future, it will likely have great value in assisting with patient care. With careful validation and development of exosome-based biomarkers, future companion diagnostic tests offer an opportunity to improve these important areas of healthcare. With their ability to pass through the blood-brain barrier and abundance in plasma, cerebrospinal fluid, and other accessible biological fluids, exosomes may be the perfect target for developing biomarkers and companion diagnostics for neurodegenerative disorders conditions. JoPM

INSET 3

Biomarkers, Diagnostics, Companion Diagnostics

The Journal recognizes biomarkers, diagnostics, and companion diagnostics assays as distinct testing designations. We make this distinction to avoid confusing specific functions for each assay in different fields, ranging from drug discovery and development to clinical applications. Biomarkers are particularly useful in the course of developing a drug or as read-outs for the state of a patient. In contrast, an FDA-approved diagnostic has utility as a test (based on biomarkers) that allows a physician to treat a patient. Companion diagnostic developers need to identify which patients are most likely to respond to a specific therapy, thereby increasing the likelihood of successfully treating patients and recommending which patients may need to seek another treatment option (or, in some cases, no follow-on treatment at all). In fact, companion diagnostics need to provide "information that is essential for the safe and effective use of a corresponding therapeutic product (from FDA definition)."

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Jonah is a product manager working with Exosome Diagnostics, a Bio-Techne Brand and supporting multiple product lines in therapeutic oncology and immunology. He earned a Master's degree in Oncology and PhD in Immunology at State University of New York at Buffalo, Roswell Park Division where he patented a novel immune oncology related biomarker. In the next phase of his work at the Harvard Stem Cell Institute, he drove research on the preclinical utilization of reprogrammed blood cells to better approach blood transplantation and reduce graft rejection. Shifting gears after some brief work in pharma and biotech he has spent the last ten years building a career focusing on commercially supporting and launching new products in areas including immunotherapy, infectious disease, oncology.

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