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Exosomes: What do we love so much about them?

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Summary

Exosomes, once thought to be biomarkers of a diseased state are now thought to be biologically active and some of the paracrine effects of stem cell therapy. While there is much excitement around their actions, there is also a growing need to better understand the role of cell source, exosome content, and exosome targeting in a quantitative manner. Better understanding of these variables and others, in a reproducible and comprehensive approach, could better inform future therapy.

Keywords

exosome; microRNA; systems biology

In recent years, exosomes have been thrust in to the spotlight for their ability to deliver a therapeutic payload to injured cells and/or regions of the cardiovascular system. As I sit here and try to come up with an analogy to showcase some of the important issues, I felt using the upcoming American Heart Association Scientific Sessions destination may have some impact. For those who have traveled to New Orleans and have been lucky enough to sample the amazing cuisine, there is one thing that springs to my mind when thinking about Cajun and Creole foods. With apologies to the vegetarians reading this, one major ingredient of many of these dishes is sausage. So yes, I am comparing sausages to exosomes, but please bear with me. Thinking out loud, they both are loved by diverse groups, the source is both contentious and also a defining factor of what we love, and they both contain lots of things in them that could be the source of the great flavor...or activity.

Now that the analogy is out of the way, let us expand a bit on all the points. Originally considered cell debris, small, membrane-derived vesicles were more appropriately characterized in 1987 and termed exosomes [1]. Exosomes are a specific subtype of secreted

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membrane-bound vesicles, with diameters ranging from 30-130 nm. They are actively and constitutively released from cells by fusion of multivesicular bodies with the cell membrane. These vesicles have been shown to carry proteins, mRNA, and microRNA, and have been implicated in intercellular communication [2]. In fact, most initial studies over the first few decades focused on the identification of cargo within the exosomes and vesicles that could be used to detect a pathophysiological condition. It was in the early-2000s when researchers found that exosomes could transfer signals from one cell to another, a novel form of paracrine and possibly autocrine signaling [3]. Nearly every cell type has been shown to secrete exosomes; some of those verified include platelets, lymphocytes, and adipocytes, and, muscle, tumor, glial, and stem cells.

While exosomes were around for quite some time, it was not until 2008 when researchers found that they carried microRNAs (miRs). Like most other circulating factors and vesicles, most studies focused on the signature of the miRs within the exosomes as a biomarker of diseased states, primarily for cancer. A key study by Hergenreider et al demonstrated that endothelial and smooth muscle cells transfer atheroprotective signals by extracellular vescicle-mediated miR transfer, ushering in a new role for vesicles, and possibly exosomes, as genetic and epigenetic regulators of cell function [4]. Since then, hundreds of studies have confirmed that exosomes and other vesicles do indeed carry non-coding RNAs and transfer these to regulate functions in target cells. Studies by Arslan et al and Ibrahim et al were then among the first to show that exosomes-derived from stem cells could be used to treat dysfunction following myocardial infarction [5]. In fact, much of the benefits of cell therapy, at least for cardiosphere-derived cells (CDCs), were linked to their exosomes as inhibition of exosome secretion led to a reduction in efficacy [6]. All of these points lead to the first issue mentioned above, which is the source of the exosomes.

As mentioned above in the above analogy, people enjoy sausages from all different sources. Similarly, we seem to love exosomes from all sources. To date, exosomes derived from embryonic stem cells [7], cardiac progenitor cells (CPCs) [8], CDCs [6], immune cells [9], mesenchymal stem cells [5], and umbilical cord blood-derived cells [10] have all improved function following myocardial infarction in animal models. While this is quite exciting, how will one determine what cell source is optimal for exosome therapy? The answer is more cross-cutting, quantitative analysis of the effects of the exosomes. For example, which exosomes alter fibrosis, improve angiogenesis, improve endogenous stem cell migration, and more metrics. While it sounds counterintuitive to do the same thing as the other studies, it is clearly the only way to compare them between studies other than using multiple sources of exosomes in the same study. Quantitative analysis regarding the magnitude of the changes is a starting point by which to compare whether certain sources of exosomes have differential, or even overlapping effects. In addition, more data on the target cells of the exosomes is needed. Exosomes contain surface proteins that many believe are not just a random cellular event, but specify where the exosome is targeted. For example, in the study by Ibrahim et al, the authors show exosome uptake in to neonatal cardiomyocytes [6]. In contrast, the study by Gray et al (and our own unpublished data) suggests that exosomes derived from c-kit positive CPCs do not get taken up by cardiomyocytes [8]. While there was a difference between the cells (one used neonatal and the other adults), there exists the potential that CDC exosomes may contain a surface protein that is recognized by cardiomyocytes while

the CPCs do not. In fact, the authors demonstrated robust uptake by cardiac fibroblasts, indicating the possibility that surface proteins may dictate that relationship. Thus a comprehensive study of the role of surface proteins included in exosomes by both donor and recipient cells is greatly needed.

To return again to our sausage reference, we all know that they contain a variety of ingredients that contribute to their appeal. Similarly, exosomes contain protein, lipids, sugars, and microRNAs. While the earliest discoveries of exosomal transfer centered on the immune system (transfer of immunologically active exosomes) [11], recent studies have focused on their ability to regulate target gene expression. The first report of exosomes containing microRNAs was in 2007, and the authors termed this “exosomal shuttle RNA” referring both to mRNA and microRNA [12]. Since this discovery, publications on this phenomenon have increased at an exponential pace, yet there are very few quantitative analyses of microRNA content and function. Most studies look for enriched microRNAs compared to a control exosome preparation and then attempt to reverse engineer importance. For example, a certain microRNA is heavily enriched and happens to negatively regulate fibrosis, and thus confirmation experiments show that treatment with the microRNA brings about the same response. But what of the other thousands of microRNAs in the exosome? The need to publish a plausible and testable mechanism drives much of this, but there needs to be a quantitative and unbiased way to determine microRNA contributions. Casting aside our obvious bias, we have used computational modeling to determine not only what microRNAs are changing, but what other ones are changing with them (covarying signals). By sequencing entire exosome contents and then quantitatively fitting them to cellular or physiological outputs (regression analysis) one can make inferences about signals that are likely to contribute to a response [8]. The obvious drawbacks are that this does not actually pinpoint a causative mechanism, and also creates a large amount of data that needs to be tested. As more datasets are collected of exosome sequencing, the need arises to build a comprehensive model that determines what the contents of each exosome are, and how likely those contents are to contribute to the response. In fact, we are currently attempting to do this with CPCs and $CD34^+$ cell exosomes [13] as both have similar effects on angiogenesis. Identifying both unique and common signals that contribute to exosome function could help predict the efficacy of other exosome sources, as well as generate synthetic exosomes with selected signals.

I will end with not another belabored sausage reference, but rather with some ideas on areas of interesting growth in this field (this is an opinion piece). What we have found, and others now echo, is that what is put in to exosomes does not always match what is in the cell. For example, we show that cells subjected to hypoxia alter what goes in the exosomes, but not all microRNAs enriched in the exosomes were upregulated in the cell. Thus, there appears to be a regulated response that places certain microRNAs in exosomes in response to stimuli. In recent review, it was suggested that perhaps RNAs interact with specific molecules on the surface of multivesicular bodies, though this has not been directly tested [14]. Understanding why some microRNAs are preferentially loaded in specific exosomes could lead to directed stimulus control of microRNA loading from a single cell to multiple different outputs. Finally, there are indeed more within exosomes than microRNAs. In fact, a recent paper demonstrated that in exosomes derived from cancer patients (and other sources) that there

was actually less than 1 microRNA molecule per exosome [15]. This is in line with the fact that many studies require large amounts of exosomes to see their effects, and also raises the possibility that, as the authors noted, exosomes are unlikely to be functional delivery vehicles for microRNA. Thus the more quantitative data that can be gathered on exosome content, the more models can be adjusted to account for these variables. As several groups are now attempting to create synthetic and designer exosomes, determining the markers that target cells, the best contents, and unbiased and quantitative ways to analyze exosome function is critical.

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