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EDITORIAL COMMENT

On Our Doorstep, A Precious Cargo From MSCs

The Role of Extracellular Vesicles in Stem Cell Therapy*

John P. Cooke, MD, PhD

Good things come in small packages. During the strange times in which we now find ourselves, sequestered from the pandemic, our days are brightened by the regular arrival of brown rectangular packages. Fairly generic in appearance, varying only in a relatively narrow range of dimensions, they arrive at our doorstep. But inside these blandly wrapped packages, we find a nearly limitless variety of cargo that maintains our connection with the rest of the world. The contents of the cargo are necessary for our activities of daily life, they sustain us, influence our behavior, rejuvenate us.

A cellular cargo that bears some similarities to our own porch parcel is featured in this issue of *JACC: Basic to Translational Science* by Wang et al. (1). Extracellular vesicles (EVs) are small packages released by cells. They range in size from nano- to micrometers and are delimited by a lipid bilayer. Under the electron microscope, these small round vesicles seem uniform and bland. However, under closer inspection, they contain a precious cargo consisting of proteins, nucleic acids, lipids, or metabolites that reflect the state of the cell from which they were derived. Cells release EVs into the local environment to influence neighboring cells, or into the systemic circulation where they travel to distant tissues. Subsequently, EVs are taken up by endocytosis, and their contents can significantly influence cell function and behavior.

Wang et al. (1) have studied EVs from mesenchymal stromal cells (MSCs). The MSCs are a form of resident stem cell that can differentiate into a narrow range of mesodermal cells, including adipose, muscle, fibroblast, and endothelial cells. The MSCs are favored in the development of stem cell therapies, as there is a substantial preclinical literature revealing that exogenously injected MSCs have beneficial immunomodulatory and angiogenic effects. For some time now, it has been recognized that any benefit of MSCs are due to a paracrine effect (rather than their differentiation into other cells and their incorporation into the tissues in which they have been introduced). These paracrine effects are mediated in part by proteins released by MSCs, such as the anti-inflammatory cytokine interleukin-10, as well as the angiogenic factor vascular endothelial growth factor (VEGF) (2). Wang et al. describe another paracrine effector, the MSC-derived EVs, and their rejuvenating effect on endothelial progenitor cells (EPCs).

Young MSCs rejuvenate aged EPCs. Murine MSCs and EPCs were isolated from bone marrow of young or aged mice. The cells were sorted using magnetic beads with antibodies specific for these cell types, and the cells were cultured for further study. The aged MSCs showed typical morphological signs of cellular senescence and grew more slowly. The authors observed that co-culture with young (but not aged) MSCs rejuvenated aged EPCs. Specifically, the investigators observed that co-culture with young MSCs decreased the expression of the cell senescence marker beta galactosidase in aged EPCs. Furthermore, the co-culture reduced the EPC levels of the cell cycle inhibitors $p16^{INK4a}$ and $P19^{ARF}$, which are also markers

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From the Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, Texas, USA.

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of senescence. Notably, in the co-culture system used, the MSCs were not in direct contact with the EPCs, so something released by the young MSCs was rejuvenating the aged EPCs. This observation is consistent with that of other investigators, in that it seems that the paracrine effects of MSCs are responsible for their rejuvenating effects. So what rejuvenating factors were being released?

Rejuvenation is mediated by EVs. As described previously, EVs are a form of cellular communication, small biological messages that travel through extracellular space to be absorbed by other cells, and thereby influence their behavior. Recently, several research groups have found that the administration of MSC-derived EVs can mimic the effects of MSC therapy (2). Therefore, the investigators collected the conditioned medium from aged and young MSCs, and isolated EVs and purified them using magnetic beads carrying an antibody against CD63, a common EV antigen. Subsequently, aged EPCs were cultured for 4 days with EVs from the aged or young MSCs added to the medium. The EVs from young (but not aged) MSCs reduced the levels of the senescence marker and cell cycle inhibitors in EPCs.

The EV cargo includes microRNAs (miRs) which are small bits of ribonucleic acid (about 22 nucleotides) that can bind to messenger RNA causing its destruction. Thus, miRs influence gene expression by regulating which messenger RNA are converted into functional or structural protein within a cell. Previously, the investigators have found that senescent endothelial cells have a characteristic miR profile. Interestingly, they found the same profile in aged MSCs, that is higher levels of miR-146a, miR-10A*, miR-21, and miR-29c, and a lower level of miR-126. When they examined the EVs from aged MSCs, they found the same alterations in the miR profile, in comparison with EVs from youthful MSCs.

Can the cargo of EVs be genetically enhanced? The investigators reasoned that, by making the EV cargo from old MSCs look more like that of young MSCs, they could restore the regenerative benefit of old MSCs. To do so, they transfected old MSCs with a lentiviral vector that overexpressed miR-126, which is known to be important in endothelial function and angiogenesis. The genetically modified MSCs produced tailored EVs (TEVs) that carried a cargo with 20-fold more miR-126 than EVs from the unmodified aged MSCs. Furthermore, when aged EPCs were treated with the TEVs, the senescence marker declined, the cells proliferated more, and the EPCs performed better in an in vitro angiogenesis assay.

To determine if TEVs could have similar benefits in vivo, the investigators used a murine model of peripheral arterial disease, in which the femoral artery is ligated to reduce blood flow, as assessed by Doppler imaging. This well-characterized model is responsive to angiogenic agents such as VEGF or a variety of stem cell therapies. In this model, the investigators found that intramuscular injection of TEVs increased perfusion by Doppler imaging, in association with an increase in tissue vascularity as assessed by histology.

The investigators propose that the angiogenic benefit of the TEVs is primarily due to an enhancement of murine EPCs. This is not likely. The TEV-induced enhancement of capillary density is more likely due to a local effect on existing endothelial cells, causing a VEGF-mediated sprouting and migration of these cells to form new capillaries. A small contribution may be made by resident fibroblasts, a subset of which can transdifferentiate into endothelial cells under the influence of innate immune activation and local angiogenic factors (3). To be sure, EPCs can contribute to neoangiogenesis in ischemic tissue, but it is not clear how locally injected TEVs would affect EPCs circulating in the blood.

Here, it is worth pointing out that the term *endothelial progenitor cell* is a misnomer. These circulating cells are of hematopoietic lineage, and the great majority of these cells do not inosculate into capillaries. Rather, they secrete angiogenic factors that contribute to angiogenesis. To be sure, you can isolate EPCs from the bone marrow, using the accepted surface markers (one of which is the hematopoietic cell marker CD45) and culture them under specific conditions, ultimately generating cells that very closely resemble endothelial cells. But under these specific conditions (angiogenic factors such as VEGF and fibroblast growth factor), you can also generate endothelial cells from fibroblasts, given a jolt of innate immune activation (4). Indeed, as demonstrated in a recent *Nature Medicine* paper, the benefit of MSCs may be due in large part to their activation of innate immune signaling (5). In tissues confronting a challenge (e.g., ischemia), activation of inflammatory signaling causes global changes in the expression and activity of epigenetic modifiers that places the chromatin into an open state configuration. Under these conditions, the cell can reach back into its genetic toolbox, and pull out what it needs to survive and adapt. Such epigenetic plasticity permits changes in cell phenotype that permit remodeling, regeneration, and repair.

Nevertheless, the work of Wang et al. (1) has expanded our knowledge of the rejuvenating effects of MSCs. The benefit of MSCs seems to be in large part due to their release of EVs. Because they may be easier to generate, characterize, and deliver, EVs might represent a more feasible therapeutic avenue for cardiovascular regeneration. Furthermore, whereas the EVs from aged MSCs do not impart rejuvenating effects on other cells, they can be genetically engineered to do so. Dr. Hare and colleagues have been pioneers in clinical trials of MSCs for cardiovascular therapy. In this latest report, to our field of regenerative medicine, they have delivered some precious cargo.

AUTHOR DISCLOSURES

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ADDRESS FOR CORRESPONDENCE: Dr. John P. Cooke, Department of Cardiovascular Sciences, Houston Methodist Research Institute, 6670 Bertner Avenue, Mail Stop R10-South, Houston, Texas 77030. E-mail: jpcooke@houstonmethodist.org.

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