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Linda Marbán, Capricor Therapeutics

PRODUCT R&D

STEM AND DELIVER

By Karen Tkach, Staff Writer

Exosomes, once considered cellular debris, are gaining a name in cancer as biopsy-free diagnostic agents, and in drug delivery for their ability to enter cells in a targeted manner. Now, Capricor Therapeutics Inc. wants to use the particles for regenerative medicine, and is preparing to announce its first program early next year.

Exosomes are membrane-enclosed particles shed by cells, that contain proteins, lipids and nucleic acids. (See "Enter Exosomes", page 9)

So far, the commercial focus for exosomes has been primarily in oncology. Exosome Diagnostics Inc. and Aethlon Medical Inc. subsidiary Exosome Sciences Inc. are developing diagnostics that aim to use the abundance of circulating exosomes in the bloodstream as a way to determine the protein and RNA signatures of cancer cells.

And last month, Codiak BioSciences Inc. was launched by former Biogen Inc. exec Douglas Williams together with Eric Lander, president and director of the Broad Institute of MIT and Harvard, to pursue exosomes as biomarkers and as delivery agents for cancer therapies.

The idea that exosomes from stem cells could have regenerative properties has been gaining ground in academia and in a small niche of regenerative medicine companies. Last year the lab of Eduardo Marbán, Capricor's scientific founder and the director of the Cedars-Sinai Heart Institute, showed exosomes from cardiac-derived stem cells promoted heart repair after myocardial infarction (MI), and published the findings in *Stem Cell Reports*.

On November 12th, at the meeting of the World Alliance Forum in San Francisco (WAFSF), Capricor CEO Linda Marbán highlighted the finding that exosomes can account for over 80% of the benefits of the company's cardiac-derived stem cells. Capricor is developing CAP-1002, its lead stem cell product, together with Johnson & Johnson, and the cells are in Phase II trials for MI and Phase I for heart failure.

"We started our exploration of the exosome as a therapeutic because we discovered it as we were looking for the mechanism of action of our cells," Linda Marbán told BioCentury.

Marbán noted that the particles have several advantages over stem cells, including low immunogenicity, even in xenograft experiments, and easier CMC. The particles can be lyophilized and stably stored, and could have lower cost off-the-shelf use than stem cells, she said.

John Sinden, CSO of ReNeuron Group plc, told BioCentury that being able to derive exosomes from a GMP, clinical-grade stem cell product is also a plus because the cells are standardized and easily scalable, which helps "in terms of building a platform."

ReNeuron is conducting preclinical studies of exosomes secreted by its lead cellular therapy, the neural stem cell line CTX, which is in Phase II trials for stroke disability and in Phase I for critical limb ischemia. Sinden said ReNeuron is pursuing exosomes in different indications from its stem cell products, in diseases "that the cells wouldn't necessarily be great in."

Danilo Tagle, associate director for special initiatives at NIH's National Center for Advancing Translational Sciences (NCATS), told BioCentury exosomes have some safety advantages over stem cell therapies. Tagle leads NCATS's Extracellular RNA Communication Consortium (ERCC), a program that connects and funds researchers working on exosomes and other extracellular vesicles.

He said the particles' benefits include "the avoidance of potential teratoma formation," as well as their transience compared with

stem cells. "When you put in stem cells, they are there pretty much for the duration of that person's life, whereas the exosomes do turn over, and so you don't have the difficulty of having to do long-term follow-ups to track the cells as they divide," he said.

CAPRICOR'S CORNER

Capricor launched its program after licensing the findings from Cedars-Sinai Medical Center last year.

In the *Stem Cell Reports* study, the Cedars-Sinai group showed exosomes secreted by Capricor's cardiac-derived stem cells could replicate many of the parent cells' therapeutic effects. The exosomes increased cardiomyocyte proliferation and viability *in vitro* and decreased scar tissue in a mouse model of MI compared with vehicle or exosomes from a control fibroblast cell line. The stem cell-secreted exosomes also increased cardiac function, viable heart mass and infarcted wall thickness.

In addition, the authors showed that inhibiting exosome secretion decreased the regenerative capacity of the stem cells in the MI model, suggesting exosomes account for most of the cells' therapeutic activity.

Although the team identified miR-146a as a key mediator of the exosomes' activity, Marbán believes there are a range of other components at play as well.

Capricor has characterized the exosomes at a molecular level, identified the best delivery routes for the particles in mice, rats and pigs, and developed standardized culture conditions, which Marbán said is key to tuning the exosome's effects.

"By altering the conditions the cells are exposed to, they can take on different flavors, such as being more pro-angiogenic or antifibrotic," she said.

To start, the company will use exosomes derived from its cardiac-derived stem cells, which Marbán thinks will have applications beyond the heart. She noted that the company is looking at opportunities in eye and skin diseases and cancer, and is "on the cusp of announcing new indications."

BUDDING FIELD

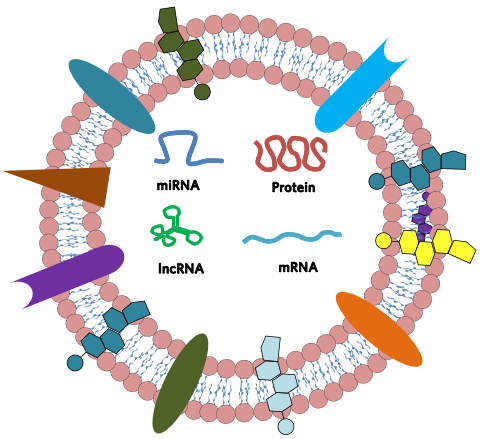
NCATS's Tagle thinks it's early days in the field, and a critical starting point should be to categorize and understand what kinds of exosomes different cells are capable of producing, before attempting *de novo* design.

"Once we have this long laundry list of different types of exosomes, looking at what makes them distinct in terms of specific delivery or capacity to take in larger molecules, then we can start thinking about engineering exosomes," he told BioCentury.

ENTER EXOSOMES

Exosomes are secreted nanovesicles that can ship molecular cargo between cells. Capricor Therapeutics Inc. is conducting preclinical studies on the regenerative properties of exosomes produced by stem cells and hopes to develop the exosomes as an off-the-shelf therapeutic for tissue repair.

The particles are about 30-100 nm in diameter, with membranes typically enriched in lipids such as cholesterol, proteins such as tetraspanins, and other cell-specific markers (**colored shapes in membrane**). In addition, glycoproteins on the exosome surface can target the vesicles to specific recipient cells. The particles encapsulate their cargo — **proteins**, lipids, or nucleic acids including **mRNA**, microRNA (**miRNA**) and long non-coding RNA (**lncRNA**) — which mediates the exosomes' effects, such as reprogramming of target cells. *Source: Reproduced from a corporate presentation, with permission from Capricor Therapeutics Inc.*



Part of that characterization has been figuring out which molecules mediate the particles' effects, and how. Tagle said research so far suggests proteins and nucleic acids play distinct roles.

"I think if you're looking for the functionality of the exosomes in terms of transforming the phenotypes of the recipient cells, it would be the nucleic acids," he said. "But if you're looking at the functionality of exosomes in relation to their specificity of delivery, then it would be the glycoproteins on the surface that determine the targeting. In the end it's a constellation of all these combinations that one needs to consider when developing exosome-based therapeutics."

Tagle added that while miRNAs in exosomes have been thought to play important roles in reprogramming, recent studies suggest they are not the only nucleic acid species involved.

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>
> Danilo Tagle, NCATS

"As our investigators are starting to do RNA sequencing analysis, we are finding out that miRNA is not the only nucleic acid that's present. There is DNA, there's long non-coding RNA, there's small nucleolar RNA. And all of those RNA molecules, as we understand more about them, tend to have some sort of regulatory role."

A lot about exosome-based therapies needs to be defined by regulatory agencies, said Tagle.

"Because they're cell derived, they could potentially be under the purview of the FDA's cellular biologics division," he told BioCentury. "But once you start putting cargo into them, whether it be protein-based vaccines, natural products or small molecules, they become treated like nanovectors. We need a few test cases."

He noted that NCATS's ERCC is trying to help scientists navigate these gaps by promoting academic-industry partnerships, which it hopes will bring exosome research closer to the clinic. "Part of NCATS's mission is really being a catalyst," he said.

COMPANIES AND INSTITUTIONS MENTIONED

Aethlon Medical Inc. (NASDAQ:AEMD), San Diego, Calif.
Biogen Inc. (NASDAQ:BIIB), Cambridge, Mass.
Broad Institute of MIT and Harvard, Cambridge, Mass.
Capricor Therapeutics Inc. (NASDAQ:CAPR), Beverly Hills, Calif.
Cedars-Sinai Heart Institute, Los Angeles, Calif.
Cedars-Sinai Medical Center, Los Angeles, Calif.
Codiak BioSciences Inc., Cambridge, Mass.
Exosome Diagnostics Inc., Cambridge, Mass.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
National Center for Advancing Translational Sciences (NCATS), Bethesda, Md.
National Institutes of Health (NIH), Bethesda, Md.
ReNeuron Group plc (LSE:RENE), Guildford, U.K.
U.S. Food and Drug Administration (FDA), Silver Spring, Md.

TARGETS AND COMPOUNDS

miR-146a – MicroRNA-146a

REFERENCES

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