iPSC Derived Exosomes and their Influence on Angiogenesis

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Introduction

Extracellular vesicles (EVs) are secreted by all cell types and facilitate intercellular communication via transfer of cargos including proteins, nucleic acids and lipids. EVs derived from induced pluripotent stem cells (iPSCs) produce therapeutically potent EVs through the delivery of these bioactive components to recipient cells. iPSCs are self renewing and therefore have utility for scalable biomanufacturing of EVs. One therapeutic property of iPSC EVs is their ability to induce angiogenesis - the sprouting of new blood vessels to replenish nutrients and oxygen, though the migration and growth of endothelial cells. HOTAIR is a long non-coding RNA (lncRNA) that has been shown to promote angiogenesis and wound healing in diabetic mice (Born, et al. 2021). My project revolves around developing strategies to load HOTAIR into iPSC EVs to enhance their angiogenic potential and therefore utility in a variety of disease applications.

Impact:

EVs possess therapeutic properties such as anti-inflammatory, anti-apoptotic and pro-angiogenic effects. Specifically, promoting angiogenesis is important for recovery after tissue damage. For example, EVs secreted from iPSCs have been demonstrated to transmit bioactive components (e.g. proteins, RNA, and DNA) that are involved in myocardial angiogenesis, myocardial fibrosis and immune inflammatory response, and thus have potential for the clinical treatment of cardiovascular disease. (Liu, et al. 2021). My project focuses on enhancing the inherent pro-angiogenic effects of iPSC EVs via cargo loading to maximize their clinical translational potential in applications such as wound healing and cardiac injury.

Site Information:

Jay Biotherapeutic Development and Delivery Lab

University of Maryland, College Park – Fischell Department of Bioengineering

Mentors: Dr. Steven M. Jay & Daniel Levy

Jay Lab Mission: “Dr. Jay’s lab aims to uncover new biological insights towards the design, production, and delivery of novel biotherapeutics and biomaterials. They strive to develop new drug delivery and biomanufacturing approaches using fundamental tools from both engineering and biology.”

Research Goals: Develop new biotechnologies to address clinical needs in would repair and cardiovascular disease, using extracellular vesicles (EVs) and engineered protein therapeutics.

Methods/Activities:

In the Jay lab, I was able to learn a wide array of modern biological laboratory techniques, including western blot, quantitative polymerase chain reaction (qPCR), EV isolation via tangential flow filtration (TFF), as well as endothelial gap closure and proliferation assays. In general, EVs derived from iPSCs have been shown to possess robust anti-inflammatory and moderate angiogenic properties, and thus their angiogenic potential can be improved. To do so, iPSCs were transfected with a plasmid overexpressing HOTAIR, a pro-angiogenic lncRNA, in order to load it into the iPSCs (figure 1). Then, the EVs containing HOTAIR were isolated using TFF. Next, qPCR was performed to determine the amount of RNA that was loaded into the EVs. Finally, a gap-closure assay was conducted to check for endothelial migration, serving as a proxy for angiogenesis (figure 2).

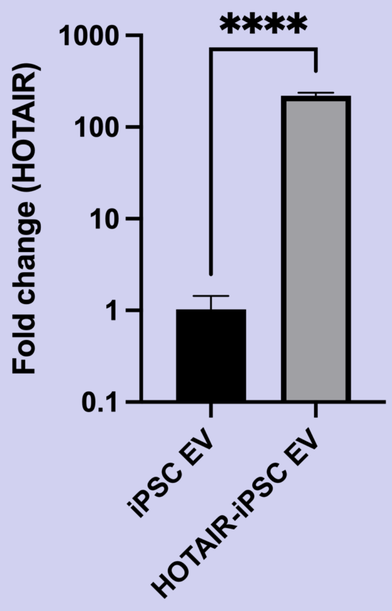

Figure 1 (left): Graph displaying RNA content, showing that the HOTAIR lncRNA is successfully loaded into EVs.

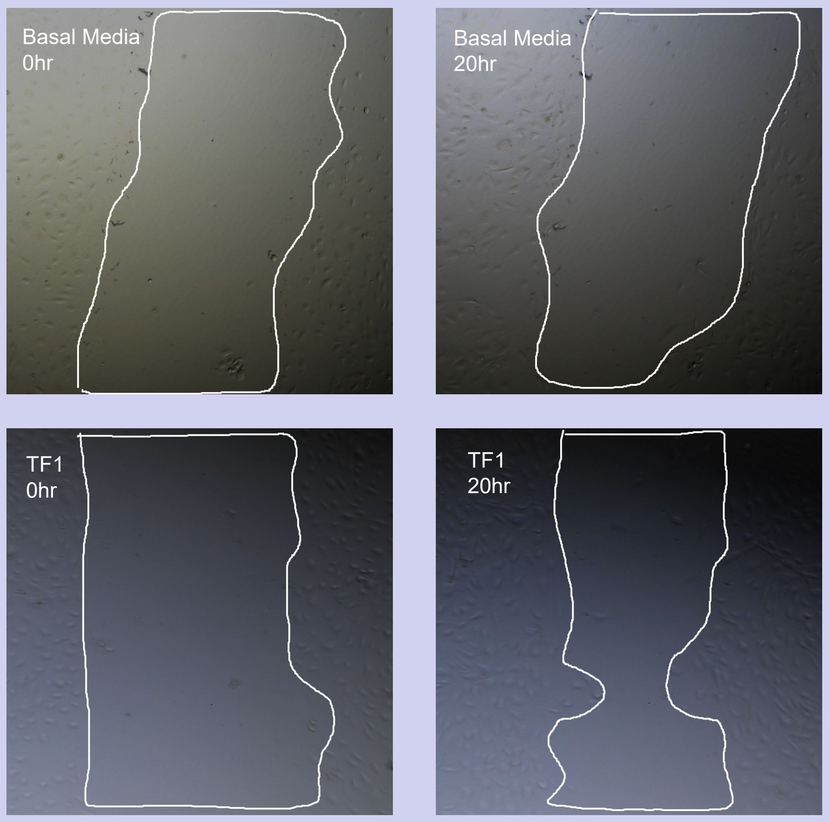

Figure 2 (right): Images from Endothelial gap closure assay. Treatment groups used: (1) Growth media control, (2) basal media control, (3) untransfected iPSC, and (4) TF1. The untransfected iPSC is the backbone, serving as negative control. TF1 is a plasmid that overexpresses HOTAIR. When HOTAIR is overexpressed in cell, there is a higher content in EVs, which would promote angiogenesis. Images were taken at 2 timepoints: 0hr (left set) and 20hr (right set). Images show gap closure for basal media (top set) compared to the TF1 treatment (bottom set).

Discussion & Future Work:

One future application would be adding zipcode sequence to HOTAIR. Zipcode sequences get trafficked to EVs and can thus be used to enrich RNA cargos, such as HOTAIR, into EVs. The combined overexpression of HOTAIR with the appendage of a zipcode sequence can facilitate increased loading of RNA into EVs. It is anticipated that loading more HOTAIR into EVs would promote an increase in angiogenesis. An additional extension would be to use these EV-localization zipcode sequences, to load other types of therapeutically-relevant RNA cargos into EVs. Finally, in terms of laboratory techniques, I am going to learn how to perform a tube formation assay to further demonstrate angiogenesis *in vitro*.

Acknowledgments:

I would like to thank Dr. Jay for giving me the opportunity to complete research on groundbreaking topics in bioengineering and for welcoming me into the lab. Additionally, I would like to thank my mentor, Dan Levy, for guiding me in the lab and allowing me to participate in his research. Finally, I want to thank Dr. Holtz and Dr. Merck for their instruction throughout the scholar's program and for making it an enjoyable experience!

References:

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