Biochemical Society

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Role of NRP1 in endothelial cell dysfunction

Endothelial cells are essential components of the vascular system, as they form a protective lining in blood vessels. These cells constantly experience shear stress due to blood flow, which significantly affects their function and overall vascular health. High-shear laminar flow encourages protective responses such as vasodilation and prevention of blood clots. Conversely, low-shear multidirectional flow can lead to endothelial dysfunction and inflammation, significantly contributing to atherosclerosis, a disease in which plaques build up in the inner lining causing thickening of an artery. Dr. Raimondi's expertise in the topic provided a clear structure to the project and we had set the plan to investigate the effects.

This research project explores the role of Neuropilin-1 (NRP1), which is a transmembrane protein crucial for stabilizing the connections between endothelial cells. When NRP1 is defective or absent, these connections can weaken, leading to an increased inflammation and risk of cardiovascular diseases. However, the specific mechanisms by which NRP1 influences endothelial responses under shear stress are not fully understood.

The study focused on comparing different forms of NRP1 (wild-type NRP1 and a mutant variant known as YSNN). By investigating these variants, the project aimed to determine whether they would lead to different outcomes of atherosclerotic disease. The goal was to understand the role of NRP1 defects in cardiovascular health and whether the YSNN mutant alters the progression of atherosclerosis. Because of Dr. Raimondi's expertise, we had a foundation of information he had previously analysed that we worked on, using Human umbilical vein epithelial cells and transfecting them was recreating the environment atherosclerosis is affected in.

To achieve these objectives, several key techniques were used. Primary human endothelial cells were cultured, providing a controlled environment for experimentation. Learning & mastering certain cell culture techniques was crucial. This included defrosting cryopreserved cells to ensure viability, as healthy cells are essential for reliable experimental results. Additionally, learning methods for splitting and seeding cells to maintain optimal growth conditions was important in executing a successful experiment. Many experiments were conducted to perfect this process, which was a challenge as cells seemed unresponsive despite the optimal environment, but after many attempts and Dr. Raimondi's guidance, the experiment was successful.

The next step was transfecting endothelial cells with various NRP1 constructs was a significant aspect of the research. Control transfections were included to provide a baseline for comparison against experimental conditions. Wild-type NRP1 was introduced to assess its role in maintaining endothelial stability and function, while the YSNN mutant form was examined to determine its influence on endothelial behaviour

and cardiovascular health.
Performing Real-time PCR (RT-PCR) analysis to confirm successful transfection and evaluate gene expression levels was essential as it allowed for the quantification of NRP1 expression in transfected cells, ensuring that the manipulations implemented were effective.
Many challenges were faced in the duration of the research, especially concerning time constraints that limited the analysis of immunostaining data, which could have provided more insights into the roles of wild-type and mutant NRP1 in maintaining endothelial cell junction stability. However, the techniques learned during transfection and PCR set groundwork for future analyses and advanced understanding of endothelial cell behaviour.

The implications of this research are important in cardiovascular health as understanding how NRP1 functions to maintain endothelial integrity under shear stress is important for developing therapeutic strategies against cardiovascular diseases. Preventing endothelial dysfunction is vital for reducing the risk of atherosclerosis. By revealing the roles of wild-type NRP1 and the YSNN mutant, the aim is to identify new strategies for stabilizing endothelial function in vulnerable areas, such as in bends of arteries.

Investigating whether the presence of the YSNN mutant alters the progression of atherosclerosis could give insights into the mechanisms of underlying endothelial dysfunction, which may guide the development of targeted therapies designed to reduce the risk of atherosclerosis.

Since cardiovascular diseases are a leading cause of death globally, the impact of this research is highly significant. Identifying molecular targets, such as NRP1, that can protect endothelial cells from dysfunction may reveal novel treatment strategies. Effective new therapies have the potential to improve patient outcomes in addition to decreasing the financial burden of cardiovascular diseases on healthcare systems and patients.

The Biochemical Society's mission to promote scientific research that addresses critical health challenges aligns with the project as investigating the fundamental mechanisms governing endothelial cell responses to shear stress shows an unexplored area of research, which contributes to a broader understanding of vascular biology, with a high potential in disease prevention and treatment.

Throughout this research, valuable skills were developed that will be useful for future endeavours in biomedical sciences. I gained the ability and the required knowledge to handle human endothelial cells, including techniques such as defrosting, splitting, and seeding, as well as transfection and RtPCR protocols, which are essential for studying gene function and protein interactions.

The challenges we faced during this project built problem-solving skills, particularly in optimizing conditions for cell culture and transfection. Although time prevented immunostaining data and analysis and it was not completed, the experience gained in

planning and conducting experiments as well as interpreting results will be beneficial for my future in research.

In summary, this project has greatly increased my understanding of the molecular mechanisms by which endothelial cells respond to shear stress. Although data analysis is ongoing, the knowledge and skills I acquired during this research will lay the groundwork for future studies in biomedical research. The implications of this work for developing therapies that target endothelial dysfunction shows potential for reducing the burden of cardiovascular diseases worldwide using new targets as treatment.

References

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