Understanding the Fate of Mesenchymal Stromal Cells Following Intravenous Administration in Mice

Student's Name

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Lay Summary

The project presents the post-IV fate and behavior of mesenchymal stromal cells (MSCs) following administration to mice, similar to the study conducted by Fischer et al. (2005) and further explored by Song, Scholtemeijer, & Shah (2020).

This study is supposed to give some insight into the involvement of MSCs with lung tissue, paying attention to their influence on the basic lung functioning, healing process, and immune response. A study by Tolomeo et al. (2023) on the biodistribution of MSC-derived extracellular vesicles can deepen understanding of MSCs' interactions within lung tissues. The research is vital to better understand the role of MSC in the treatment of lung and kidney diseases, which can potentially advance into application in regenerative medicine.

Abstract

"In vivo fate of human umbilical cord-derived mesenchymal stromal cells" (hUC-MSCs) in the lungs of mice is what is studied in this research. The study reports the interaction of MSCs and lung macrophages, as detailed in the work of Zhou and Moore (2017) and Jiao et al. (2023), to understand the therapeutic potential and immunomodulatory effects of MSCs.

This study aims to give a full insight into the behavior of MSCs post-intravenous administration, majorly their biodistribution within lung tissue in mice. Hematoxylin and Eosin (H&E) staining and lectin targeting for macrophages are being applied for MSC effects on the functionality of the lung and for their immunomodulation. Hernandez Pichardo et al. (2022) Discusses the inflammatory response in the lung due to intravenous administration of human umbilical cord MSCs, aligning with my focus on MSCs' interaction with lung macrophages.

Methodology

In our study, we've delved into several techniques to gain insights into lung structures and cellular interactions. Here's a glimpse into our approach:

- **H&E Staining**: We started by taking frozen lung sections from mice and applying H&E staining. This classic technique paints a vivid picture, with haematoxylin highlighting the nuclei and eosin giving the cytoplasm its color. This method, rooted in the work of Fischer et al. (2005), lays the foundational groundwork for understanding basic lung structures. Recent studies have utilized similar techniques for detailed lung tissue analysis, such as a study on "Sigmar1 ablation leads to lung pathological changes associated with pulmonary fibrosis, inflammation, and altered surfactant proteins levels" which uses H&E staining to observe structural organizations of lung tissue, providing insight into the effects of Sigmar1 ablation on lung pathology.
- **Immunofluorescence**: Next up, we employed immunofluorescence, a method that's like a high-tech game of tag. Here, antibodies are used to precisely target and illuminate hUC-MSCs and their interactions within lung tissues. We brought in the big guns—fluorescent dyes like Alexa Fluor 488 and Alexa Fluor 594—to spotlight and track different cell groups. This technique, inspired by the research of Zhou and Moore (2017), is a game-changer in tracing cellular dynamics. Huang et al. (2022) demonstrated the concept of transplanted MSCs as an endocrine reservoir, which provides insights into systemic effects. Another study, "Rapid en-bloc hematoxylin-eosin staining for human lung cancer tissue for fluorescence micro-optical sectioning tomography," describes a methodology for H&E staining in lung cancer and paracancerous tissues, which includes detailed steps for tissue collection, fixation, staining, and consecutive sectioning, as well as the use of fluorescence imaging with propidium iodide staining to observe cellular structures.
- **Microscopy**: To actually see these fascinating interactions, we turned to microscopy. It's not just about looking closely; it's about seeing differently. By employing various illumination methods such as brightfield and darkfield, we've

been able to enhance the details of the MSCs mingling with lung tissues. Again, Zhou and Moore's (2017) insights were instrumental here.

● **Image Analysis**: Once we had our stunning microscopic images, it was time to dive deeper. We used ImageJ software for the heavy lifting in quantitative analysis. This stage is all about measuring and understanding how MSCs interact with macrophages. The software helps merge and dissect the images from the fluorescent microscope, a technique bolstered by Nagai-Okatani and team's (2019) research.

● **Statistical Analysis**: Last but certainly not least, we crunched the numbers using R studio. This part involves statistical wizardry like Welch Two Sample t-tests to get those all-important p-values. This step, critical for interpreting our results, draws from the methodology used by Song and others (2020), and aligns with recent practices in statistical analysis of biological data, such as the study "Exosomal PGE2 from M2 macrophages inhibits neutrophil recruitment and NET formation through lipid mediator class switching in sepsis," which investigates the role of M2 macrophage-derived exosomes in modulating inflammatory responses during sepsis-related acute lung injury (ALI). It includes methods like H&E staining, immunofluorescence, and immunohistochemistry to examine lung tissue injury and neutrophil infiltration.

Results

        The degree of autofluorescence in the lung verifies the degree of autofluorescence presented in this study. Thus, this study has established the ability to analyze MSC interactions by using H&E staining to identify key anatomical features in the mouse lung. These data hint at the interaction of hUC-MSCs with lung tissue of mice, aligning with findings from similar studies like those conducted by Song, Scholtemeijer, & Shah (2020). No substantial difference was found in the total number of macrophages between treatment groups, indicating that hUC-MSCs did not significantly impact macrophages' total numbers compared to the control group. Here, Qian et al. (2021)'s Findings about alleviating renal failure in diabetic mice with intravenous administration of bone marrow MSCs will be compared to my study's observations on lung and kidney disease. Then, the role of MSC-derived extracellular vesicles in neuroinflammation will be discussed in relation to MSCs' immunomodulatory effects Hornillo Mellado et al. (2023). Moreover, the work justifies the course of further study in regenerative medicine and immunomodulation.

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