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Research Plan - Mitochondrial Transfer Examination in Cell Co-Cultures

A. RATIONALE: The concept of mitochondrial transfer has become increasingly well established and understood in recent years. Despite the recent resurgence in studies investigating this particular process, the general understanding of the scientific community surrounding mitochondrial transfer is lacking compared to other cellular processes. Preliminary findings have displayed that mesenchymal progenitor cells (mAo) enhance phagocytosis while expressing the VCAM-1 gene in co-culture. Cellular interaction between mAo and macrophage cells (MΦ) was indicated by the development of processes protruding from cells that appeared to extend towards other cells, in time resulting in a distinct overlap between several cells suggesting an interaction.

B. HYPOTHESIS/EXPECTED OUTCOMES: As it is not particularly well-studied, the importance of mitochondrial transfer is not yet entirely understood. Past research has suggested that mitochondrial transfer may play a significant part in triggering the regenerative abilities of various types of cell tissue while also considering artificial mitochondrial transfer to be a potential therapeutic option in the future for those with mitochondrial diseases. Despite these hypotheses, mitochondrial transfer therapy is far from being implemented on the medical level due to the general ambiguity surrounding it. By enhancing the understanding of this process, the scientific community can better implement this understanding in order to bring medical applications of mitochondrial transfer closer to reality.

The specific aim of this study is to confirm that the initially recognized intercellular interaction examined in prior phagocytosis studies is indeed mitochondrial transfer as well as to examine the possibility of mitochondrial transfer between different cell types, specifically mAo and MΦ. Additionally, it seeks to determine the potential involvement of the VCAM1 gene in mitochondrial transfer, as that particular gene was noted to be upregulated in the prior phagocytosis study in trials in which the aforementioned cell extensions were noted.

C. RESEARCH METHODS:

Procedures: To determine interaction occurring between cells MΦ cells (#CRL-2471) will be acquired from American Type Culture Collection, and mAo cells from freezer stock will be used. One group which will be used for analysis will contain only mAo cells, just as the ones used in the initial phagocytosis study. Another experimental group will consist of a co-culture of mAo cells and MΦ cells. The mAo cells will be cultured in Dulbecco's Modified Eagle's Medium with 10% FBS for both groups. Initially, the MΦ cells will be cultured in a similar medium, however, also being supplemented with 4 mM glutamine containing 1.5 g/L sodium bicarbonate and 4.5 g/L glucose. Following this culture in 100mm dishes, the MΦ cells will be added to the mAo in 6 wells of a 24-well plate with coverslips as well as an entire 4 chamber dish. A separate 6 wells of said 24 dish, as well as a second 4 chamber dish, will be used for examination of the group containing solely mAo. Fluorescence microscopy will be used for analysis and recognition of mitochondrial transfer. In preparation for microscopy, MitoTracker Green will be applied to the cells along with a red fluorescent linker in order to identify mitochondrial location and activity. Additionally, through the application of a VCAM1

monoclonal antibody to these groups, the prevalence of VCAM1 will be noted through light microscopy. Images will be taken at one, three, five, and twenty-four hour intervals under fluorescent light which allows for the identification of the MitoTracker and linker.

Risk and Safety: Being in the lab, it is of course possible that one may be exposed to dangerous substances. This particular study is rather low-risk, as both cell lines used in the study are considered to be at BSL-1 level risk. The lab itself has been considered to be at BSL-2 level risk. With that said, precautions must still be taken in order to ensure safety in the lab. As a result, a lab coat and gloves will be used at all times, with culture being carried out in a BSL-2 level safety cabinet. All cultured material and potentially hazardous agents will be disposed of in specialized biohazard disposal bins.

Data analysis: Image analysis will be conducted using Fiji software, allowing for the enhancement of color in images as well as the creation of composition images in order to form the clearest evidence of mitochondrial interaction. Fiji will also be used in order to measure the area of the aforementioned cell extensions which have been noted in order to better formulate a timeline of this intracellular interaction.

3. POTENTIALLY HAZARDOUS BIOLOGICAL AGENTS RESEARCH: Considering live cells are being cultured and examined in this study, some precaution does need to be taken. The macrophages used in the study were acquired through purchase from the American Type Culture Collection (#CRL-2471) (Manassas, VA). mAo cells will be taken from preexisting

freezer stock. While care must be taken in using these cells, no atypical extra precaution must be taken. The biosafety level is determined based upon risk assessment, as well as former analysis by other scientists. As the cells are considered to be rather low-risk, they are assigned a biosafety level of BSL1, the lowest possible score when measuring the risk of using such cells. That said, when disposed of, all cells still are discarded in a special biohazard deposit bin that will be disposed of properly by the institution's department of health and safety.

4. HAZARDOUS CHEMICALS:

MitoTracker:
Applied to cell cultures in order to increase mitochondrial visibility. Considered very low-risk, it does not have any major negative effect with any human contact. Still, gloves will always be used and used cultures with MitoTracker will be disposed of in biohazard disposal bins.

Hoechst Dye:
Applied to cell cultures in order to increase cell nucleus visibility. Considered very low-risk, it does not have any major negative effect with any human contact. Still, gloves will always be used and used cultures with Hoescht dye will be disposed of in biohazard disposal bins.

VCAM 1 Primary antibody:
Applied to cell cultures in order to visualize VCAM 1 expression. Considered very low-risk, it does not have any major negative effect with any human contact. Still, gloves will always be used and used cultures with the VCAM 1 primary antibody will be disposed of in biohazard disposal bins.

Cell fixative (paraformaldehyde):
2% paraformaldehyde diluted in PBS will be used as a fixative. Considering the solution contains paraformaldehyde, a potential carcinogen, this can be considered slightly higher risk than other substances used in the study. However considering the low concentration of paraformaldehyde, risk was still minimal and only normal lab safety procedures are required. Cells which the fixative will be applied to will be disposed of in biohazard bins.

Blocking solution:
Used following cell fixation. Considered very low-risk, it does not have any major negative effect with any human contact. Still, gloves will always be used and cells that blocking solution are applied to were disposed of in biohazard bins.

Culture mediums:
The culture mediums which will be used are Dulbecco's modified Eagle's medium (DMEM) for progenitor cells, while macrophages will used DMEM supplemented with 4 mM glutamine containing 1.5 g/L sodium bicarbonate and 4.5 g/L glucose. Both of these are considered to be rather low-risk and require no extra safety precautions. Still, gloves will always be used and cells that blocking solution are applied to were disposed of in biohazard bins.

Bibliography

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THERE ARE NO ADDENDUMS TO THIS RESEARCH PLAN