Mitochondrial Transfer From Mesenchymal Progenitor Cells to Macrophages

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Abstract

Preliminary findings have indicated that increased expression of VCAM 1 is indicative of the ability for mesenchymal progenitor cells to support macrophage phagocytosis. The phenomenon of mitochondrial transfer, a process which has been proven to display reparative tendencies in several cellular processes, including aerobic respiration, is a potential explanation for the examined increased phagocytic activity. In this study, the potential for mitochondrial transfer between mesenchymal progenitor cells and murine macrophages was examined in order to determine if mitochondrial transfer from mesenchymal progenitor cells could serve to rescue phagocytic function. The possibility of a role for VCAM 1 in this mitochondrial transfer was examined as well due to the previous finding that VCAM 1 expression was critical in increasing phagocytosis. In order to do this, the prevalence of the VCAM 1 protein and mitochondria in mesenchymal progenitor cell and macrophage co-culture was examined through fluorescence microscopy. Fluorescent agents were applied in order to maximize the visibility of both the mitochondria as well as the VCAM 1 protein. This co-culture was compared with a control culture consisting of only progenitor cells. It was believed that mitochondrial transfer would be confirmed in the co-culture and that the role of VCAM 1 would be confirmed through clear prevalence of the VCAM 1 protein as well as mitochondria at locations of interest, generally where tunneling nanotubes were present. Ultimately, it was noted that there was clear natural mitochondrial transfer between mAo cells growing in culture. It was also noted that VCAM 1 likely plays a role in this transfer, as it was significantly seen to be prevalent along with the developing tunneling nanotubes which initiated the intracellular interaction. This interaction could not, however, be confidently confirmed within the macrophage and progenitor cell co-culture, suggesting that mitochondrial transfer from mesenchymal progenitor cells to macrophage cells requires further research. As this research is furthered, it is critical to recognize that mitochondrial transfer has been recognized to have promising prospects in various fields of therapeutic medicine due to its tendency to rescue cellular functions.

Introduction

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 still lacking compared to other cellular processes. Preliminary findings have displayed that mesenchymal progenitor cells (mAo) cells enhance phagocytosis during co-culture when VCAM 1 is expressed by the mAo cells. It has also been noted that mesenchymal progenitor cells transfer mitochondria to one another through cellular protrusions observable through microscopy.

The phenomenon of these protrusions has been recognized by researchers as tunneling nanotubes which act as a form of transport between cells (Lou et al, 2012). While this was more prevalent within mAo cell cultures, it was also found in MΦ cell cultures. VCAM 1 is known to have influence in cell adhesion within various types of leukocytes, including macrophages (Nagata et al, 1998). The prevalence of the VCAM 1 protein was ultimately examined as it was found to be beneficial to the phagocytic functions of macrophages, with these functions of course being their main purpose within the body.

Mitochondrial transfer between different cell types has also been recognized, a concept which was central to this study. If transfer between the mAo cells and MΦ cells is found, there may be potential for recovery of suppressed phagocytic activity due to macrophage damage, as prior studies have displayed that mitochondrial transfer in co-culture led to distinct cellular repair (Spees et al, 2006). In fact, Li and colleagues suggested that mesenchymal stem cells may be able to reverse lung damage caused by cigarette smoke specifically (Li et al, 2014). The current understudied state of mitochondrial transfer relative to other topics can likely be attributed to the ambiguity surrounding the process, as the scientific community has not managed to find potential medical applications for the process.

With this is mind, researchers have now begun to develop several testable theories on how this transfer actually takes place, such as through tunneling nanotubes and cellular fusion (Torrabla et al. 2016). Recent research has suggested that mitochondrial transfer may play a

significant role in triggering the regenerative abilities of various types of stem cells, an important discovery considering the growing field of stem cell treatment (Paliwal et al, 2018). Additionally, other research has suggested that artificial mitochondrial transfer could be a potential therapeutic option in the future, targeting specific damaged cells in those with mitochondrial diseases (Islam et al, 2012). Despite these hypotheses, mitochondrial transfer therapy is far from being implemented on the medical level. By enhancing the understanding of this process, medical applications of mitochondrial transfer can be brought closer to reality.

The goal of this particular study was to confirm the potential mitochondrial transfer between mAo and MΦ cells, as well as determine the possible involvement of the VCAM 1 gene in mitochondrial transfer through fluorescence microscopy, as this particular gene was noted to be upregulated in the preliminary study where it's expression by mAo enhanced macrophage phagocytosis. Consequently, it was hypothesized that mitochondrial transfer through tunneling nanotubes would be observed in a mAo and MΦ co-culture. It was also believed that VCAM 1 may play a role in the possible mitochondrial transfer between macrophages and somatic cells, specifically concerning the tunneling nanotubes which facilitate mitochondrial transfer.

Materials and Methods

Cell Culture

The murine macrophage cells used in the study were acquired through purchase from the American Type Culture Collection (#CRL-2471). They were cultured in 100mm dishes with Dulbecco's modified Eagle's medium (DMEM) with 4 mM glutamine containing 1.5 g/L sodium bicarbonate and 4.5 g/L glucose. Additionally, the MΦ medium was supplemented with 4 mM glutamine containing 1.5 g/L sodium bicarbonate and 4.5 g/L glucose in order to facilitate cell growth. The mAo cells were obtained from lab freezer stock. These were cultured in 100mm dishes as well, however, the medium did not contain the supplements that the MΦ medium did as they would be detrimental to mAo cell proliferation. Cell culture mediums were purchased through Invitrogen (Carlsbad, CA).

Preparation of 24 Well Plate

A 24 well plate was used in order to prepare the cells for fluorescence microscopy. The plate was exposed to ultraviolet radiation under the hood for 10 minutes in order to ensure sterility. Following this, slide coverslips were dipped in 75% ethanol, dried, and placed into each of the 12 wells used in preparation for microscopy. Following cell fixation, these slips would be placed onto slides and used in microscopy.

Replating Into 24 Well Plate

Once the cells were deemed ready for examination, they were resuspended through centrifugation and replated onto a 24 well plate. Six wells were plated with mAo cells, and six wells were plated with a co-culture of mAo cells and MΦ cells. MitoTracker Green purchased from ThermoFisher (Waltham, MA) was introduced to the first two columns, and the third was examined without MitoTracker. The VCAM1 primary antibody and anti-mouse secondary antibody were incubated in preparation for inoculation into the cells. The VCAM1 antibody was inoculated into the first and third columns in order to ensure that it could be examined in both the mAo only group as well as the co-culture. This was indicated by a red fluorescence under the microscope. Hoechst stain dye, indicated by blue fluorescence, was also applied to every well in order to maximize the visibility of the cell nuclei.

Cell Fixation

Prior to examination of the cells, they were fixed on the coverslips within each well. Following aspiration of the medium 2% paraformaldehyde diluted in PBS was used as a fixative. Blocking solution (10% fetal bovine serum, 0.25% bovine serum albumin 7.5%) was then added to the wells containing coverslips and the plate was incubated at room temperature and shielded from light overnight.

Fluorescence Microscopy/Cell Imaging and Analysis

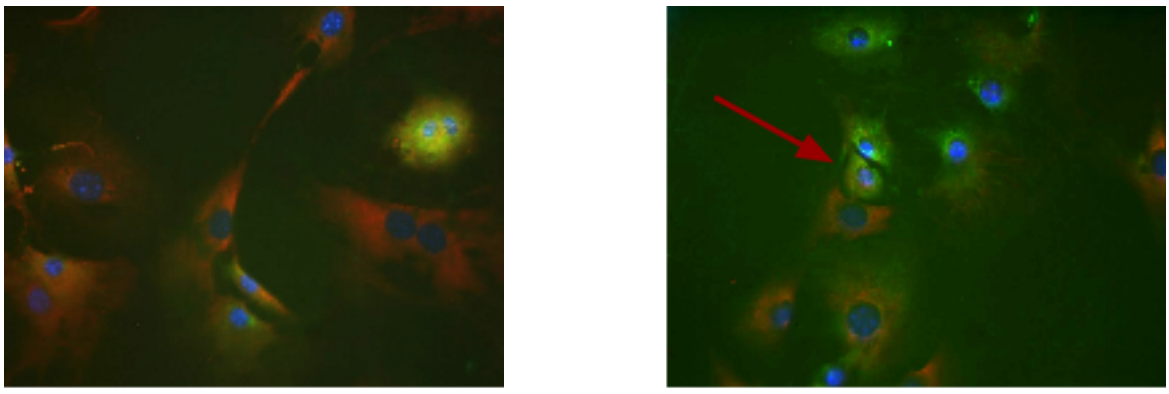
Infinity analyze software was used for cell imaging. Cells were observed and imaged over a 24 hour period under bright field light as well as under blue, red, and green fluorescence

filters. Following imaging under each fluorescence filter, images were colored using Fiji ImageJ software. Composite images combining all fluorescence filter images were also created through Fiji. Evidence of mitochondrial transfer noted through the intracellular overlap of MitoTracker Green was recorded, as well as the prevalence of VCAM 1 at transfer locations.

Results

Mitochondria and VCAM 1 are Both Localized at Tunneling Nanotubes

Following examination of the images, it was determined that there was mitochondrial interaction and transfer within mAo culture groups. This was not unexpected, as trials from prior experiments had displayed similar interaction between mAo cells. VCAM 1 was also extremely prominent throughout the cell processes which developed throughout the experiment, often overlapping areas with high concentrations of mitochondria. While the observed correlation of VCAM 1 antibody prevalence and tunneling nanotubes concentrated in mitochondria did vary across several images, it must be noted that it was still realized among all images from the mAo group. Images from the mAo group can be observed in figure 1.

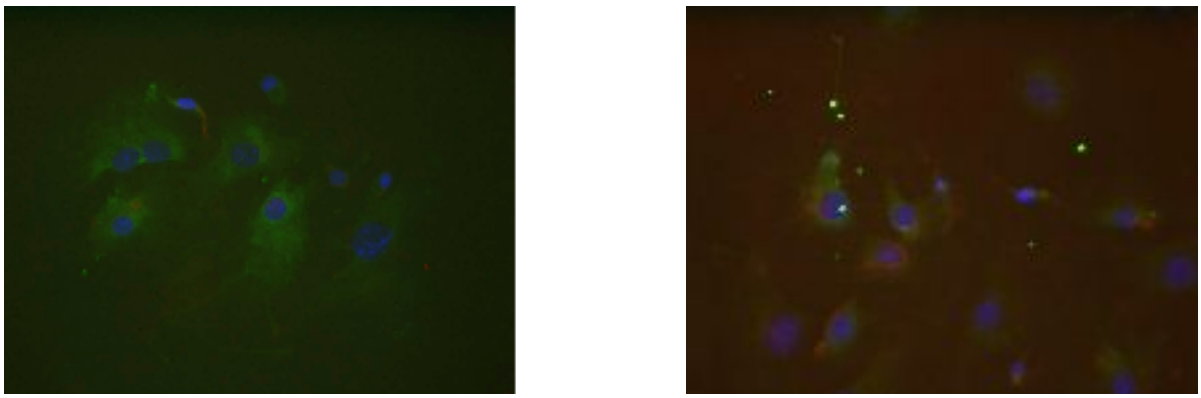


(A) **(B)**

Figure 1A & 1B: Examples of images taken from the mAo culture groups. As can be seen, there is an overlap of red and green fluorescence in both Figure 1A and 1B, indicating a positive correlation of VCAM 1 activity as well as mitochondrial transfer. Tunneling nanotubes from each cell are also noted to be extending towards other cells, more notable in Figure 1A, indicating an active cell action promoting this transfer.

Mitochondrial Transfer and VCAM 1 Prevalence Unclear in Co-Culture

Throughout imaging of the mAo and MΦ co-culture, similar tunneling nanotubes as recognized in the mAo group were noted to be protruding from both the mAo and MΦ cells. However, co-culture images were also seen to have some background green fluorescence in several cases along with several points of fluorescence disconnected to cells. With this in mind, VCAM 1 did seem to still have a significant presence, being consistently fluorescent along with noted mitochondrial cell processes in both cell types. However, the VCAM 1 was far less prevalent in this group as opposed to images from the co-culture group can be seen in figure 2.



(A) **(B)**

Figure 2A & B: Examples of images taken from the co-culture groups. As can be seen, there are less tunneling nanotubes towards other cells as well as some points of green fluorescence which are not connected to cells at all. VCAM 1 is somewhat active in tunneling nanotubes, though to a lesser extent than in the mAo group.

Discussion

This study was able to confirm the incidence of the suspected mitochondrial transfer between mAo cells in simple cell culture. Additionally, it was also concluded that VCAM 1 may play a major role in the creation of the recognized tunneling nanotubes which conducted mitochondrial transfer in mAo, as VCAM 1 was consistently prevalent along the lines of the noted nanotubes. However, while the results suggest that there is potential for mitochondrial transfer from mesenchymal progenitor cells to MΦ cells, the background fluorescence that was

realized indicates a need for further investigation of this topic. While there is already an understanding of mitochondrial transfer between different cells of the same type and differing types, a concept that has been mentioned in cancer therapy treatment, there is still a lack of understanding concerning somatic cells and phagocytes (Pasquier et al. 2013). Some studies have indicated that somatic cell treatment may play a protective role for drugs against chemotherapy, thus eliminating the inhibition of drug activity by chemotherapy (Wang et al. 2018). The results of this particular study do suggest that mitochondrial transfer into and repair of phagocytes is plausible, however, it was unable to confirm this.

It can be said with confidence that the VCAM 1 protein has a role in mitochondrial transfer, or at least the formation of tunneling nanotubes which facilitate this transfer between somatic cells. While the exact role is not entirely clear, it should be further studied in order to determine which part of the process it plays a more significant role in. Although the same can not necessarily be said for VCAM 1 in the co-culture due to the complications that came with the co-culture analysis, there is some potential for VCAM 1 activity which warrants further research. It should also be noted that VCAM 1 is known to play a significant role in fetal development processes, suggesting that there may be some significant level of mitochondrial transfer in this life process (Gurtner et al., 1995).

As a whole, there were far too many complications in the co-culture group to draw any distinct conclusion. This can primarily be attributed to the background fluorescence noted in the co-culture groups, which is potentially due to a misstep in the protocol for dyeing the cells. Throughout the study, a new protocol was used in which all three fluorescent dyes were applied to the cells as opposed to just one dye as was done prior. However, this procedure seemed to work for the mAo culture groups, suggesting that a separate protocol may be required for the co-culture groups. Due to time constraints, this could not be completed. Additionally, due to time limits in the lab more time passed between dye application and imaging than was optimal, leading to increased exposure to light in each sample although measures were made to minimize light exposure. This light exposure ultimately led to photobleaching of the dyes, making the images more difficult to examine. In future research, maintenance of potency of each fluorescence agent should be emphasized. As a whole, however, future research should seek to

confirm mitochondrial transfer between somatic cells and phagocytes in order to determine a potential role in repair of the phagocytic process. As some research has suggested that actual repair due to transfer only occurs in situations where no or very few healthy mitochondria exist, research should focus on optimizing this process to ensure it occurs whenever required while also seeking to confirm this prospect (Cho et al., 2012). Future research should also continue to examine the potential applications for mitochondrial transfer therapy in medicine as well. The applications of this technique are not limited to only phagocytic rescue, which should still be confirmed by future research, but could potentially include nearly all cell processes as the possibility that mitochondrial transfer can prove to be a promoter for phagocytosis suggests that it may also be a promoter for other processes which do not explicitly use mitochondria.

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