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Altered metabolism for neuroprotection provided by mesenchymal stem cells

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Abstract:

Mesenchymal stem cells (MSCs) are multipotent adult stem cells which have become popular research targets for their use in cellular therapy for tissue repair. While recent advancements in research have shown the MSCs have immunomodulatory functions which are altered in response to host inflammatory molecules, how these stimuli produce different functional outcomes is not understood. Here, we evaluate research examining how the proinflammatory cytokine interferon-γ (IFN-γ) affects the immunomodulatory functions of MSCs by altering their metabolism. This study indicates that IFN-γ causes an increase in glycolytic activity and uncoupling of glycolysis to tricarboxylic acid cycle and hence, the glycolytic metabolites and intermediates can be funneled toward the production of anti-inflammatory modulators indoleamine-2,3-dioxygenase and PGE2. A complete understanding of how MSCs' cellular metabolism affects their function is necessary for their employment in cellular therapy, as MSCs have been demonstrated to have pro- and anti-inflammatory functions. These findings are a large step forward in the understanding of the regulation of MSCs and toward their eventual use in cellular therapy, specifically for stroke recovery, in which MSCs have been shown to have powerful neuroprotective and neurogenerative effects.

Keywords:

Akt, cell therapy, glycolysis, indoleamine-2,3-dioxygenase, interferon-γ, mammalian target of rapamycin proteins, mesenchymal stem cells, metabolism, neuroprotective, PGE2, stroke treatment, uncoupling

Mesenchymal Stem Cells: The Stars of Modern Cell Therapy Research

Mesenchymal stem cells (MSCs) are multipotent adult stem cells which have the capacity to differentiate into adipocytes, chondrocytes, and osteoblasts.[1] Since their discovery, MSCs have been a popular area of research for their potential use in cell therapy. This is largely based on their easy accessibility, genetic stability allowing for *in vitro* cultural expansion, and the absence of ethical issues in obtaining them compared with other forms of stem cells.[2] Cells exhibiting the characteristics of MSCs have been harvested from a wide variety of adult tissues, ranging all the way from teeth[3] to the skin,[4] as well as alternative sources such as amniotic fluid[5] and Wharton's Jelly.[6]

Following their discovery, MSCs were primarily being investigated as agents of cell therapy for connective tissue disorders. Being that MSCs were able to differentiate into most types of connective tissue, it was hypothesized that they would aid in tissue repair by proliferating and differentiating into the tissue which they were repairing.[7] However, it was recently discovered that MSCs have potent immunomodulatory properties in response to inflammatory stimuli, prompting much research into MSCs for the treatment of inflammatory

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diseases and processes.[8] Treatments and therapies utilizing MSCs are currently being developed for stroke recovery,[9] multiple sclerosis,[10] and amyotrophic lateral sclerosis[11] among many others.[12]

Unlocking the Therapeutic Potential of Mesenchymal Stem Cells

The investigation has already begun into many of the potential therapeutic uses of MSCs, despite poor knowledge for the mechanisms by which MSCs exert their immunomodulatory effects. To fully utilize the therapeutic potential of MSCs, it is necessary to have a complete understanding of how their immunomodulatory effects are controlled. Most of the research into therapeutic uses of MSCs have been focused on human MSCs (hMSCs). A recent study has shown that one of the primary determinants for the functional fate of each hMSC is its metabolism, which both provides energy and substrates for growth and regulates effector functions that generate specific secretory profiles and immune responses.[1] The hMSCs' functions are determined by their metabolic state, with different metabolic phenotypes producing different effector functions.[13] Understanding exactly how metabolism affects the function of hMSCs is critical to being able to fully utilize their therapeutic value.

In their previous studies, Liu and Ma had established that hMSCs are metabolically plastic and that hMSC phenotype was regulated by metabolic reconfiguration occurring in response to environmental cues.[14,15] For example, hMSCs exposed to interferon-γ (IFN-γ) respond by suppressing the proliferation and function of T-cells.[16] In response to inflammatory stimuli, hMSCs have been shown to exert regulatory effects on T-cells, B-cells, macrophages, dendritic cells, and natural killer cells; although the potency of their suppression of T-cells has been shown to be the key indicator for their immunomodulatory properties.[17] Treatment with IFN-γ causes hMSCs to exert their immunomodulatory effects through activation of the indoleamine-2,3-dioxygenase (IDO) enzyme, the first enzymatic step in the conversion of tryptophan to kynurenine.[18] Although both IDO1 and IDO2 are present in hMSCs, it is IDO1 that exerts its immunosuppressive role on T-cells by increasing the O2-dependent catabolism of tryptophan and the production of toxic tryptophan metabolites, resulting in cell cycle arrest and apoptosis of T-cells.[19] This same IDO is also associated with the re-education of immune cells into their immunosuppressive phenotypes, including M2 macrophages and T regulatory cells.[16]

At this point, hMSCs present an exciting area of discovery and opportunity in cell therapy. hMSCs, in response to environmental stimuli such as cytokines, exert immunomodulatory effects on the host immune system by altering the proliferation, survival, and function of host immune cells.[1] The environmental stimuli act on the hMSCs by altering their cellular metabolism, driving them toward the production of immunomodulating molecules. The enzyme indoleamine-2,3-dioxygenase (IDO) is one of the primary immunomodulating molecules originating from hMSCs.[1] It exerts its immunomodulatory effects by suppressing T-cell proliferation and reeducating host immune cells into their immunosuppressive phenotypes.[1]

The Missing Piece to the Mesenchymal Stem Cell Puzzle

To effectively utilize hMSCs clinically for endogenous tissue repair, a complete understanding of how their immunomodulatory effects are regulated is necessary. It has been previously established that endogenous inflammatory factors, such as IFN-γ and tumor necrosis factor-α, affect the metabolism of nearby hMSCs which, in turn, affects the immunomodulatory functions of said hMSCs.[8]

Recent research has attempted to bridge the gap and determine what changes to hMSC metabolic activity, produced by IFN-γ activation of hMSCs, led to the increased IDO production by hMSCs and therefore to immunomodulatory effects. The study, done by Dr. Yijun Liu and associates at Florida State University, included a thorough analysis of the metabolism of hMSCs cultured with or without IFN-γ, as well as how their metabolism affects their function.[1] Cultures were assessed for IDO and PGE2 production, glycolysis rate, mitochondrial complex I and III activity, oxygen consumption rate, extracellular acidification rate, and mitochondrial reactive oxygen species (mROS) and total reactive oxygen species (ROS) levels.[1] This allowed researchers to isolate the activity of each significant step of cellular metabolism and hence that they might identify what specific modifications in metabolism produce what modifications in function.

Liu's study was able to isolate what specific metabolic changes occurred in hMSCs in response to IFN-γ. They discovered that the presence of IFN-γ caused a reconfiguration of hMSCs' energy metabolism, resulting in the uncoupling of aerobic glycolysis from the tricarboxylic acid (TCA) cycle and oxidative phosphorylation.[1] In response to IFN-γ exposure, an increase in glycolysis and a decrease in TCA cycle activity was observed along with an increase in the secretion of kynurenine and PGE2 and an increase in production of IDO and COX2.[1] It was also discovered that this increase in the production of IDO and COX2 observed with IFN-γ exposure is only noted in glucose-rich mediums, and

not those rich in pyruvate, the metabolic product of glycolysis that enters the TCA cycle.[1] Together, these findings suggest that exposure to IFN-γ causes hMSCs to shift their metabolism to aerobic glycolysis. This shift increases the availability of glycolytic intermediates, which provide substrates for the production of the immunomodulatory factors produced by hMSCs.[1]

This finding exposes a key step in the regulation of hMSCs function, but it still does not explain how IFN-γ leads to an increase in glycolysis and uncoupling from the TCA cycle. To determine this, Liu *et al*. examined how IFN-γ affected other portions of hMSC metabolism. They found that hMSCs treated with IFN-γ showed inhibition of the mitochondrial electron transport chain (ETC).[1] This inhibition causes an increase in the production of mROS, which the researchers were able to mechanistically link to increased glycolysis and IDO production. To support this connection between IFN-γ, the ETC, and glycolysis, the researchers also examined how inhibitors of the ETC modulated the effects of IFN-γ on glycolysis. They found that inhibitors of both complex I and complex III, when co-administered with IFN-γ, enhanced the production of mROS and therefore, the increase in glycolysis.[1]

The researchers also examined the role of the intracellular signaling mTOR proteins and its effector Akt. mTOR activation has been associated with an increase in translation of glycolytic enzymes and their transcriptional regulators.[20] Akt has been shown to increase glycolysis by stimulating expression of GLUT1, the primary glucose uptake transporter, and activating the enzymes H2K and PFK, which provide overall control over the glycolytic activity.[21] In the current study, it was demonstrated that the inhibition of mTOR activity caused a reduction in glucose consumption and IDO production.[1] It should be noted that the mTOR inhibitor used here was rapamycin, a drug commonly used for immune suppression, which may be contraindicative to future cell therapies utilizing hMSCs.[1] While the exact role of mTOR and Akt signaling was not established in this study, it is clear that it is a necessary piece of the complex puzzle that is the regulation of hMSCs' immunomodulatory functions.

Liu *et al*. study revealed a great deal about how cellular metabolism regulates the immunomodulatory functions of hMSCs. To summarize, the proinflammatory cytokine IFN-γ interacts with hMSCs in two ways. One of these ways appears to be the activation of mTOR protein signaling cascade, which, in turn, increases glycolytic activity. IFN-γ also interacts with the hMSCs' mitochondria, where it inhibits the ETC and causes an increase in the generation of mROS.[1] These mROSs themselves further enhance glycolytic activity.[1] This large increase in glycolytic activity coincides with a downregulation of the TCA cycle and oxidative phosphorylation.[1] This unusual uncoupling, along with the increase in IDO and PGE2 synthesis that comes with an increase in glycolysis, suggests that intermediates and metabolites of glycolysis serve as substrates for the synthesis of IDO and PGE2.[1]

Areas for Further Investigation

While this study revealed a great deal about how hMSCs' immunomodulatory functions are regulated, there are still plenty of questions to answer. One of these questions lies in the role of mitochondrial metabolites such as citrate and succinate. In Dr. Liu *et al*.'s study, an accumulation of citrate, ATP, and succinate were noted in IFN-γ treated hMSCs, but the role of these metabolites was not established.[1] Previous studies have demonstrated that an accumulation of succinate in macrophages leads to induction of the glycolytic metabolic phenotype through activation of hypoxia-inducible factor-1α,[22] and that in activated dendritic cells accumulation of citrate led to its rerouting into fatty acid synthesis to expand the endoplasmic reticulum and Golgi apparatus, which are essential components of protein production.[23] As of now, the role of citrate and succinate in hMSCs remains unclear.

It should also be noted that hMSCs have also been shown to modulate inflammation through the engagement of programmed cell death protein-1 (PD-1) and its ligands PD-L1 and PD-L2 through either cell-cell contact or secreted factors.[24] How, or even whether, this function is controlled through cellular metabolism remains an ongoing area of the study.

Mesenchymal Stem Cells as Stroke Therapy

Ischemic stroke is one of the leading causes in the USA of death and disability, but the available treatments for it are extremely limited.[25] Ischemic strokes most commonly occur when blood flow to an area of the brain is obstructed, resulting in neuronal cell death. Ischemic strokes can cause varied focal neuronal deficits depending on the area of the infarct. Currently, the only clinically available treatment for ischemic stroke is alteplase, a recombinant tissue plasminogen activator. Intravenous alteplase works by promoting thrombolysis and hopefully restoring blood flow to the brain, but it is only clinically effective 4.5 h after stroke occurs.[26] Because of stroke's sudden onset and difficult to recognize symptoms, patients often do not present for treatment until after the therapeutic window for alteplase. New treatments that can prevent areas of ischemic infarct from growing or potentially repair damaged areas are desperately needed.

MSCs are being investigated for their use in stroke therapy because of their neuroprotective and neurogenic actions. Part of what makes strokes so devastating is the delayed neuronal death that occurs following the acute ischemic episode. Immediately following stroke, endogenous inflammatory processes are upregulated to levels which destroy hypoxic tissue local to the area of insult, induce apoptosis, and initiate a feedback loop of inflammatory cascades that can expand the original area of damage.[9] Research has shown that the neuroprotective factors of MSCs dampen the inflammation typically present in the subacute phase of a stroke, significantly decreasing the extent of delayed cell death.[27] It has also been shown that IFN-γ from the spleen migrates to the area of infarction following a stroke and contributes to inflammation.[28] The new study by Liu *et al*. illuminating exactly how MSCs react to IFN-γ through immunomodulation [Figure 1], provides excellent evidence for why MSCs have the potential to be utilized for the treatment of stroke outside of the 4.5-h window in which standard treatments are effective.[1]

Evidence also exists for the utility of MSCs during the chronic treatment phase following stroke. Another reason that strokes are so devastating lies in the central nervous system's extremely poor ability to regenerate and repair. Delivery of MSCs during the chronic phase of stroke has been shown to activate regenerative mechanisms, such as angiogenesis, neurogenesis, and synaptogenesis, which can help restore cerebral function.[29,30] Although these functions of MSCs are not the focus of this review, they are worth mentioning to highlight just how powerful of a therapeutic option for the treatment of stroke that MSCs are shaping up to be. Many clinical trials testing the safety and efficacy of MSCs for the treatment of stroke have already shown promising results via improvement of stroke patients on neurological function scales.[25,31,32]

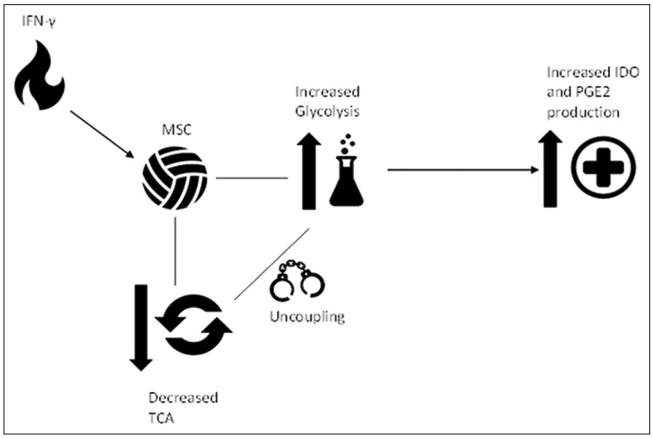

Figure 1: Mesenchymal stem cell-induced therapeutic metabolism for stroke neuroprotection. Transplantation with mesenchymal stem cells can alter brain metabolism towards improved bioenergetics thereby mounting neuroprotective effects against neurological disorders, such as stroke

It should also be noted that the clinical applications of MSCs are not limited to the treatment of stroke. Basic research has shown potential for MSCs to have far-reaching uses across the expansion of medicine, like Kin *et al*.'s recent study revealing potential uses of MSCs in treating depression.[33] A recent review of clinical trials based on MSCs found that 493 such trials had been completed and added to the NIH database, and many more have likely followed in the years since the review.[12] These trials span the entire practice of medicine, with focuses in hematological disease, graft-versus-host disease, organ transplantation, diabetes, inflammatory disease, diseases of the liver, kidney, and lung, cardiovascular diseases, diseases of the bone and cartilage, neurological diseases, and autoimmune diseases.[12] This laundry-list of disease and dysfunction, spanning just about every organ system, serves to highlight just how vital MSCs appear to be to future of medicine, and why understanding how they work is of critical importance.

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Conflicts of interest

There are no conflicts of interest.

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