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High glucose microenvironment and human mesenchymal stem cells behavior

Mateen MA *et al*. HG microenvironment and MSCs

Abstract

High glucose (HG) culture conditions *in vitro* and persistent exposure to hyperglycemia in diabetic patients are detrimental to the stem cells, analogous to any other cell type in our body. It interferes with diverse signaling pathways, *i.e.,* mammalian target of rapamycin (mTOR)-phosphoinositide 3-kinase (PI3K)-Akt signaling, to impact physiologic cellular functions, leading to low cell survival and higher cell apoptosis rates. While elucidating the underlying mechanism responsible for apoptosis of adipose tissue-derive mesenchymal stem cells (MSCs), a recent study has shown that HG-culture conditions dysregulate mTOR-PI3K-Akt signaling in addition to mitochondrial malfunctioning due to defective mitochondrial membrane potential (MtMP) that lowers ATP production. This organelle-level dysfunction energy-starves the cells and increases oxidative stress and ultra-structural abnormalities. Disruption of mitochondrial electron transport chain defects produces altered mitochondrial NAD+/NADH redox state, as evidenced by a low NAD+/NADH ratio that primarily contributes to the reduced cell survival in HG. Some previous studies have also reported altered mitochondrial membrane polarity (causing hyperpolarization) and reduced mitochondrial cell mass, leading to perturbed mitochondrial homeostasis. The hostile microenvironment, thus created due to HG exposure, creates structural and functional changes in the mitochondria, altering their bioenergetics and reducing their capacity to produce ATP. These are significant data as MSCs are extensively studied for tissue regeneration and restoring their normal functioning in cell-based therapy. Therefore, MSCs from hyperglycemic donors should be cautiously used in clinical settings for cell-based therapy due to concerns about their poor survival rates and higher rate of proliferation post-engraftment. As hyperglycemia alters the bioenergetics of the donor MSC, rectifying the loss of MtMP may be an excellent target for future research to restore the normal functioning of MSCs in hyperglycemic patients.

Key Words: Adipose tissue; Apoptosis; Bioenergetics; Cell survival; Cell therapy; Hyperglycemia; Mitochondria; Mesenchymal stem cells, Stem cells

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Core Tip: High glucose (HG) conditions, seen *in vitro* as well as in diabetic patients, adversely affect stem cells by disrupting the mammalian target of rapamycin-phosphoinositide 3-kinase-Akt signaling, resulting in reduced cell survival and increased apoptosis. A recent study on adipose tissue-derived mesenchymal stem cells (MSCs) found dysregulation of this signaling pathway and defective mitochondrial membrane potential (MtMP) under HG conditions. This leads to decreased ATP production, heightened oxidative stress, and structural abnormalities, causing diminished cell survival. Altered mitochondrial NAD+/NADH redox state and disrupted mitochondrial homeostasis worsen the hostile microenvironment induced by HG exposure. These findings are a note of caution for using MSCs from hyperglycemic donors in cell-based therapy due to their poor survival and proliferation rates. Future research targeting MtMP restoration may enhance the therapeutic efficacy of MSCs in hyperglycemic patients.

INTRODUCTION

Chronic exposure to a high glucose (HG) microenvironment *in vitro* and *in vivo* is detrimental to the cells and has physiological and pathological consequences (Figure 1). At the cellular level, the damaging effects of HG exposure for a prolonged period can cause glucose cytotoxicity that invariably affects every body cell, encompassing red blood cells to the stem cells[1-3].

Insulin resistance, pancreatic beta cell damage, and decreased insulin production lead to hyperglycemia that drastically affects the whole body at the organ and cellular levels. An uncontrolled hyperglycemic state will lead to chronic systemic inflammation that will bring about morphological and functional changes in the body cells, including stem cells[4]. This persistent uncontrolled hyperglycemia also produces bone marrow (BM)

microenvironmental changes, which cause functional impairment of stem cells[5]. Nguyen *et al*[6] have reported a reduced proliferation rate besides an increased expression of stress-associated genes, activating transcription factor 4, and C/EBP homologous protein in mesenchymal stem cells (MSCs). On the same note, Kim *et al*[7] reported defective osteogenic differentiation but increased adipogenic differentiation rate in BM-derived MSCs. MSCs from streptozotocin (STZ)-induced diabetic rats show a slow proliferation rate and poor myogenic potential[8]. These studies show that hyperglycemia causes changes in progenitor cell biology and affects their normal behavior and functions during tissue repair[9]. Hence, attempts have been made in some cases to pre-differentiate MSCs into insulin-producing cells before transplantation in hyperglycemic experimental animal models[10].

The anti-hyperglycemic therapy to regain glucose homeostasis can also interfere with the quality and efficacy of MSC treatment. Hsiao *et al*[11] reported that metformin caused apoptosis of MSCs *via* the AMP-activated protein kinase (AMPK)-mammalian target of rapamycin (mTOR) pathway. Interestingly, the authors observed that hyperglycemia could protect the cells from metformin-induced apoptosis. In another study involving a rat model of diabetic cardiomyopathy, Ammar *et al*[12] observed impaired angiogenesis and higher myocardial fibrosis in response to concomitant treatment with metformin and MSCs compared to MSCs treated animals. These data were attributed to impaired MSCs' functionality in the presence of metformin treatment.

HG CULTURE- AND HYPERGLYCEMIA-INDUCED SIGNALING

Hyperglycemic conditions *in vivo* are simulated *in vitro* by culturing the cells in HG conditions to study the effects of hyperglycemia. HG culture conditions have been shown to cause rapid cellular dysfunction by promoting transcriptional changes[13]. Some of the essential mechanisms involved therein include the formation of advanced glycation products (AGEs), PKC activation, mTOR/Akt dysregulation, *etc*., that lead to elevated reactive oxygen species (ROS) stress, increased pro-inflammatory cytokines

production, growth factors, abnormally high gas transmitters, altered cell bioenergetics, *etc.*

For example, Aguiari *et al*[14] have reported that muscle-derived stem cells and adipose tissue-derived stem cells under HG-culture conditions preferentially adopted adipogenic phenotype in response to ROS accumulation and activation of PKC-β in the cells. They supported their findings by treating the cells with oxidizing agents and silencing PKC-β in the cells to inhibit their adipogenic differentiation. In a later study, human aortic endothelial cells' HG-culturing has been reported to cause significant pathway changes during the first four hours, with distinct clusters of genes showing altered transcriptional profiles unique to HG conditions[13]. Temporal co-expression and causal network analysis showed a relationship between type 2 diabetes mellitus and activation of growth factor signaling pathways, including signal transducer and activator of transcription 3 and nuclear factor-kappaB. On the same note, MSCs in HG culture undergo senescence mediated by Akt/mTOR dysregulation[3]. However, some studies report that for the detrimental effects of HG culture conditions, the cells may need persistent long-term exposure because they may resist short-term exposure to HG culture conditions[15]. It was interesting to note that MSCs from healthy donors had shorter doubling time under HG culture conditions compared to the diabetic donors' MSCs, thus implying that the difference in their responsiveness is more a function of the pathophysiology of diabetes. On the same note, changes observed in diabetic donor-derived MSCs' respiration capacity were responsible for their compromised cellular functions[6]. There is reportedly a decreased angiogenic paracrine activity, which was evident from reduced secretion of pro-angiogenic growth factors, *i.e.*, vascular endothelial growth factor-A (VEGF-A), angiopoietin-1 (Ang-1), and Ang-2, and VEGF-C in the HG MSCs[7].

Chronic HG culture conditions also drive glycation reactions through the receptor for advanced glycation end products, resulting in the formation of AGEs and endogenous inflammatory mediators[16,17]. It has been reported that stimulation with AGE-bovine serum albumin induced the generation of ROS and attenuated the proliferation and

migration of MSCs *via* activation of ROS-p38 mediated pathway[18]. Another study reported that HG reduces the regeneration ability of BM-MSCs through the activation of glycogen synthase kinase-3beta, which plays a vital role in inhibiting the proliferation of BM-MSCs *via* the inhibition of C-X-C chemokine receptor type 4[19].

Continuing their efforts to study the effects of hyperglycemia on MSCs' functionality, Abu-El-Rub *et al*[20] report interesting comparative data *in vitro* by culturing human adipose tissue-derived MSCs (AD-MSCs) under low glucose and HG conditions in a parallel set of experiments. It is pertinent to mention that the authors use *in vitro* culture conditions for exposure to HG. Hence, the term "hyperglycemia" in the aims, conclusion, and elsewhere in the manuscript does not reflect the experimental design. The authors have primarily focused on three endpoints, cell viability, cell apoptosis, and mitochondrial energetics, to share their findings supported by some mechanistic studies that will be discussed in the following sections.

CELL VIABILITY AND APOPTOSIS

Besides cellular dysfunction and suppression of proliferation, an HG microenvironment activates signaling pathways that direct MSCs' apoptosis. However, these signaling pathways need to be studied and established further. Tumor necrosis factor-α expression changes significantly affect MSCs' proliferation and death in a STZ-induced type 1 diabetic mouse model[21]. In contrast, in another interesting study, human BM-MSCs in diabetic serum showed higher cellular death and decreased angiogenic response caused by the induction of autophagy signaling with a high-level expression of p62[22].

Endoplasmic reticulum stress-induced autophagy is another mechanism contributing to the inactivation of the mTOR, which reduced p-S6 (a marker of mTOR activity)[23]. Building on these data, Abu-El-Rub *et al*[20] revealed higher apoptosis in human AD-MSCs (hAD-MSCs) cultured in HG using low glucose culture as control. Elucidating the mechanism causing poor survival of MSCs in an HG microenvironment *via* impairment of the phosphoinositide 3-kinase (PI3K)/mTOR axis, they found significantly increased

tuberous sclerosis 1 (TSC1) protein. It is now well-established that mTOR is an essential regulator of mitochondrial dynamics *via* generating the required mitochondrial potential to produce ATP[24]. As a part of the mechanism, TSC1 binding inactivates mTOR, while PI3K, a known activator of mTOR, is needed to remove the inhibitory effects of TSC1[25]. Furthermore, the downregulation of mTOR significantly reduced complex I, IV, and V in HG-cultured hAD-MSCs. These molecular data suggest impacting the mitochondrial oxidative phosphorylation and inducing mitophagy and massive oxidative stress[26]. Although data from the Abu-El-Rub *et al*[20] provide a better understanding of the activation of proapoptotic signaling in hAD-MSCs in the HG microenvironment, it would have been interesting to see if similar signaling was activated in MSCs from other tissue sources as well as from other species to delineate any tissue or species-specific differential responsiveness to HG culture conditions. Also, the mechanistic data could have been more convincing if the authors had used gain-of-function and loss-of-function studies to establish a causal relationship between mTOR, PI3K, Akt, and TSC1. There is no mention of TSC2, which forms a physical and functional complex *in vivo*[27]. The evidence is based on western blotting alone, showing TSC1 expression with simultaneous loss of PI3K and mTOR in the HG cultured cells. There needs to be more evidence to prove their dependence/relationship with each other. Intriguingly, the authors designed the studies for stipulated time points of 3, 7, and 14 d; they provided data only for the day 7 time point. It would have been interesting to include day three and day 14 data in the results or at least as supplementary data to show how early these molecular and organelle level changes occurred and continued in the HG culture. Similarly, it would have been interesting if the cells were returned to normoglycemic conditions at each time point to observe any possible reversibility of the changes at each time point. There are better methods to observe cell viability than the Trypan blue dye exclusion method to exclude the researcher's bias. Another mechanism suggested by the authors for MSCs' low viability is the drop in NAD+/NADH ratio in hAD-MSCs, correlated with impairment of the

inner mitochondrial membrane potential (MtMP) that will be discussed in the next section.

MITOCHONDRIAL CHANGES IN RESPONSE TO HYPERGLYCEMIC MICROENVIRONMENT

Before discussing the impairment of MtMP as a part of cells' response to hyperglycemia, the readers need to understand the basic functioning of mitochondria. A continual, uninterrupted energy supply is critical for cellular processes, *i.e.,* growth, repair, maintenance, *etc.*, for which robust intracellular mechanisms are in place[28]. Mitochondria play a crucial role in supporting these cellular functions with ATP production during normal mitochondrial bioenergetics, besides contributing to other processes such as aging, ion homeostasis, and apoptosis[29]. The mitochondrial intermembranous space houses the enzymes involved in the electron transport chain, capturing energy carried by electrons in NADH and FADH2 to generate ATP. The flux of electrons creates a stable MtMP facilitated by proton pumps, *i.e.,* complexes I, III, and IV. Contingent upon the cell's energy needs, mitochondria undergo fusion or fission such that the process stimulates and inhibits ATP synthesis, respectively[30]. At the molecular level, AMPK enables mitochondrial fusion *via* mitofusin 1 (Mfn1), Mfn2, and optic atrophy 1, while dynamin-related protein 1 (Drp1) and fission protein 1 control mitochondrial fission. More recent studies have shown that mitochondrial functions go far beyond energy-producing organelle, *i.e.,* cell differentiation and their regenerative potential[31-33].

HG culture conditions *in vitro* and hyperglycemia in diabetic patients cause mitochondrial dysfunction due to altered MtMP, thus lowering ATP production. A low NAD+/NADH ratio is observed in cardiac dysfunction in diabetic hearts. At the same time, it also changes mitochondrial membrane polarity and reduces mitochondrial cell mass, leading to perturbed mitochondrial homeostasis in human mononuclear cells[34,35]. Hyperglycemia also causes mitochondrial fragmentation with upregulation of Drp1 (promoting fission) or down-regulation of Mfn1/2 (inhibiting fusion), thus further

reducing mitochondrial ATP synthesis[35]. It creates structural and functional changes in the mitochondria, altering their bioenergetics and thus jeopardizing their survival[36-38]. There is also an increase in ROS stress in the cytosol and mitochondria[39]. Abu-El-Rub *et al*[20] have attributed reduced NAD+/NADH ratio in hAD-MSCs exposed to an HG environment as responsible for driving the cells towards apoptosis *via* dysregulation of mitochondrial complexes I, IV, and V. They have supported their findings with MtMP changes in hAD-MSCs assessed by the MtMP assay kit. All these factors confirm dysfunction in mitochondrial bioenergetics in the cells, resulting in low survival and higher apoptosis in HG culture conditions. Table 1 summarizes some of the studies from the published literature reporting the effect of HG culture conditions.

One of the main limitations of the proposed mechanism is that there needs to be an attempt to extrapolate these data *in vivo* using experimental animal models. This is important before use as a novel target to improve the survival of MSC in diabetic patients. Moreover, it would have been interesting if the authors had used cellular preconditioning using preconditioning mimetics or a sub-cellular preconditioning approach to stabilize the MtMP, which can enhance cell survival and reduce apoptosis in HG culture conditions[43-45]. The authors have already successfully used a sub-cellular preconditioning approach for cytoprotection of donor stem cells for heart-cell therapy to enhance their survival post-engraftment[46,47]. Underscoring the mechanism, the authors have shown that mito-Cx43 gene targeting was cytoprotective *via* a shift of mitochondrial Bak and Bcl-xL balance.

CONCLUSION

In conclusion, it is evident that HG conditions have detrimental effects on the different cell types, including cancer cells, and may also change their normal functions, *i.e.*, migration potential and invasiveness[1,2,48-50]. Hence, understanding the mechanism of apoptosis by which chronic exposure to HG, both *in vitro* as well as *in vivo*, will help combine preconditioning strategy, especially the sub-cellular preconditioning approach, will go a long way in promoting donor cell survival post-engraftment in diabetic

patients and vice versa in the clinical settings wherein MSCs have already progressed to advanced phases of assessment[51,52].

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