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Research Article

Hypoxic Preconditioning Prevents Oxidative Stress-Induced Cell Death in Human Hair Follicle Stem Cells

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Background: Cell therapy, involving the transplantation of viable cells for therapeutic purposes, offers immense promise but faces challenges related to cell survival and functionality post-transplantation. Preconditioning strategies, particularly hypoxic preconditioning, have emerged as a means to enhance cell adaptability and resilience.

Objectives: This study investigated the impact of hypoxic preconditioning on the survival and oxidative stress tolerance of nestin-expressing hair follicle stem cells (hHFSCs) and SH-SY5Y neuroblastoma cells, two crucial cell types for central nervous system therapies. The study also examined the relative expression of three key genes, HIF1α, BDNF, and VEGF following hypoxic preconditioning.

Materials and Methods: hHFSCs were isolated from human hair follicles, characterized, and subjected to hypoxia for up to 72 hours. SH-SY5Y cells were similarly preconditioned for up to 72 hours. Cell viability under hypoxic conditions and oxidative stress was assessed. The relative expression of key genes was evaluated using qRT-PCR.

Results: hHFSCs exhibited remarkable resilience to hypoxic conditions, while SH-SY5Y cells displayed lower tolerance. Hypoxic preconditioning improved the viability of both cell types under oxidative stress. HIF1α mRNA was significantly downregulated, and VEGF transcripts increased after preconditioning, suggesting adaptations to prolonged hypoxia.

Conclusion: Hypoxic preconditioning enhances the survival and oxidative stress resilience of hHFSCs and SH-SY5Y cells, offering potential benefits for central nervous system cell therapy. The differential responses observed emphasize the need for tailored preconditioning strategies for specific cell types. These findings underscore the importance of hypoxic preconditioning and warrant further research into the underlying mechanisms, bringing us closer to effective neurological disorder treatments.

Keywords: EPI-NCSCs, HAP stem cells, Hypoxia, Priming

1. Background

Cell therapy, also referred to as cytotherapy, cell transplantation or cellular therapy, represents an innovative therapeutic approach involving the transplantation of viable cells into patients to elicit beneficial medicinal effects. Within the realm of cell

therapy, a wide array of treatments exists, ranging from those involving stem cells to non-stem cell-based interventions, and encompassing single or multiple cell types. These therapies exhibit distinct mechanisms of action, isolation methodologies, immunophenotypic profiles, and regulatory considerations (1). Despite its tremendous promise, cell therapy faces formidable challenges, primarily centered around issues related to cell homing and post-transplantation survival. A pivotal challenge arises from the relatively short lifespan and low survival rates of transplanted cells at the target site, often due to the loss of their native supportive environment. This environmental shift can activate apoptotic signaling pathways, ultimately leading to cell death. Moreover, therapeutic cells often exhibit limited proliferation within the patient's body, which limits their overall therapeutic efficacy (2).

In response to these challenges, a range of approaches collectively known as "preconditioning strategies" have emerged. These strategies employ various techniques to enhance the performance and adaptability of cells during ex vivo expansion, thereby increasing their chances of survival and functionality post-transplantation (3, 4). While the field of preconditioning in cell therapy continues to evolve, recent research underscores its growing importance, if not necessity, for successful cell transplantation. Stem cell preconditioning, in particular, is poised to garner increased attention, both within the basic research community dedicated to preconditioning and in the realm of translational stem cell research. Preconditioned cells typically exhibit significantly improved survival rates, enhanced differentiation potential, augmented paracrine effects leading to increased trophic support, and superior homing abilities to lesion sites. Additionally, transplantation of pre-conditioned cells contributes to the suppression of inflammatory factors and immune responses, fostering functional recovery (5, 6).

Preconditioning methods available to researchers encompass hypoxia, incubation with pharmacological/ chemical agents, exposure to trophic factors/cytokines, physical factor-based preconditioning, and genetic modification (7). Among these, hypoxic preconditioning has garnered substantial attention in recent years. Hypoxia equips cells with the ability to adapt to their external microenvironment, reducing oxidative stress, enhancing proliferation and differentiation, maintaining stemness, and improving motility, all of which are advantageous in the context of cell therapy following transplantation (8-10).

2. Objectives

In light of these considerations, the present study aimed to assess the impact of hypoxic preconditioning on cell survival and tolerance to oxidative stress in two distinct categories of human cells: nestin-expressing hair follicle stem cells (hHFSCs) and SH-SY5Y neuroblastoma cells. These cell types are widely used in cell therapy for central nervous system conditions. Moreover, the relative expression of three pivotal genes: brain derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and hypoxia-inducible factor 1-alpha (HIF1α) were evaluated following hypoxic preconditioning. This investigation seeks to evaluate the effects of hypoxic preconditioning across diverse cell types and shed light on the potential benefits of this approach, thereby advancing cell therapy outcomes.

3. Materials and Methods

3.1. Cell Preparation and Ethical Statement

The human SH-SY5Y neuroblastoma cell line was purchased from the Pasteur Institute (#C611, 23430, Tehran, Iran) and cultured in Dulbecco's modified Eagle medium/ Ham's F-12 supplemented with fetal bovine serum and penicillin/streptomycin. To isolate and culture nestin-expressing hair follicle stem cells, hair follicles were microdissected from pubic skin punches obtained from a 22-year-old brain-dead patient. The patient's legal guardian provided signed informed consent for the skin donation. The hair follicles were then cut into several pieces and explanted onto collagen-coated cell culture plates, following previously described procedures (11, 12). Hair follicles were nourished with minimum essential medium alpha modification containing fetal bovine serum, chick embryonic extract, and penicillin/ streptomycin until stem cell migration occurred. Migrated stem cells at passages 3 to 5 were used in this study. This research was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (Approval Number: IR.SUMS. REC.1401.279) and the study was performed in accordance with Declaration of Helsinki

3.2. Verification of Nestin and SOX10 Expression by Migrated hHFSCs

To evaluate the expression of nestin and SOX10, which are among the markers of neural crest stem cells and neural crest cells (13, 14), immunofluorescent staining was conducted. Following fixation and blocking steps, cells were incubated overnight at 4 °C with rabbit anti-nestin antibody (1:50; Proteintech, #19483–1-AP) or rabbit anti-SOX10 antibody (1:100; Proteintech, #10422–1-AP). After washing and reblocking, the targeted proteins were visualized through a 2-hour incubation with goat anti-rabbit IgG AlexaFluor488 secondary antibody (1:1000; Abcam, #ab150085). Nuclei were eventually counterstained with Hoechst 33342 (Sigma, #B2261), and the ZOE fluorescent cell imager was employed to capture images.

3.3. Verification of Major Cell Surface Markers Expression by Migrated hHFSCs

Flow cytometry assays were employed to examine the cell surface markers expressed by hHFSCs. In brief, hHFSCs from the third passage were initially blocked with fetal bovine serum and subsequently exposed to specific antibodies at 4 °C, including CD29-PerCP (#080,210, ImmunoSteps), CD45-FITC/CD34-PE (#341,071, BD Biosciences), CD44-APC (#103,012, BioLegend), CD73-PE/Cyanine7 (#344,009, BioLegend), CD90-APC (#328,113, BioLegend), and CD105-PE (#323,205, BioLegend). Following the necessary washing steps, fluorescence was quantified using a BD FACSCalibur.

3.4. Hypoxic Induction

To induce a hypoxic environment, hHFSCs and SH-SY5Y cells were initially seeded in 4-well plates and subsequently placed within a modular incubator chamber (MIC-101, Billups-Rothenberg). This chamber was thoroughly purged with a gas mixture consisting of 5% CO_2 and 95% N2. Subsequently, the sealed chamber was positioned within an incubator set to a constant temperature of 37 °C for durations of 24, 48, or 72 hours. It's important to note that for each of these time points, there was a corresponding normoxic control group in which cells were cultured under standard conditions, exposed to ambient air containing 5% CO_2 at 37 °C.

3.5. Live-Dead Staining

Following hypoxic induction, live-dead staining analysis was performed using a triple staining method involving propidium iodide (PI, #P4170, Sigma), fluorescein diacetate (FDA, #F7378, Sigma), and Hoechst 33342 (Sigma, #B2261). In this assay, PI served as an indicator for both necrotic and late apoptotic cells, while FDA was utilized to identify vital, living cells, and Hoechst 33342 was employed for nuclei staining. The PI-FDA-Hoechst working solution was prepared by adding 5 µL of PI stock solution (2 mg. mL^{-1}), 5 µL of FDA stock solution (5 mg. mL^{-1}), and 5 µl of Hoechst stock solution (2 mg. mL^{-1}) to one milliliter of phosphate-buffered saline. Cells were exposed to this mixture for 5 minutes and the ZOE fluorescent cell imager was employed to capture images. Four independent replicates were conducted in both the normoxic and hypoxic groups.

3.6. Oxidative Stress Induction

The hHFSCs and SH-SY5Y cells were initially seeded in 96-well plates. After the desired incubation time in either a normoxic or hypoxic environment, oxidative

Table 1: List of primers

Gene	Sequence	Amplicon (bp)
HIF1α	F- CCTGCTTGGTGCTGATTTGTGAAC R- CTGGCTCATATCCCATCAATTCGG	150
BDNF	F- GGAGGCTATGTGGAGTTGGCATTG R- TGTAGGCACTTAAAGCACGAGGTC	195
VEGF	F- GGCAGAAGGAGGAGGGCAGAATC R- GGCACACAGGATGGCTTGAAGATG	150
B2M	F- AGGCTATCCAGCGTACTCCAAAGA R- AACCCAGACACATAGCAATTCAGG	94

stress was induced by exposing both hHFSCs and SH-SY5Y cells to hydrogen peroxide (H_2O_2) for 24 hours. Hydrogen peroxide was used at concentrations ranging from 200 to 2000 micromolar, and it was diluted in a complete medium (15). There were eight independent replicates for each dose of H_2O_2 treatment in both the normoxic and hypoxic groups.

3.7. Cell Viability Assay

After inducing oxidative stress, a cell viability assessment was conducted using 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). To perform this assessment, a solution of 0.5 mg. mL^{-1} MTT (Sigma, USA) was prepared in the culture medium and added to each well. Following a 3-hour incubation period, the MTT solution was removed, and isopropanol was added to dissolve the blue formazan crystals. Finally, the resulting color was measured at 570 nm using a microplate reader (BioTek, USA).

3.8. Evaluation of Target Genes

The qRT-PCR technique was employed to assess the relative expression levels of HIF1α, BDNF, and GDNF after hypoxic preconditioning, following a set of standard procedures (16). Total RNA was extracted from three independent replicates in accordance with the recommended protocols of the manufacturer. To ensure the purity of the RNA samples and eliminate any DNA contamination, DNase I treatment was conducted, following the manufacturer's instructions. Subsequently, reverse transcription was performed to convert the RNA into complementary DNA (cDNA), following the provided guidelines. The qRT-PCR reactions were conducted using the first-strand cDNA samples, along with specific primer pairs and RealQ Plus 2x Master Mix Green (Ampliqon, Denmark), on an Applied Biosystems StepOne system (ABI, USA). Specific primer pairs were designed for the target genes as follows: HIF1α: forward- CCTGCTTGGTGCTGATTTGTGAAC, reverse- CTGGCTCATATCCCATCAATTCGG; BDNF: forward-GGAGGCTATGTGGAGTTGGCATTG, reverse- TGTAGGCACTTAAAGCACGAGGTC; VEGF: forward- GGCAGAAGGAGGAGGGCAGAATC, reverse- GGCACACAGGATGGCTTGAAGATG. The amplification conditions were as follows: an initial denaturation at 95 °C for 15 minutes, followed by 40 cycles of denaturation at 95 °C for 20 seconds, and annealing/extension at 60 °C for 60 seconds.

To normalize the expression data, the house-keeping gene beta-2 microglobulin (forward- AGGCTATCC AGCGTACTCCAAAGA, reverse- AACCCAGACA CATAGCAATTCAGG) was used (**Table 1**). Primer specificity was confirmed by analyzing the melting curves, which yielded a single peak. Additionally, to ensure the absence of genomic contamination, negative controls were included, consisting of samples without reverse transcriptase and non-template controls. Finally, data analysis was carried out using the $2^{-\Delta\Delta CT}$ method to calculate fold changes in gene expression.

3.9. Statistical Analysis

The data were subjected to analysis using GraphPad Prism® 9.0, and the results were presented as the mean ± SEM (standard error of the mean). To compare cell viability data, a two-way analysis of variance (ANOVA) was employed, followed by a Bonferroni post hoc test. For the analysis of relative expression data, an unpaired t-test was applied. Statistical significance was determined at a threshold of $P < 0.05$.

4. Results

4.1. Verification of Migrated hHFSCs

Human hair follicles sourced from pubic skin (**Fig. 1A**) were isolated and transferred to culture plates. In the days following the plating, stem cells originating from these follicles migrated (**Fig. 1B**) and began to proliferate gradually (**Fig. 1C**). Notably, immunofluorescent staining confirmed that almost all of the migrated hHFSCs exhibited robust expression of nestin **(Fig. 1D**), and SOX10 (**Fig. 1E**), which are among the markers of neural crest stem cells and neural crest cells (13, 14). Furthermore, previous research had already established that stem cells derived from the bulge region of hair follicles express specific surface markers, including CD105, CD90, CD73, CD44, and CD29, underscoring their identity as multipotent stem cells. It's important to note that while CD34 is typically expressed in murine follicular stem cells within the bulge, this marker did not label the corresponding region in human follicles, aligning with our findings. Similarly, the results from our flow cytometry analysis demonstrated that in vitro-

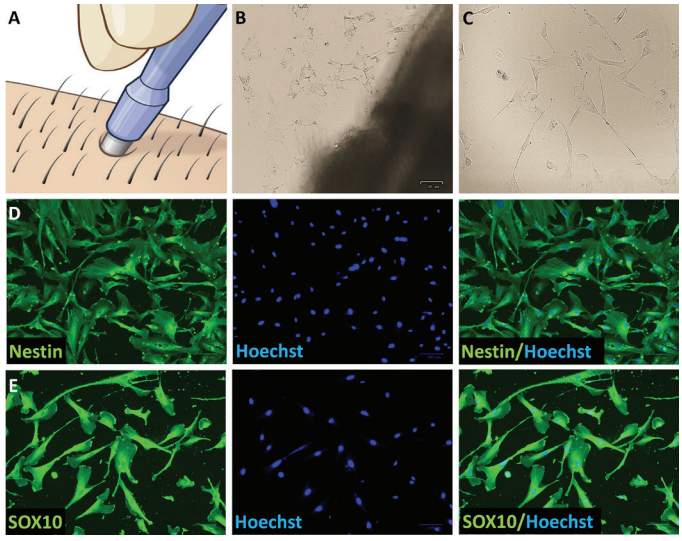

Figure 1: Migration and characterization of human hair follicle stem cells (HFSCs). Using skin punch to obtain human pubic skin **A**). Migration of stem cells from the hair follicles **B and C**). Expression of nestin **D**) and SOX10 (E) by in vitro cultured HFSCs.

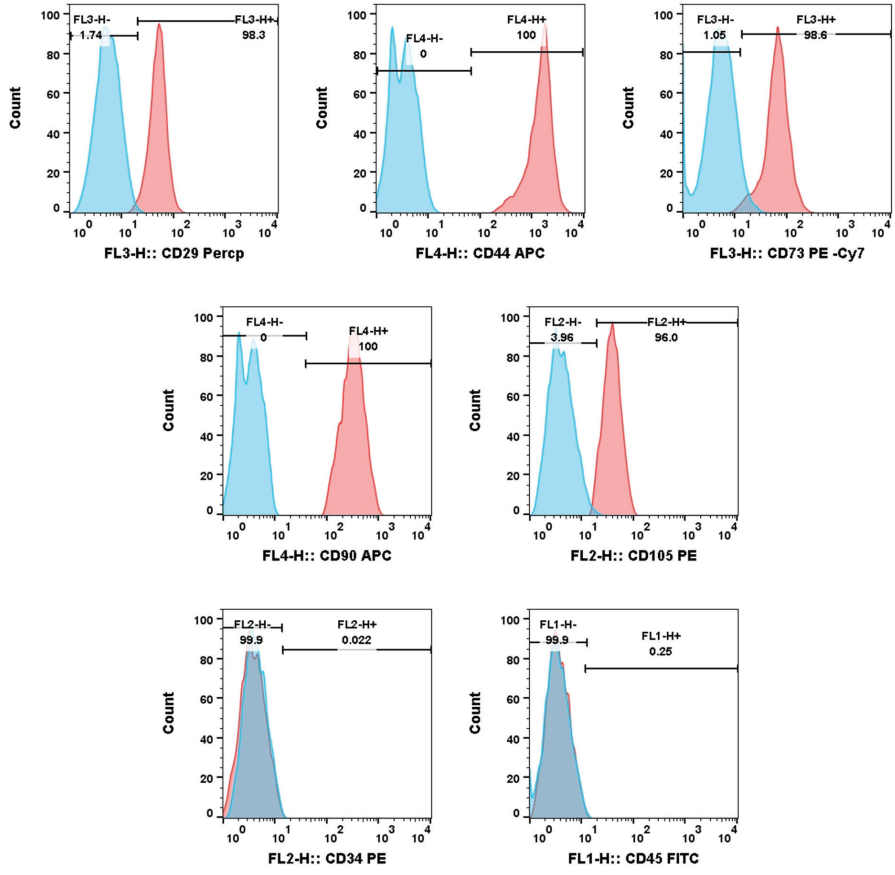

Figure 2: Surface marker expression by human hair follicle stem cells (HFSCs). Expression of CD29, CD44, CD73, CD90 but not CD34 and CD45 surface markers by in vitro cultured HFSCs.

cultured hHFSCs strongly expressed CD29, CD44, CD73, CD90, and CD105 (**Fig. 2**), while lacking the expression of CD34 and CD45 (**Fig. 2**), which are typically associated with hematopoietic cells.

4.2. Hypoxic Conditions and Live-Dead Analysis

The hHFSCs were subjected to a hypoxic environment for durations of 24, 48, and 72 hours. Following each time interval, a live-dead analysis was conducted utilizing triple staining with PI, FDA, and Hoechst dyes. Our findings revealed that the hHFSCs exhibited remarkable resilience even when exposed to hypoxic conditions for up to 3 days, as no signs of cell death were observed at any of the hypoxic time points (**Fig. 3**). As expected, under normal oxygen conditions (normoxia), no cell death was detected in the hHFSCs either (**Fig. 3**).

In contrast, SH-SY5Y cells were also cultured under hypoxic conditions for the same time intervals as the hHFSCs. Unlike hHFSCs, a 3-day exposure to

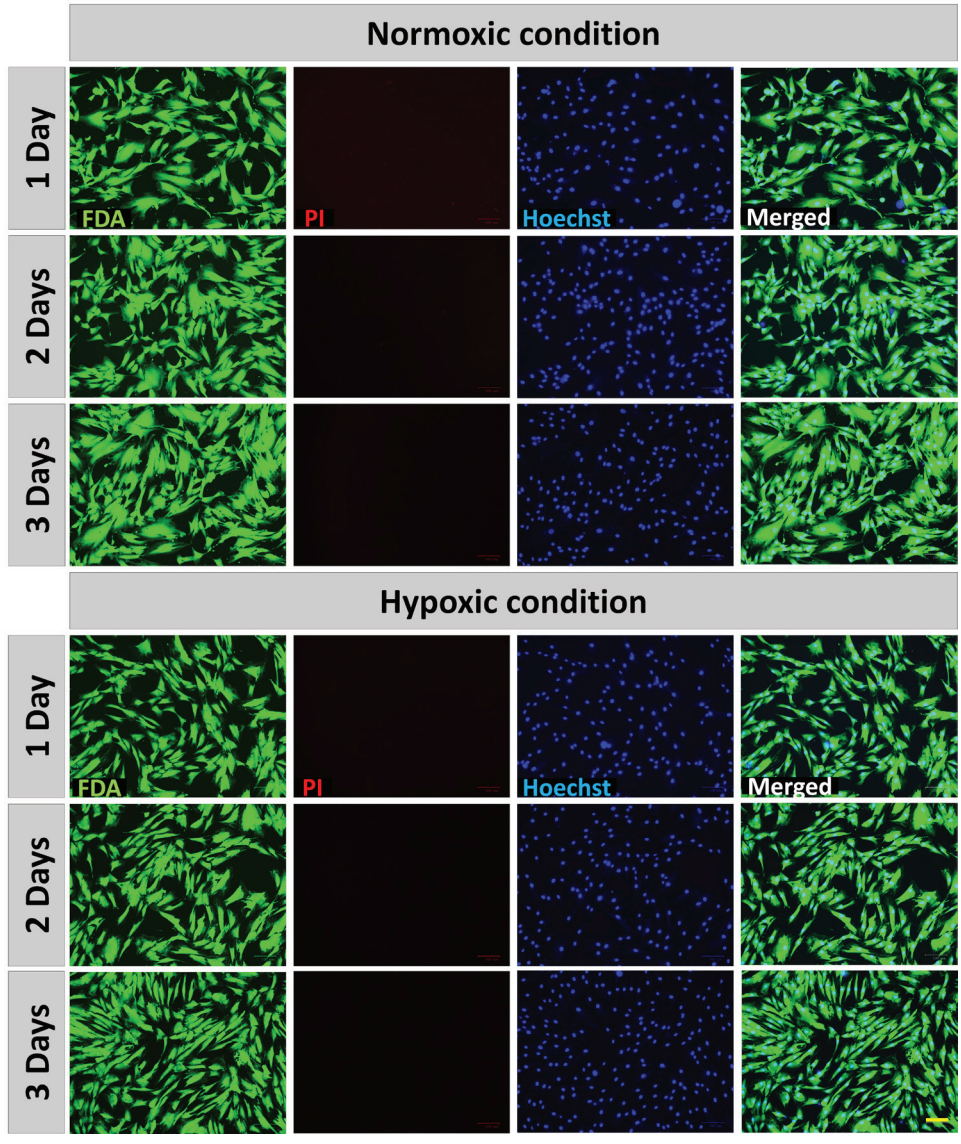

Figure 3: Effects of normoxic and hypoxic conditions on the viability of human hair follicle stem cells (HFSCs). Using propidium iodide (PI, red), fluorescein diacetate (FDA, green), and Hoechst 33342 (blue) to stain HFSCs cultured under either normoxic conditions (upper part) or hypoxic conditions (lower part).

hypoxia resulted in the death of nearly all SH-SY5Y cells. Although the number of live SH-SY5Y cells was higher following a 2-day exposure to hypoxia, more than half of these cells succumbed to cell death. However, no evidence of cell death was observed after just 1 day of hypoxia (**Fig. 4**).

Consequently, we selected a 72-hour hypoxic preconditioning period for hHFSCs and a 24-hour hypoxic preconditioning period for SH-SY5Y cells based on these findings.

4.3. Hypoxic Preconditioning and Resilience to Oxidative stress

Based on the results discussed earlier, hHFSCs underwent 72 hours of hypoxic preconditioning, while SH-SY5Y cells were subjected to 24 hours of hypoxic culture. Subsequently, both hypoxia-preconditioned cell types were exposed to varying concentrations of H_2O_2, ranging from 200 to 2000 micromolar, for a duration of 24 hours. Cell viability assays were then conducted for each group, including corresponding normoxic control

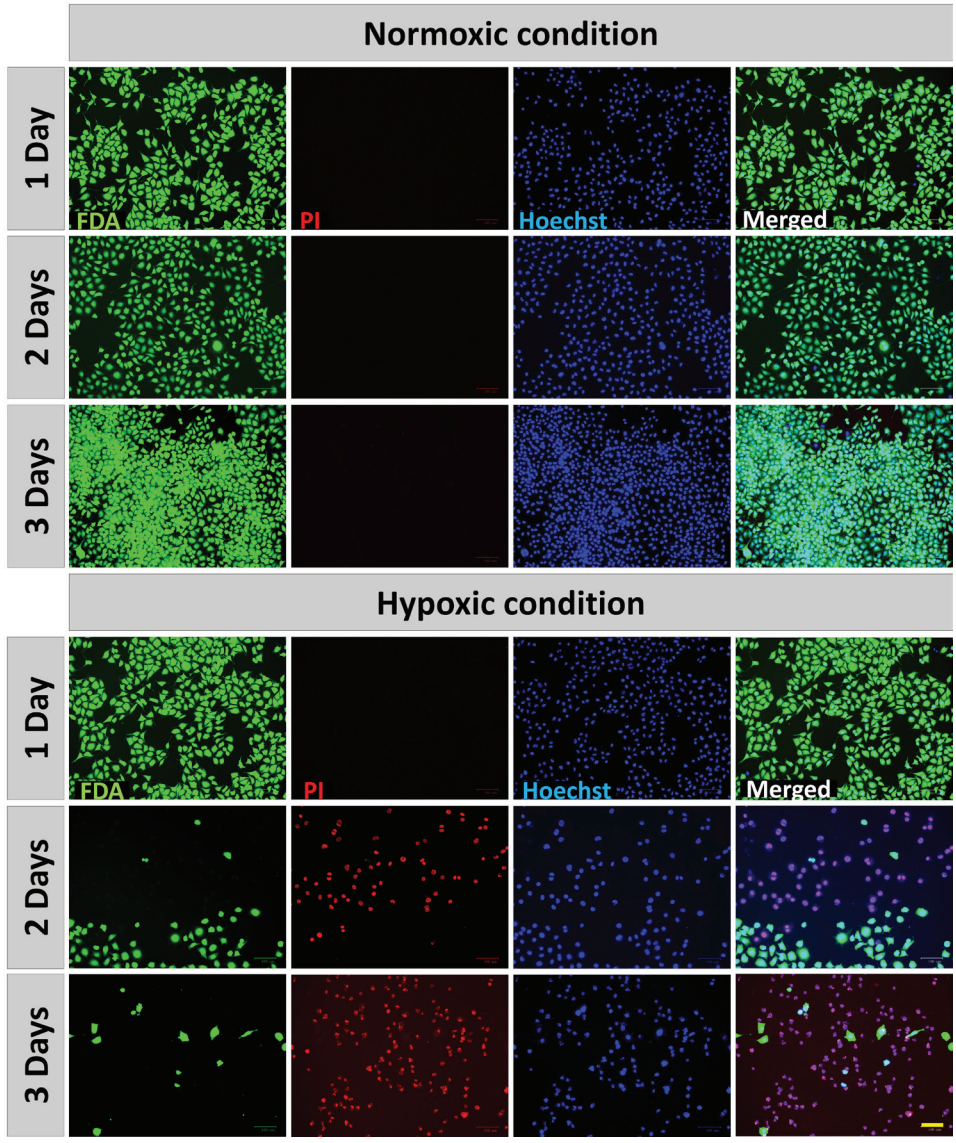

Figure 4: Effects of normoxic and hypoxic conditions on the viability of SH-SY5Y neuroblastoma cell line. Using propidium iodide (PI, red), fluorescein diacetate (FDA, green), and Hoechst 33342 (blue) to stain SH-SY5Y neuroblastoma cells cultured under either normoxic conditions (upper part) or hypoxic conditions (lower part).

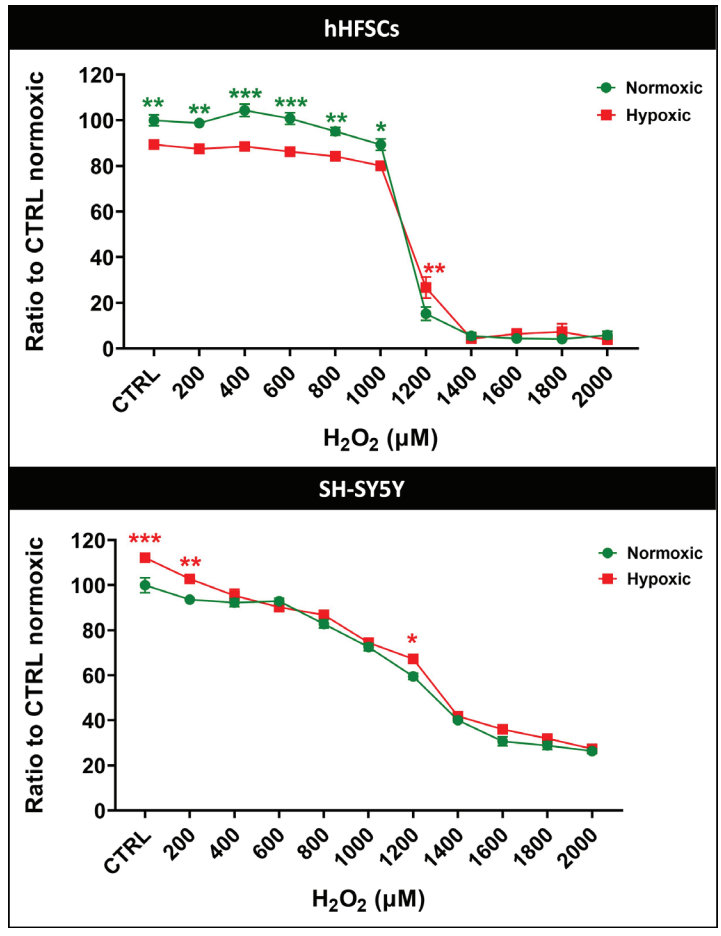

Figure 5: Effects of normoxic and hypoxic preconditioning on the viability of human hair follicle stem cells (HFSCs) and SH-SH5Y cells exposed to H_2O_2. Viability of normoxic and hypoxic preconditioned HFSCs and SH-SY5Y cells following exposure to varying concentrations of H_2O_2. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

groups. The outcomes of our study demonstrated that hypoxic preconditioning exhibited a protective effect in both hHFSCs (P=0.002) and SH-SY5Y (P=0.02) when exposed to 1200 micromolar H_2O_2 (**Fig. 5**).

4.4. Hypoxic Preconditioning and Expression of Target Genes

The relative expression levels of HIF1α, BDNF, and VEGF were assessed in both hypoxia-preconditioned cell types. Interestingly, we observed remarkably similar alterations in the expression of these targeted genes. One-day hypoxic preconditioning in SH-SY5Y cells and three-day hypoxic preconditioning in hHFSCs resulted in significant downregulation of HIF1α mRNA (P<0.01; **Fig. 6**), with a concurrent approximately 600% increase in VEGF transcripts (P<0.001; **Fig. 6**). However, the impact of hypoxic preconditioning on BDNF expression was not statistically significant (P>0.1; **Fig. 6**).

5. Discussion

Over the past two decades, nestin-expressing hair follicle stem cells (also known as epidermal neural crest stem cells and hair-follicle associated pluripotent stem cells) have emerged as a promising source of stem cells for treating neurological disorders (17-26). Several factors contribute to their growing significance in this field, including their ease of isolation and cultivation (27), their expression of nestin as a marker for neural stem and progenitor cells (28), and their remarkable

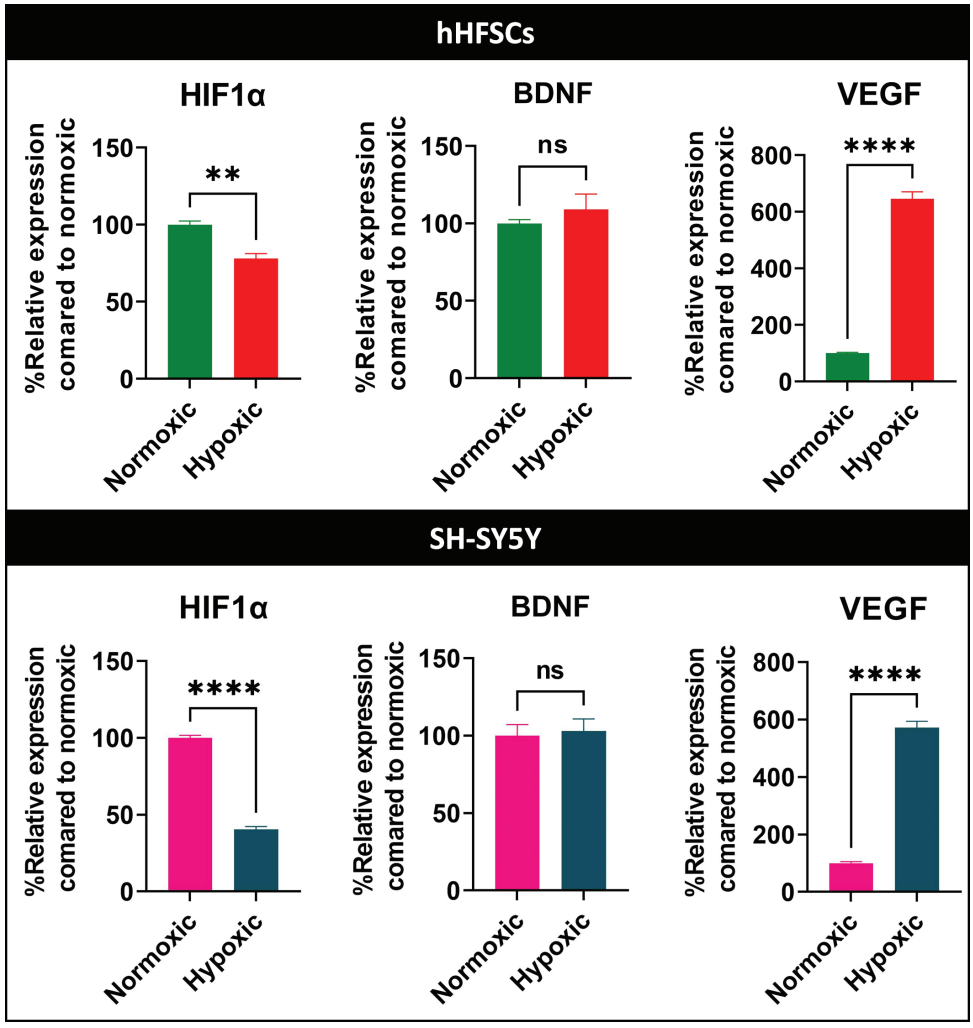

Figure 6: Expression levels of HIF1α, BDNF, and VEGF in normoxic and hypoxic preconditioned human hair follicle stem cells (HFSCs) and SH-SY5Y cells. Relative expression levels of hypoxia-inducible factor 1-alpha (HIF1α), brain-derived neurotrophic factor (BDNF), and vascular endothelial growth factor (VEGF) in hypoxic preconditioned HFSCs (upper part), and the SH-SY5Y neuroblastoma cell line (lower part) compared to normoxic cultured cells. $^{**}P<0.01$ and $^{****}P<0.001$; ns: non-significant.

ability to differentiate into both neuronal and glial cells (29-31). Additionally, these stem cells have shown the capacity to secrete various neurotrophic factors (32, 33), further enhancing their therapeutic potential. Moreover, SH-SY5Y cell lines have played a pivotal role in the study of neurological disorders for many years. They are widely utilized in neurobiology and neuroscience research due to their ability to differentiate into neuron-like cells and their relevance in modeling a variety of neurological conditions, such as Alzheimer's and Parkinson's diseases (34, 35). Hence, in this study, we investigated the impact of hypoxic preconditioning on the survival and resilience of these two distinct human cell types. This investigation is a crucial step towards enhancing the efficacy of cell therapy for future applications. Additionally, we examined the alterations in the expression of specific genes (HIF1α, BDNF, and VEGF) following hypoxic conditioning.

Our results demonstrate a stark contrast in the resilience of hHFSCs and SH-SY5Y cells to hypoxic conditions. Hypoxia is known to equip cells with the ability to adapt to their external microenvironment (36). In line with this

expectation, hHFSCs exhibited remarkable resilience even when exposed to hypoxic conditions for up to 3 days. This suggests that hypoxic preconditioning could be a valuable strategy for enhancing the survival and adaptability of hHFSCs in a transplant scenario, making them a promising candidate for cell therapy in the context of central nervous system conditions. Conversely, SH-SY5Y neuroblastoma cells displayed a much lower tolerance to hypoxic conditions. A 3-day exposure to hypoxia resulted in a significant decrease in cell viability. While a 2-day exposure showed a comparatively better outcome, with more live cells, this indicates that the optimal duration for hypoxic preconditioning varies depending on the cell type. This discrepancy emphasizes the importance of selecting the appropriate preconditioning duration for specific cell types in therapeutic applications.

Our study also investigated the impact of hypoxic preconditioning on cells' resilience to oxidative stress, a pivotal consideration in cell therapy where transplanted cells confront environments with elevated levels of reactive oxygen species (ROS). Oxidative stress presents a formidable challenge in cell therapy, inducing cell death through diverse mechanisms including lipid peroxidation, protein oxidation, DNA damage, mitochondrial dysfunction, activation of cell death pathways, and inflammation (37). ROS, such as superoxide anion, hydrogen peroxide, and hydroxyl radical, play pivotal roles in initiating damage to lipids, DNA, and proteins, ultimately culminating in cellular demise (38, 39). Our findings underscore the effectiveness of hypoxic preconditioning in mitigating oxidative stress-induced damage in both hHFSCs and SH-SY5Y cells, as evidenced by a notable improvement in cell viability, especially under the challenge of 1200 micromolar H_2O_2. While several feasible mechanisms can be proposed for this observed effect, they were not evaluated in the present study. Hypoxic preconditioning has been observed to upregulate the antioxidant defenses within stem cells (40, 41). These enhanced antioxidants can effectively scavenge ROS, neutralizing their harmful effects and preserving cellular homeostasis, thereby enabling stem cells to withstand oxidative insults more effectively and promoting cell survival (40, 41).

Considering the susceptibility of mitochondria to oxidative stress due to their involvement in ROS production (42), hypoxic preconditioning emerges as a crucial protective mechanism (43). By stabilizing mitochondrial function under conditions of oxidative stress, hypoxic preconditioning preserves ATP pro-duction, maintains membrane potential, and prevents the release of pro-apoptotic factors, thereby contributing to the survival of preconditioned stem cells (44).

Furthermore, hypoxic preconditioning has been shown to modulate cell death pathways, favoring cell survival amidst oxidative stress. This modulation may involve the suppression of pro-apoptotic signaling cascades or the activation of pro-survival pathways, effectively tipping the balance away from cell death and towards cell survival (45). Inflammatory responses triggered by oxidative stress exacerbate cellular damage and promote cell death (46). However, hypoxic preconditioning may attenuate inflammatory signaling pathways, dampening inflammation-induced cell death and promoting stem cell survival under oxidative stress conditions (44).

Then, our finding suggests that hypoxic preconditioning could serve as a method to enhance the resistance of transplanted cells to the oxidative environment in the host tissue. This is particularly promising in the context of neurological disorders, where oxidative stress plays a significant role in the progression of diseases (47, 48). The enhanced viability of SH-SY5Y cells and hHFSCs under oxidative stress conditions indicates the potential for improved therapeutic outcomes in central nervous system cell therapy.

Finally, the relative expression of HIF1α, BDNF, and VEGF was assessed following hypoxic preconditioning to shedding light on potential molecular mechanisms underlying these cellular responses. Notably, we observed significant downregulation of HIF1α mRNA in both SH-SY5Y cells (after 1-day hypoxic preconditioning) and hHFSCs (after 3-day hypoxic preconditioning). Simultaneously, we noted a subs-tantial increase in VEGF transcripts.

HIF1α pathway plays a crucial role in cellular adaptation to low oxygen levels. Under normoxic conditions, HIF1α is hydroxylated and targeted for degradation by the von Hippel-Lindau protein. However, under hypoxic conditions, the hydroxylation process is inhibited, leading to HIF1α stabilization and subsequent translocation to the nucleus. Here, it acts as a transcription factor, promoting the expression of genes involved in cell survival and angiogenesis

(36). In the context of our study, the significant downregulation of HIF1α mRNA after hypoxic preconditioning may seem counterintuitive. This downregulation could potentially be attributed to a negative feedback loop, where prolonged exposure to hypoxia triggers the degradation of HIF1α, limiting excessive gene expression (49). As a result, it might regulate the cellular response to prolonged hypoxia, contributing to the resilience observed in hHFSCs and SH-SY5Y cells. The observed findings could also be ascribed to an elevated translation rate. In mammals, the rate of transcription is notably lower compared to that of translation. Consequently, it is conceivable that the reduced HIF1α mRNA levels observed following prolonged hypoxia exposure, were concurrent with an upsurge in HIF1α protein levels. Nevertheless, due to the highly complex and multifaceted nature of HIF1α regulation, further investigations in subsequent studies are necessary to explore this phenomenon in greater detail.

VEGF is a central player in angiogenesis and tissue repair. VEGF and HIF1α are interconnected in a complex regulatory network and it are a well-documented phenomenon that hypoxia triggers VEGF expression, mainly through HIF1α (50). An increase in VEGF transcripts following hypoxic preconditioning could be the result of the cell's adaptive response to hypoxia. Intriguingly, our study revealed a concomitant downregulation of HIF-1α mRNA levels, adding a layer of complexity to the cellular response to prolonged hypoxia.

6. Conclusion

In summary, our study demonstrates that hypoxic preconditioning has a differential impact on hHFSCs and SH-SY5Y cells, providing valuable insights into the optimization of cell therapy strategies for central nervous system conditions. The enhanced resilience to hypoxic conditions, improved resistance to oxidative stress, and modulation of key genes observed in this study underscore the potential of hypoxic preconditioning as a crucial component in future cell therapy approaches. Further research will be essential to elucidate the precise mechanisms governing these cellular responses and to translate these findings into clinical applications, bringing us closer to the promise of effective treatments for neurological disorders.

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