RESEARCH ARTICLE

**Hypoxic Preconditioning Promotes Survival of Human Adipose Derived Mesenchymal Stem Cell [version 4; peer review: 3 approved]**

Previously titled: Hypoxic preconditioning promotes survival of human adipocyte mesenchymal stem cell via expression of prosurvival and proangiogenic biomarkers

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**Abstract**

**Background:** Contributing factors for improved survival of human adipocytes mesenchymal stem cells (h-AMSCs) cultured through hypoxia preconditioning, in example apoptosis inhibition involving BCL2 and HSP27 expression, trigger signal expression (VEGF), SCF expression, OCT-4 expression, and CD44+ expression. The objective of this study was to explain the mechanism and role of hypoxic preconditioning and the optimal duration of hypoxic preconditioning exposure to improve survival of h-AMSCs.

**Methods:** An experimental laboratory explorative study (*in vitro*) with hypoxic preconditioning in h-AMSCs cultures. This research was conducted through four stages. First, isolation of h-AMSCs culture from adipose tissue of patients. Second, the characterization of h-AMSCs from adipose tissue by phenotype (flowcytometry) through CD44+, CD90+ and CD45-expression before being pre-conditioned for hypoxic treatment. Third, the hypoxic preconditioning in h-AMSCs culture (*in vitro*) was performed with an oxygen concentration of 1% for 24, 48 and 72 hours. Fourth, observation of survival from h-AMSCs culture was tested on the role of CD44+, VEGF, SCF, OCT-4, BCL2,
HSP27 with Flowcytometry and apoptotic inhibition by Tunnel Assay method.

**Results:** The result of regression test showed that time difference had an effect on VEGF expression ($p<0.001; \beta=-0.482$) and hypoxia condition also influenced VEGF expression ($p<0.001; \beta=0.774$). The result of path analysis showed that SCF had effect on OCT-4 expression ($p<0.001; \beta=0.985$). The regression test results showed that time effects on HSP27 expression ($p<0.001; \beta=0.398$) and hypoxia precondition also affects HSP27 expression ($p<0.001; \beta=0.847$). Pathway analysis showed that BCL2 expression inhibited apoptosis ($p=0.030; \beta=-0.442$) and HSP27 expression also inhibited apoptosis ($p<0.001; \beta=-0.487$).

**Conclusion:** Hypoxic preconditioning of h-AMSC culture has proven to increase the expression of VEGF, SCF, OCT-4, and BCL2 and HSP27. This study demonstrated and explained the existence of a new mechanism of increased h-AMSC survival in cultures with hypoxic preconditioning (O2 1%) via VEGF, SCF, OCT-4, BCL2, and HSP 27.

**Keywords**
apoptosis, h-AMSCs, BCL-2, HSP27, SCF, VEGF expression
Abbreviations
AMSC: Adipose Mesenchymal Stem Cells
ATP: Adenosine Triphosphate
BAX: BCL-2-associated X protein
BCL2: B-Cell Lymphoma 2
CD44: Cluster of Differentiation 44
h-AMSC: human Adipose Mesenchymal Stem Cells
HSF1: Heat Shock Factor 1
HSP27: Heat Shock Protein 27
ITD: Institute of Tropical Diseases (Universitas Airlangga)
MANOVA: Multivariate Analysis of Variance
OCT4: Octamer-binding transcription factor 4
PK2: Protein k-2
PKC: Protein kinase C
ROS: Reactive oxygen species
SCF: Stem Cell Factor
SLF: Steel Factor
SPSS: Statistical Package for Social Sciences
VEGF: Vascular Endothelial Growth Factor

Introduction
Several literatures provide abundant information that human adipose derived mesenchymal stem cell (h-AMSCs) is an attractive resource for therapeutics and have beneficial effects in regenerating injured cardiomyocytes due to their self-renewal ability and broad differentiation potential under physiological and pathological conditions.\(^1\)–\(^3\)

Despite the impressive potential of the h-AMSC-based therapy, several obstacles (e.g., the difficulty of maintaining self-renewal and poor survival due to apoptosis and/or necrosis at the administration site) have been encountered.\(^4\) Some studies suggest that more than 90% of transplanted stem cells, either intravenously, intramyocardially, and intracoronary delivery, have necrosis and apoptosis and only about 5% transplanted stem cells can survive up to 14 days in infarcted myocardium.\(^5\) The survival of stem cells transplantation is so poor because high percentage of dead cells due to factors such as limited availability of blood, hypoxia, oxidative stress, inflammatory processes, loss of extracellular cell buffer matrix (anoic), non-conducive microenvironment to myocardial infarction, structural damage to blood vessels and lack of nutritional support.\(^6\)

Therefore a particular strategy is needed to improve survival, increase proliferation, migration, maintain the potential for differentiation and viability of stem cells in environments with low oxygen levels. One of those strategies is to precondition hypoxic precursors \textit{in vitro} on oxygen concentrations mimicking the stem cells’ niche.\(^7\)–\(^8\) Contributing factors for improved survival of h-AMSCs cultured through hypoxia preconditioning, i.e., apoptosis inhibition involving BCL2 and HSP27 expression, trigger signal expression (VEGF), SCF expression, OCT-4 expression, and CD44 + expression.\(^9\)

In detail, it has never been explained how far the role of hypoxic preconditions in inhibiting apoptosis of h-AMSCs culture \textit{in vitro}, in order to enhance survival and increase proliferation, maintain multi-potency, stemness and inhibition of apoptosis. Based on the description above, we consider it is necessary to conduct a research to explain the increased survival of h-AMSCs through the treatment of sub-lethal hypoxia precondition (oxygen concentration of 1%) for 24, 48, and 72 hours by looking at the expression of inhibition on apoptosis and HSP27 expression, and BCL2. In addition, it is necessary to observe the role of hypoxic preconditions in the proliferation process through the expression of SCF, OCT-4, and BCL2.
**Objective**
A study was conducted to explain and confirm the mechanism and role of hypoxic preconditioning and the optimal duration of hypoxic preconditioning exposure to improve survival of h-AMSCs so that it could be used as a benchmark for h-AMSCs culture strategy before transplantation. This study was an experimental laboratory explorative study (*in vitro study*) with hypoxic preconditioning in human adipose derived mesenchymal stem cell (h-AMSCs) cultures.

**Methods**

**Ethical approval**
The use of human subjects in this study had been obtaining an ethical approval from research ethics committee of Dr. Soetomo Academic General Hospital - Faculty of Medicine, Airlangga University (Number: 264/Panke.KKE/IV/2017) issued on April 6th, 2017 under the name of I Gde Rurus Suryawan as principal investigator.

**Study design**
This study is an exploratory laboratory experimental study (*in-vitro study*) with hypoxic preconditions in the culture of human-adipose derived mesenchymal stem cell (h-AMSCs) derived from human adipose tissue. The aim of this study was to obtain stem cells that have high survival so that they are not only viable but also have high adaptability to the environment when the stem cells are transplanted. This type of experiment is a true experimental post-test only control group design accompanied by phenotypic h-AMSCs characterization against CD44+, CD90+ and CD45- before being given treatment.

**Study setting**
This research was conducted at the Center for Research and Development of Stem Cell - Institute Tropical Disease (ITD) Universitas Airlangga, Dr. Soetomo Academic General Hospital and the Faculty of Medicine, Airlangga University, Surabaya. The implementation of this study lasted for 2-3 months.

**Sample size**
The sample size in this study was obtained using the Federer's formula for sample size.\(^{10}\) This formula is used as a control for the degree of freedom in MANOVA. The formula description is as follows:

\[
\text{Sample size: } (r-1) (K-1) \geq 15
\]

\(r\) = replication (experimental unit sample size per group)

\(K\) = number of subject group observations

\(K = 6\)

\((r-1) (K-1) \geq 15\)

\((r-1) (6-1) \geq 15\)

\((r-1) 5 \geq 15\)

\(r-1 \geq 3\)

\(r = 4\)

Then the number of replications for each group is 4, so that the total sample is 24 plate culture.

**Materials**

**Experimental Unit:**

1. h-AMSCs, namely human-adipose derived mesenchymal stem cell from adipose tissue obtained from minimally invasive surgery with small incisions (3-5 cm) under local anaesthesia in the lower abdominal area by a surgeon (*Figure 1*). These materials came from patients who were prepared for clinical application of stem cell therapy at the Network Bank Dr. Soetomo General Hospital, Surabaya. All procedures were approved by the relevant ethics committees, and written informed consent was obtained from all study participants. The h-AMSCs experimental unit was taken from adult patients who were in a stable state who were not taking...
anti-platelets or anti-coagulants and then multiplied \textit{in vitro} at the 5\textsuperscript{th} passage to 24 units. A total of 24 units were divided into two groups, namely control and treatment. The control group (P0) had 12 culture units in normoxic conditions (21\% O\textsubscript{2} concentration). The treatment group (P1) was 12 units pre-conditioned to hypoxia (1\% O\textsubscript{2} concentration). Both treatment groups were observed for survival (CD44+, VEGF, SCF, OCT-4, BCL2, HSP27, and apoptotic inhibition at 24, 48 and 72 hours of cell culture). Observation of apoptotic inhibition based on the expression of BCL2 and HSP27 along with the percentage of apoptosis that occurred.

2. Washing buffer (phosphate-buffered saline, PBS, Sigma-Aldrich, Milan, Italy, 0.1\% sodium azide, and 0.5\% bovine serum albumin (BSA), Radnor, USA) was used for all washing steps (3 ml of washing buffer and centrifugation, 400 g for eight minutes at 4\(^\circ\)C). Briefly, 5\times10\textsuperscript{5} cells/sample were incubated with 100 ml of 20 mM ethylene-diaminetetraacetic acid (EDTA, Sigma-Aldrich) at 37\(^\circ\)C for 10 minutes and washed.
3. Recombinant human VEGF Monoclonal Antibody (JH121) (1:50 tested dilution; Thermo Fisher Scientific Cat# MA5-13182, RRID: AB_10981661) and F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Thermo Fisher Scientific Cat# A48282, RRID:AB_2896345).

4. SCF Monoclonal Antibody (OTI5F6) (1:100 tested dilution; Thermo Fisher Scientific Cat# MA5-26328, RRID: AB_2725298) and F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Thermo Fisher Scientific Cat# A48282, RRID:AB_2896345).

5. OCT4 Monoclonal Antibody (9B7) (1-2 μg/mL dilution; Thermo Fisher Scientific Cat# MA1-104, RRID: AB_2536771) and F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Thermo Fisher Scientific Cat# A48282, RRID:AB_2896345).

6. Bcl-2 Monoclonal Antibody (100/D5) (1:50 tested dilution; Thermo Fisher Scientific Cat# MA5-11757, RRID: AB_10978135) and F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Thermo Fisher Scientific Cat# A48282, RRID:AB_2896345).

7. HSP27 Polyclonal Antibody (OTI5F6) (1:1000 tested dilution; Thermo Fisher Scientific Cat# PA1-017, RRID: AB_2120942) and F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Thermo Fisher Scientific Cat# A48282, RRID:AB_2896345).

Experimental procedures
This research was conducted in four stages as follows:

1. Isolation and culture of h-AMSCs from the patient's adipose tissue (human) (Figure 1).

2. Characterization of h-AMSCs from adipose tissue phenotypically (Flowcytometry) through identification of CD44+, CD90+ and CD45- before being treated with hypoxic preconditions.

3. Hypoxic precondition in in vitro h-AMSCs culture was carried out with an oxygen concentration of 1% for 24, 48 and 72 hours.

4. Observation of survival of h-AMSCs in the form of CD44+, VEGF, SCF, OCT-4, BCL2, HSP27 expression, and apoptotic inhibition:

   a. Phenotype expression of CD44 + was carried out by the flowcytometric method.

   b. Immunocytochemical expression of VEGF

   c. Immunocytochemical expression of SCF from h-AMSCs culture

   d. Phenotype of OCT-4 expression (Immunocytochemistry and Immunofluorescence)

   e. Apoptotic inhibition, based on the expression of BCL2 and HSP27 by immunocytochemistry accompanied by a low percentage of apoptosis through the Tunnel Assay method (Figure 2).

Cell culture
Cells were isolated from patient’s adipose tissue (human) obtained from minimally invasive surgery with small incisions (3-5 cm) under local anaesthesia in the lower abdominal area by a surgeon. Cells were passaged every 2 days. Passage 5 was used for these experiments. Then, h-AMSCs were isolated using an immuno-magnetic separation technique from the cell culture. Following isolation, all cells were cultured at 37°C in a 5% CO2/95% air atmosphere in a humidified incubator (NuAire; Plymouth, MN). Under normal condition, cells were grown to 70% sub-confluence, and treated with complete DMEM, under normoxia or hypoxia condition, for 24, 48, and 72 hours. A total of 24 units were divided into two groups, namely control and treatment. The control group (P0) had 12 culture units in normoxic conditions (21% O2 concentration). The treatment group (P1) was 12 units pre-conditioned to hypoxia (1% O2 concentration). For treatment of cells with hypoxia, a CO2-enriched environment was generated with a BBL™ GasPak Pouch system (Becton Dickinson; Franklin Lakes, NJ). The level of hypoxia obtained with these pouches is <1% O2.
Immunocytochemistry

Quantification of VEGF, SCF, OCT4, Bcl-2, and HSP27 were performed by immunocytochemistry. Assessment of the expression of the differentiation marker of VEGF, SCF, OCT4, Bcl-2, and HSP27 in h-AMSCs culture at this stage using the FITC-labelled immunocytochemical method, was carried out on 24, 48, and 72 hours after the cells were exposed to hypoxic precondition. H-AMSCs cultures were incubated with a target unmasking fluid (Accurate Chemical & Scientific Corp., Westbury, NY, USA) for 15 min using a microwave oven to retrieve the antigens.

The slides were reconstituted in phosphate buffer saline (PBS), pH 7.4 and blocked with 10% normal goat serum (v/v). For double staining, the slides were incubated with Recombinant human VEGF Monoclonal Antibody (JH121) (1:50 tested dilution; Thermo Fisher Scientific Cat# MA5-13182, RRID: AB_10981661), SCF Monoclonal Antibody (OTI5F6) (1:100 tested dilution; Thermo Fisher Scientific Cat# MA5-26328, RRID: AB_2725298), OCT4 Monoclonal Antibody

Figure 2. Observation of survival of h-AMSCs in the form of CD44+, VEGF, SCF, OCT-4, BCL2, HSP27 expression, and apoptotic inhibition:

A. Phenotype expression of CD44+ was carried out by the flowcytometric method.
B. Immunocytochemical expression of VEGF.
C. Immunocytochemical expression of SCF from h-AMSCs culture.
D. Phenotype of OCT-4 expression (Immunocytochemistry and Immunofluorescence).
(9B7) (1–2 μg/mL dilution; Thermo Fisher Scientific Cat# MA1-104, RRID: AB_2536771), Bcl-2 Monoclonal Antibody (100/DS) (1:50 tested dilution; Thermo Fisher Scientific Cat# MA5-11757, RRID: AB_10978135), HSP27 Polyclonal Antibody (OTISF6) (1:1000 tested dilution; Thermo Fisher Scientific Cat# PA1-017, RRID: AB_2120942) at 4°C overnight and then washed thrice with PBS. Some of the sections were incubated with 1% BSA as negative controls. Next, the sections were incubated with F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Thermo Fisher Scientific Cat# A48282, RRID:AB_2896345) respectively, for 1 h at 25°C (room temperature).

This was followed by incubation with DAPI (Vector Laboratories). The incubated monolayer cells were fixed with 4% formalin buffer for 15 minutes, then the object glass was washed with PBS and dried. Followed by blocking with 10% PBS for 15 minutes. Immunocytochemistry in the h-AMSCs preparation was intended to observe the expression of VEGF, SCF, OCT4, Bcl-2, and HSP27 where the cells were then given fluorophore-labeled secondary antibody with F(ab')2-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Thermo Fisher Scientific Cat# A48282, RRID:AB_2896345). Then, images were observed under a light microscope under a 200x objective lens. ImageJ (NIH) software was used for quantitative analysis. The number of positively stained cells was counted in the specimen areas, and eight sequential images per specimens in each group were analysed.

Quantification
We measured mean fluorescence intensity (MFI) in a region of interest (ROI) with ImageJ (NIH) software. We were simply measuring automatic cell counting based on the total fluorescent intensity across the entire image that lead to the potentially erroneous conclusion that the sample with the smaller area/fewer cells exhibits less staining for the probe of interest than one that is larger in area or has more cells, when the reality is that they actually exhibit similar staining levels per tissue area.

Data analysis
Statistical tests were performed with SPSS software (version 24.0; SPSS Inc). Data were collected, processed and statistically tested with several stages. The first stage is an Assumption Test in the form of a normality test to ensure that the data is normally distributed. Since all data were normally distributed, the results were presented as the mean ± standard deviation. Furthermore, a comparison test was carried out between the treatment group and the control group were using Multivariate Analysis of Variance (MANOVA). Furthermore, path analysis is carried out to determine the pathway mechanism of the influence of the independent variables on the dependent variable by using multiple linear regression statistical tests. Path modelling were performed using Run PLS-PM in Excel with the XLSTAT software. The statistical analysis was used to explain the effect of time (24, 48 and 72 hours) and hypoxic conditions on the expression of VEGF, SCF, OCT-4, CD44+, BCL2, HSP27 and the number of cells undergoing apoptosis. Differences having a p value < 0.05 were considered significant.

Results
The results showed that the time difference test on CD44+ expression was 24 hours with 48 hours (p=0.017), 24 hours with 72 hours (p=0.004), and 48 hours with 72 hours (p=0.801). The result of regression test showed that time difference had a effect on expression of CD44+ (p=0.002, β=-0.582) and hypoxia condition had no effect to CD44+ expression (p=0.066, β=0.317) (Table 1)(Figure 3).

The result of time difference test on VEGF expression is between 24 hours with 48 hours (p<0.001), 24 hours with 72 hours (p<0.001), and 48 hours with 72 hours (p=0.047). The result of regression test showed that time difference had an effect on VEGF expression (p<0.001; β=-0.482) and hypoxia condition also influenced VEGF expression (p<0.001; β= 0.774) (Table 2)(Figure 4).

Table 1. Results on CD44+ expression.

| Time (hour) | Experimental group | Mean±SD | p     |
|------------|--------------------|---------|-------|
| 24         | Normoxia           | 72.07±2.985 | 0.149 |
|            | Hypoxia            | 82.42±12.14 |       |
| 48         | Normoxia           | 67.61±3.158 | 0.370 |
|            | Hypoxia            | 69.48±2.203 |       |
| 72         | Normoxia           | 65.85±1.321 | 0.446 |
|            | Hypoxia            | 67.64±4.184 |       |
The result of time difference test on SCF expression is between 24 hours with 48 hours ($p=0.283$), 24 hours with 72 hours ($p<0.001$), and 48 hours with 72 hours ($p<0.001$). The result showed that hypoxic preconditioning influenced the expression of SCF ($p<0.001$; $\beta=0.889$) (Table 3) (Figure 5).

**Figure 3.** Flowcytometry results from human AMSCs based on cell culture for CD44+ CD90+ CD45- expression.

**Table 2. Results on VEGF expression.**

| Time (hour) | Experimental group | Mean±SD | p    |
|------------|--------------------|---------|------|
| 24         | Normoxia           | 0.175±0.074 | 0.000 |
|            | Hypoxia            | 0.766±0.123 |      |
| 48         | Normoxia           | 0.103±0.018 | 0.000 |
|            | Hypoxia            | 0.425±0.036 |      |
| 72         | Normoxia           | 0.075±0.014 | 0.000 |
|            | Hypoxia            | 0.291±0.033 |      |
Figure 4. Immunohistochemical Characteristic of h-AMSCs based on VEGF expression at: A) normoxic condition for 24 hours; B) normoxic condition for 48 hours; C) normoxic condition for 72 hours; D) hypoxic condition for 24 hours; E) hypoxic condition for 48 hours; F) hypoxic condition for 72 hours.

Table 3. Results on SCF expression.

| Time (hour) | Experimental group | Mean±SD | p   |
|------------|--------------------|---------|-----|
| 24         | Normoxia           | 0.084±0.019 | 0.000 |
|            | Hypoxia            | 0.990±0.013 |       |
| 48         | Normoxia           | 0.093±0.014 | 0.000 |
|            | Hypoxia            | 0.901±0.082 |       |
| 72         | Normoxia           | 0.075±0.024 | 0.000 |
|            | Hypoxia            | 0.596±0.087 |       |

Figure 5. Immunohistochemical Characteristic of h-AMSCs based on SCF expression at: A) normoxic condition for 24 hours; B) normoxic condition for 48 hours; C) normoxic condition for 72 hours; D) hypoxic condition for 24 hours; E) hypoxic condition for 48 hours; F) hypoxic condition for 72 hours.
Table 4. Results on OCT4 expression.

| Time (hour) | Experimental group | Mean±SD     | p    |
|-------------|--------------------|-------------|------|
| 24          | Normoxia           | 0.148±0.018 | 0.000|
|             | Hypoxia            | 0.793±0.034 |      |
| 48          | Normoxia           | 0.110±0.007 | 0.000|
|             | Hypoxia            | 0.673±0.047 |      |
| 72          | Normoxia           | 0.099±0.025 | 0.000|
|             | Hypoxia            | 0.457±0.151 |      |

Figure 6. Immunohistochemical Characteristic of h-AMSCs based on OCT-4 expression at: A) normoxic condition for 24 hours; B) normoxic condition for 48 hours; C) normoxic condition for 72 hours; D) hypoxic condition for 24 hours; E) hypoxic condition for 48 hours; F) hypoxic condition for 72 hours. Immunofluorescence assay of h-AMSCs based on OCT-4 expression at: G) normoxic condition for 24 hours; H) normoxic condition for 48 hours; I) normoxic condition for 72 hours; J) hypoxic condition for 24 hours; K) hypoxic condition for 48 hours; L) hypoxic condition for 72 hours.
The result of time difference test on OCT-4 expression is between 24 hours with 48 hours ($p<0.001$), 24 hours with 72 hours ($p<0.001$), and 48 hours with 72 hours ($p<0.001$). The result showed that hypoxic preconditioning had an effect on OCT-4 expression ($p<0.001$; $\beta=0.985$) (Table 4) (Figure 6).

The results of time difference test on BCL2 expression between 24 hours with 48 hours ($p=0.223$), 24 hours with 72 hours ($p=0.295$), and 48 hours with 72 hours ($p=0.982$). The results showed hypoxic preconditioning effect on BCL2 expression ($p<0.001$; $\beta=0.878$) (Table 5) (Figure 7).

The results of the time difference test on HSP27 expression between 24 hours with 48 hours ($p=0.040$), 24 hours with 72 hours ($p<0.001$), and 48 hours with 72 hours ($p<0.001$). The regression test results showed that time effects on HSP27

| Time (hour) | Experimental group | Mean±SD   | $p$      |
|------------|--------------------|-----------|---------|
| 24         | Normoxia           | 0.100±0.010 | 0.000   |
|            | Hypoxia            | 0.714±0.073 |         |
| 48         | Normoxia           | 0.093±0.025 | 0.020   |
|            | Hypoxia            | 0.505±0.185 |         |
| 72         | Normoxia           | 0.141±0.012 | 0.026   |
|            | Hypoxia            | 0.479±0.229 |         |

| Time (hour) | Experimental group | Mean±SD   | $p$      |
|------------|--------------------|-----------|---------|
| 24         | Normoxia           | 0.156±0.024 | 0.000   |
|            | Hypoxia            | 0.967±0.018 |         |
| 48         | Normoxia           | 0.157±0.106 | 0.000   |
|            | Hypoxia            | 0.773±0.132 |         |
| 72         | Normoxia           | 0.055±0.036 | 0.000   |
|            | Hypoxia            | 0.389±0.037 |         |

**Figure 7.** Immunohistochemical Characteristic of h-AMSCs based on BCL2 expression at: A) normoxic condition for 24 hours; B) normoxic condition for 48 hours; C) normoxic condition for 72 hours; D) hypoxic condition for 24 hours; E) hypoxic condition for 48 hours; F) hypoxic condition for 72 hours.
Table 7. Results on number of apoptotic cell amount.

| Time (hour) | Experimental group | Mean±SD     | p    |
|-------------|--------------------|-------------|------|
| 24          | Normoxia           | 0.945±0.034 | 0.000|
|             | Hypoxia            | 0.088±0.026 |      |
| 48          | Normoxia           | 0.777±0.043 | 0.000|
|             | Hypoxia            | 0.148±0.027 |      |
| 72          | Normoxia           | 0.881±0.096 | 0.000|
|             | Hypoxia            | 0.183±0.021 |      |

Figure 8. Immunohistochemical Characteristic of h-AMSCs based on HSP27 expression at: A) normoxic condition for 24 hours; B) normoxic condition for 48 hours; C) normoxic condition for 72 hours; D) hypoxic condition for 24 hours; E) hypoxic condition for 48 hours; F) hypoxic condition for 72 hours.

Figure 9. Immunohistochemical Characteristic of h-AMSCs based on number of apoptotic cell amount at: A) normoxic condition for 24 hours; B) normoxic condition for 48 hours; C) normoxic condition for 72 hours; D) hypoxic condition for 24 hours; E) hypoxic condition for 48 hours; F) hypoxic condition for 72 hours.
expression ($p<0.001; \beta=-0.398$) and hypoxic preconditioning also affects HSP27 expression ($p<0.001; \beta=0.847$) (Table 6) (Figure 8).

The results of time difference test on number of apoptotic cell amount between 24 hours with 48 hours ($p=0.004$), 24 hours with 72 hours ($p=0.562$), and 48 hours with 72 hours ($p<0.001$). Results showed that hypoxic preconditioning inhibited apoptosis via BCL2 ($p=0.030; \beta=-0.442$) and HSP27 ($p<0.001; \beta=-0.487$) (Table 7) (Figure 9).

In this study, the hypoxic preconditioning may elevate the expression of studied variables, such as the number of apoptosis through BCL2 and HSP27 expression, trigger signal through VEGF expression, proliferation through SCF expression, and multipotency through OCT-4 expression. Hypoxic preconditioning significantly affects VEGF, VEGF affects SCF expression, SCF affects OCT-4 expression, OCT-4 affects BCL2 expression, but hypoxia also affects HSP27 expression. BCL2 and HSP27 have proven inhibiting apoptosis thus enhancing h-AMSCs survival (Figure 10). In conclusion, hypoxic preconditioning of h-AMSC culture has proven to increase the expression of VEGF, SCF, OCT-4, and BCL2 and HSP27. This study demonstrated and explained the existence of a new mechanism of increased h-AMSC survival in cultures with hypoxic preconditioning ($O_2 1\%$) via VEGF, SCF, OCT-4, BCL2, and HSP 27. But CD 44+ did not play a role in the mechanism of survival improvement of human AMSC survival.

Discussion
Over the last few years, with the gradual increase in awareness of the critical role that hypoxia-induced signalling could play as a tool for generating angiogenesis on demand, two distinct approaches have emerged, as promising strategies to achieve this goal. On one hand, researchers have explored the possibility of pre-conditioning cells or grafts to hypoxia in vitro, in order to upregulate the required signalling that can then initiate angiogenesis in vivo upon transplantation. The second approach relies on direct induction of hypoxia-mediated signalling in vivo, by pharmacological means or gene
A further distinction can be made on whether the therapy involves transplantation of hypoxia pre-conditioned or genetically modified cells, or if the effect is mediated directly through gene transfer or cell-free delivery of hypoxia-induced protein factors.

The low survival of h-AMSCs after transplanting the heart muscle with myocardial infarction has limited the effectiveness of stem cell therapy. This is presumably because the transplanted stem cells are difficult to adapt to a new environment that is different from the environment during the in vitro culture process if it is carried out under normoxic conditions (2% oxygen concentration), while the niche of h-AMSCs in adipose tissue is actually under hypoxic conditions (oxygen concentration between 2-8%). The mechanism underlying the decreased effectiveness of stem cells when transplanted is thought to be because many transplanted stem cells undergo apoptosis. Therefore, a strategy is needed to increase the resistance of transplanted stem cells, one of which is the hypoxic adaptation process during in vitro culture.

The role of sub lethal hypoxia during the in vitro culture process is to provide hypoxic preconditions so that the support niche is compatible with the hypoxic environment in vivo in myocardial infarction. Hypoxic precondition will trigger Vascular Endothelial Growth Factor (VEGF) which then binds to VEGF Receptor-1 (VEGFR-1) in the cytosol. The presence of VEGF - VEGFR-1 bonds is thought to occur in a series of signalling which activates Stem Cell Factor (SCF) or Steel Factor (SLF) in the interstitial. Interstitial SCF expression will be recognized by the SCF receptor so that an SCF-receptor complex is formed in the cell nucleus and nuclear β1-integrin expression will activate Octamer-4 (OCT-4) so that stem cells experience proliferation, self-renewal but still have the potential for differentiation.

On the other hand, hypoxic conditions are thought to have an effect on mitochondria in increasing the expression of Reactive Oxygen Species (ROS). The increased ROS due to hypoxic conditions is thought to be the cause of the increase in free radicals formed through mitochondrial-mediated pathways. This triggers protein kinase-C (PKC) and protein K-2 (PK2) which then triggers the p53 gene so that there is an increase in p53 protein expression which will activate proapoptotic members such as BAX. Increased expression of p53 causes mitochondrial damage which causes pores to open in the membrane, so that Cytochrome-C and other molecules that act as APAF-1 will exit the mitochondria. CD44 is a polymorphic family that is immunologically related to proteoglycans and cell surface glycoproteins as markers of h-AMSCs. Apart from being a marker for h-AMSCs, CD44 + has a signalling function that plays a role in cell survival and motility.

The role of sub lethal hypoxia during the in vitro culture process is to provide hypoxic preconditions so that the support niche is compatible with the hypoxic environment in vivo in myocardial infarction. Hypoxic precondition will trigger Vascular Endothelial Growth Factor (VEGF) which then binds to VEGF Receptor-1 (VEGFR-1) in the cytosol. The presence of VEGF - VEGFR-1 bonds is thought to occur in a series of signalling which activates Stem Cell Factor (SCF) or Steel Factor (SLF) in the interstitial. Interstitial SCF expression will be recognized by the SCF receptor so that an SCF-receptor complex is formed in the cell nucleus and nuclear β1-integrin expression will activate Octamer-4 (OCT-4) so that stem cells experience proliferation, self-renewal but still have the potential for differentiation.

On the other hand, hypoxic conditions are thought to have an effect on mitochondria in increasing the expression of Reactive Oxygen Species (ROS). The increased ROS due to hypoxic conditions is thought to be the cause of the increase in free radicals formed through mitochondrial-mediated pathways. This triggers protein kinase-C (PKC) and protein K-2 (PK2) which then triggers the p53 gene so that there is an increase in p53 protein expression which will activate proapoptotic members such as BAX. Increased expression of p53 causes mitochondrial damage which causes pores to open in the membrane, so that Cytochrome-C and other molecules that act as APAF-1 will exit the mitochondria. This condition will activate procaspase 9 to become caspase-9 and followed by activation of procaspase 3 to become active caspase-3 which affects DNase so that DNA fragmentation occurs, and ends with cell death through the apoptotic process.

However, the low sublethal oxygen concentration is thought to activate cells for protection in the form of repair. The repair process can be done through the activation of heat shock factor-1 (HSF-1) so that the formation of several Heat Shock Proteins (HSPs) occurs. HSPs are the product of several gene families contained in the cell nucleus which act as chaperone molecules that play a role in cell survival during the stress process. Some of the HSPs that were thought to be involved were HSP70, HSP90α and HSP27. However, in hypoxic conditions that cause the glycolysis process. This glycolysis process will further affect Krebs’s cycles so that ATP synthesis decreases. This decrease in ATP concentration is thought to cause a decrease in the function of HSP70 and HSP90α. This is because HSP70 and HSP90α are ATP-dependent chaperone molecules, thus the two HSPs (HSP70 and HSP90α) do not have the ability to act as chaperones in protecting, protecting and repairing cells under stress. The role of chaperone molecules in hypoxic conditions is carried out by HSP27, because HSP27 is ATP-independent chaperone. In addition, hypoxic precondition can maintain multipotential properties through OCT-4 expression compared to normoxic conditions.

**Conclusion**

From this study, it can be concluded that the hypoxic preconditioning of h-AMSC culture has proven to increase the expression of VEGF, SCF, OCT-4, and BCL2 and HSP27. This study demonstrated and explained the existence of a new
mechanism of increased h-AMSC survival in cultures with hypoxic preconditioning (O2 1%) via VEGF, SCF, OCT-4, BCL2, and HSP 27.

Data availability

Underlying data

Figshare: Raw Data - Hypoxic Preconditioning Promotes Survivals of Human Adipocyte Mesenchymal Stem Cell via Expression of Prosurvival and Proangiogenic Biomarkers. https://doi.org/10.6084/m9.figshare.15029016.v1.37

This project contains the following underlying data:

- Data file 1. Raw Data.xlsx

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgement

We would also like to show our gratitude to Dr. Andrianto and Dr. Meity Ardiana for sharing their pearls of wisdom with us during the writing process, and we thank for anonymous residents and staffs for their so-called insights. We are also immensely grateful to all professors and consultants from Department of Cardiology and Vascular Medicine – Faculty of Medicine, Universitas Airlangga for their comments on an earlier version of the manuscript, although any errors are our own and should not tarnish the reputations of these esteemed persons.

A previous version of this paper is available at bioRxiv 2021.01.18.427057; https://www.biorxiv.org/content/10.1101/2021.01.18.427057v1.

References

1. Stegniewski J, Tomczyk M, Andrysiak K, et al.: Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes, in Contrast to Adipose Tissue-Derived Stromal Cells, Efficiently Improve Heart Function in Murine Model of Myocardial Infarction. Biommedicines. 2020; 8(12):1-21. PubMed Abstract | Publisher Full Text | Free Full Text

2. Acquistapace A, Bru T, Lesault PF, et al.: Human mesenchymal stem cell reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer. Stem Cells. 2011; 29(5): 812-824. PubMed Abstract | Publisher Full Text | Free Full Text

3. Lee J, Lee S, Lee CY, et al.: Adipose-derived stem cell-released osteoprotegerin protects cardiomyocytes from reactive oxygen species-induced cell death. Stem Cell Res Ther. 2017; 8(1): 4-9. PubMed Abstract | Publisher Full Text | Free Full Text

4. Bagger-Hosseiniabadi Z, Mesbah-Namin SA, Salehinejad P, et al.: Fibrin scaffold could promote survival of the human adipose-derived stem cells during differentiation into cardiomyocyte-like cells. Cell Tissue Res. 2018; 372(3): 571-589. PubMed Abstract | Publisher Full Text

5. Laxuvuori AT, Kell P, Wiiberg M, et al.: Characterization of human adipose tissue-derived stem cells with enhanced angiogenic and adipogenic properties. J Tissue Eng Regen Med. 2017; 11(9): 2490-2502. PubMed Abstract | Publisher Full Text

6. Green LJ, Zhou H, Padmanabhan V, et al.: Adipose-derived stem cells promote survival, growth, and maturation of early-stage murine follicles. Stem Cell Res Ther. 2019; 10(1): 1-13. PubMed Abstract | Publisher Full Text | Free Full Text

7. Yue Y, Zhang P, Liu D, et al.: Hypoxia preconditioning enhances the viability of ASCs to increase the survival rate of ischemic skin flaps in rats. Aesthetic Plast Surg. 2013; 37(1): 159-170. PubMed Abstract | Publisher Full Text

8. Wang JW, Qu YR, Fu Y, et al.: Transplantation with hypoxia-preconditioned mesenchymal stem cells suppresses brain injury caused by cardiac arrest-induced global cerebral ischemia in rats. J Neurosci. 2017; 95(10): 2059-2070. PubMed Abstract | Publisher Full Text

9. Wang XY, Liu CL, Li SD, et al.: Hypoxia precondition promotes adipose-derived mesenchymal stem cells based repair of diabetic erectile dysfunction via augmenting angiogenesis and neuroprotection. PLoS One. 2015; 10(3): 1-18. PubMed Abstract | Publisher Full Text | Free Full Text

10. Noordej M, Dekker FW, Zoccali C, et al.: Sample size calculations. Nephron - Clin Pract. 2011; 118(4): 319-323. PubMed Abstract | Publisher Full Text

11. Nouri F, Nematollahi-Mahani SN, Sharifi AM: Preconditioning of mesenchymal stem cells with non-toxic concentration of hydrogen peroxide against oxidative stress induced cell death: The role of hypoxia-inducible factor-1. Adv Pharm Bull. 2019; 9(1): 76-83. PubMed Abstract | Publisher Full Text | Free Full Text

12. Hu C, Li L: Preconditioning influences mesenchymal stem cell properties in vitro and in vivo. J Cell Mol Med. 2018; 22(3): 1428-1442. PubMed Abstract | Publisher Full Text | Free Full Text

13. Hou J, Wang L, Long H, et al.: Hypoxia preconditioning promotes cardiac stem cell survival and cardiogenic differentiation in vitro involving activation of the HIF-1α/apelin/AF axis. Stem Cell Res Ther. 2017; 8(1): 1-12. PubMed Abstract | Publisher Full Text | Free Full Text

14. Mas-Bargues C, Sanz-Ros J, Román-Domínguez A, et al.: Relevance of oxygen concentration in stem cell culture for regenerative medicine. Int J Mol Sci. 2019; 20(5). PubMed Abstract | Publisher Full Text | Free Full Text

15. Liu H, Xue W, Ge G, et al.: Hypoxic preconditioning advances CXCR4 and CXCR7 expression by activating HIF-1α in MSCs. Biochem Biophys Res Commun. 2010; 401(4): 509-515. PubMed Abstract | Publisher Full Text

16. Theus MH, Wei L, Cui L, et al.: In vitro hypoxic preconditioning of embryonic stem cells as a strategy of promoting cell survival and functional benefits after transplantation into the ischemic rat brain. Exp Neurol. 2008; 210(2): 656-670. PubMed Abstract | Publisher Full Text

17. Wei ZZ, Zhu YB, Zhang JY, et al.: Priming of the cells: Hypoxic preconditioning for stem cell therapy. Chin Med J (Engl). 2017;
24. Liang G, Li S, Du W, et al.: Vascular endothelial growth factor signaling in hypoxia and inflammation. J Neuroimmune Pharmacol. 2014; 9(2): 142-160.

25. Sun Z, Guo SS, Fassler R: Integrin-mediated mechanotransduction. 2016; 215(4).

26. Misra S, Hascall VC, Markwald RR, et al.: Interactions between hyaluronan and its receptors (CD44, RHAMM) regulate the activities of inflammation and cancer. Front Immunol. 2015; 6(MAY).

27. Sena IA, Chandel NS: Physiological roles of mitochondrial reactive oxygen species. Mol Cell. 2012; 48(2): 158-167.

28. Schieber M, Chandel NS: ROS function in redox signaling. Curr Biol. 2014; 24(10): 453-462.

29. Volpe CMO, Villar-Delfino PH, Dos Anjos PMF, et al.: Cellular death, reactive oxygen species (ROS) and diabetic complications review-Article. Cell Death Disc. 2018; 9(2).

30. Garrido C, Galluzzi L, Brunet M, et al.: Mechanisms of cytochrome c release from mitochondria. Cell Death Differ. 2006; 13(9): 1423-1433.

31. Darby JA, Hewitson TD: Hypoxia in tissue repair and fibrosis. Cell Tissue Res. 2016; 365(3): 553-562.

32. Jacob P, Hirt H, Bendahmane A: The heat-shock protein/chaperone network and multiple stress resistance. Plant Biotechnol J. 2017; 15(4): 405-414.

33. Shende P, Bhandarkar S, Prabhakar B: Heat Shock Proteins and their Protective Roles in Stem Cell Biology. Stem Cell Rev Reports. 2019; 15(5): 637-651.

34. Ryall JG, Gilad T, Dalton S, et al.: Metabolic Reprogramming of Stem Cell Epigenetics. Cell Stem Cell. 2015; 17(6): 651-662.

35. Wang Q, Li X, Wang Q, et al.: Heat shock pretreatment improves mesenchymal stem cell viability by heat shock proteins and autophagy to prevent cisplatin-induced granulosa cell apoptosis. Stem Cell Res Ther. 2019; 10(1): 1-12.

36. Hernandez I, Baio JM, Tsay E, et al.: Short-term hypoxia improves early cardiac progenitor cell function in vitro. Am J Stem Cells. 2018; 9(1): 1-17.

Reference Source

37. Suryawan IGR, Pikir BS, Rantam FA, et al.: Raw Data - Hypoxic Preconditioning Promotes Survivals of Human Adipocyte Mesenchymal Stem Cell via Expression of Prosurvival and Proangiogenic Biomarkers. F1000Research. 2024.
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Current Peer Review Status:  ✔  ✔  ✔

Version 4

Reviewer Report 08 August 2024

https://doi.org/10.5256/f1000research.169042.r303635

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Ming Pei  
Department of Orthopaedics, West Virginia University, Morgantown, WV, 26506-9196, USA

I approve the revision and response letter from the authors. I have not further comments to make. Thanks.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: adult stem cell and tissue regeneration

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 3

Reviewer Report 11 July 2024

https://doi.org/10.5256/f1000research.167902.r295867

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Ming Pei  
Department of Orthopaedics, West Virginia University, Morgantown, WV, 26506-9196, USA

The conclusion is not appropriate, which also reflects in the title. I suggest to delete “via expression of prosurvival and proangiogenic biomarkers” from the title. For the results, I did not find (1) the data (Table 3) (Figure 5) to support “VEGF influenced the expression of SCF” (page
8/23), (2) the data (Table 5) (Figure 7) to support “OCT-4 effect on BCL2 expression”, and (3) the data (Table 7) (Figure 9) to support “BCL2 expression inhibited apoptosis” and “HSP27 expression also inhibited apoptosis”.

Is the work clearly and accurately presented and does it cite the current literature?  
Partly

Is the study design appropriate and is the work technically sound?  
Partly

Are sufficient details of methods and analysis provided to allow replication by others?  
Partly

If applicable, is the statistical analysis and its interpretation appropriate?  
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?  
Partly

Are the conclusions drawn adequately supported by the results?  
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** adult stem cell and tissue regeneration

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 Jul 2024

**Ricardo Adrian Nugraha**

The conclusion is not appropriate, which also reflects in the title.

**Authors response:** thank you for your suggestion. We decided to change the conclusion statement according to the results with statement “From this study, it can be concluded that the hypoxic preconditioning of h-AMSC culture has proven to increase the expression of VEGF, SCF, OCT-4, and BCL2 and HSP27. This study demonstrated and explained the existence of a new mechanism of increased h-AMSC survival in cultures with hypoxic preconditioning (O₂ 1%) via VEGF, SCF, OCT-4, BCL2, and HSP 27.”

I suggest to delete “via expression of prosurvival and proangiogenic biomarkers” from the title.

**Authors response:** thank you for your suggestion. We have deleted “via expression of
prosurvival and proangiogenic biomarkers” from the title.

For the results, I did not find (1) the data (Table 3) (Figure 5) to support “VEGF influenced the expression of SCF” (page 8/23), (2) the data (Table 5) (Figure 7) to support “OCT-4 effect on BCL2 expression”, and (3) the data (Table 7) (Figure 9) to support “BCL2 expression inhibited apoptosis” and “HSP27 expression also inhibited apoptosis”.

Authors response: thank you for your very critical feedback. After intense discussion with all the authors, we decided to change the statement into “hypoxic preconditioning influenced the expression of SCF (based on Table 3), OCT4 (based on Table 4), BCL2 (based on Table 5), HSP27 expression (based on Table 6) and apoptosis (based on Table 7). We decided to move the Figure 10 (path analysis) in the last subsection of the results section.

Competing Interests: No competing interests were disclosed.

Reviewer Report 26 June 2024
https://doi.org/10.5256/f1000research.167902.r292484

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Suat Cheng Tan
Universiti Sains Malaysia, Kelantan, Malaysia

The authors had answered my comments.

Is the work clearly and accurately presented and does it cite the current literature? Partly

Is the study design appropriate and is the work technically sound? Partly

Are sufficient details of methods and analysis provided to allow replication by others? Partly

If applicable, is the statistical analysis and its interpretation appropriate? Partly

Are all the source data underlying the results available to ensure full reproducibility? Partly

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Stem cell biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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**Version 2**

Reviewer Report 12 June 2024

https://doi.org/10.5256/f1000research.137196.r288323

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Suat Cheng Tan

Universiti Sains Malaysia, Kelantan, Malaysia

This manuscript described the roles of hypoxic preconditioning in promoting survival of human adipose derived mesenchymal stem cell via expression of prosurvival and proangiogenic biomarkers. It is well written, data is sufficient and can be considered for approval if the authors can provide more detail for the methodologies and statistical analysis protocol.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes
**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Stem cell biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Author Response 13 Jun 2024**

Ricardo Adrian Nugraha

Thank you for your feedback and suggestion. We added several explanation regarding the methodologies, cell cultures subsection and data analysis subsection.

**Competing Interests:** None declared

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**Reviewer Report 08 February 2024**

https://doi.org/10.5256/f1000research.137196.r153685

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Gun-Jae Jeong

School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

I think this manuscript in now acceptable for indexing.

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Mesenchmal stem cells, tissue engineering

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 27 April 2022

https://doi.org/10.5256/f1000research.58917.r128761

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Gun-Jae Jeong
School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

The article entitled “Hypoxic preconditioning promotes survival of human adipocyte mesenchymal stem cell via expression of prosurvival and proangiogenic biomarkers” by I Gde Rurus Suryawan et al. (2021) reported enhanced survival of human adipocyte mesenchymal stem cell with hypoxic preconditioning. This study is well designed, and several experiments are performed well. However, this manuscript needs to be modified in certain minor aspects:

Minor comments:
1. Please revise the adipocyte mesenchymal stem cell as adipose derived mesenchymal stem cell or other appropriate word. The word adipocyte already contains the meaning of cell (-cyte). Therefore, the meanings are overlapped in a word.

2. Please provide more information of immunocytochemistry methods and antibody information in materials and methods section.

3. Please provide detailed quantification methods for each quantification. What is the measurement of each quantification experiment?

4. Overall figures: please include scale bars for each figure set. It looks like they have different magnifications.

5. Overall figures: please resize each subset figures as same size in one figure.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
No

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Mesenchmal stem cells, tissue engineering

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Author Response 27 Apr 2022**

**Ricardo Adrian Nugraha**

Dear Prof. Gun Jae Jeong

Thank you for your comment and suggestion. We are happy to get valuable comment regarding our work.

Author Response Letter to Reviewer Comments:

1. Please revise the adipocyte mesenchymal stem cell as adipose derived mesenchymal stem cell or other appropriate word. The word adipocyte already contains the meaning of cell (-cyte). Therefore, the meanings are overlapped in a word. -> We will revised as your suggestion.

2. Please provide more information of immunocytochemistry methods and antibody information in materials and methods section. -> We will provide more information regarding the immunocytochemistry methods and antibody information

3. Please provide detailed quantification methods for each quantification. What is the measurement of each quantification experiment? -> We will provide detailed quantification methods
4. Overall figures: please include scale bars for each figure set. It looks like they have different magnifications. -> Unfortunately, scale bars is difficult to achieve since we got it from the third party

5. Overall figures: please resize each subset figures as same size in one figure. -> We will resize our figure in the same size

**Competing Interests:** None declared

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Author Response 07 Aug 2022

**Ricardo Adrian Nugraha**

The article entitled “Hypoxic preconditioning promotes survival of human adipocyte mesenchymal stem cell via expression of prosurvival and proangiogenic biomarkers” by I Gde Rurus Suryawan et al. (2021) reported enhanced survival of human adipocyte mesenchymal stem cell with hypoxic preconditioning. This study is well designed, and several experiments are performed well. However, this manuscript needs to be modified in certain minor aspects:

Minor comments:

1. Please revise the adipocyte mesenchymal stem cell as adipose derived mesenchymal stem cell or other appropriate word. The word adipocyte already contains the meaning of cell (-cyte). Therefore, the meanings are overlapped in a word.

**Author Response:** Thank you for your suggestion, author have already replace adipocyte mesenchymal stem cell with adipose derived mesenchymal stem cell

1. Please provide more information of immunocytochemistry methods and antibody information in materials and methods section.

**Author Response:** Thank you for your suggestion. We have provided additional information regarding the immunocytochemistry methods and antibody information

1. Please provide detailed quantification methods for each quantification. What is the measurement of each quantification experiment?

**Author Response:** We measured mean fluorescence intensity (MFI) in a region of interest (ROI) with ImageJ software. We were simply measuring automatic cell counting based on the total fluorescent intensity across the entire image that lead to the potentially erroneous conclusion that the sample with the smaller area/fewer cells exhibits less staining for the probe of interest than one that is larger in area or has more cells, when the reality is that they actually exhibit similar staining levels per tissue area

1. Overall figures: please include scale bars for each figure set. It looks like they have different magnifications.

**Author Response:** Figure 3-10 had similar magnification. Unfortunately, figure 1 and 2...
had different magnification. We try to add scale bare but it couldn't work well.

1. Overall figures: please resize each subset figures as same size in one figure. 
Author Response: Figure 3-10 had been resize as same size in one figure. Unfortunately, we try to resize figure 1 and 2 but it couldn't be better.

**Competing Interests:** No conflict of interest regarding this publication

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