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Original Article

The Effect of Adipocyte-Derived Stem Cell Media on Stem Cell Markers in Neuroblastoma Cells

Adiposit Kaynaklı Kök Hücre Ortamının Nöroblastom Hücrelerindeki Kök Hücre Belirteçleri Üzerindeki Etkisi

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ABSTRACT

Objective: Different effects of stem cell media have been reported in different types of cancer. Neuroblastoma (NB) is the most common extracranial tumour of childhood. To be knowledgeable about pathophysiology of the disease is crucial for its effective treatment. The study aimed to determine the efficacy of human adipocyte-derived stem cell media (ADSC-M) on stem cell markers in NB cells.

Method: In the study, SH-SY5Y/N-MYC(-) and KELLY/N-MYC(+) cells were grown and adipocyte-derived stem cell conditioned medium was applied. Flow cytometry was used to identify changes in the expressions of stem cell markers in cells' own proliferative, and normal conditioned medium (NCM) and ADSC-M.

Results: While CD14, CD33, CD44, CD73 expressions disappeared in KELLY cells, CD34 levels in SH-SY5Y neuroblastoma cells decreased in ADSC-M rather than NCM. CD90 and CD106 expressions increased in KELLY and decreased in SH-SHY5Y when incubated in ADSC-M. Expression levels of HLA-ABC decreased twofold in SH-SY5Y when incubated in ADSC-M rather than NCM. Again, incubation of SH-SY5Y cells in ADSC-M decreased the expression of HLA-DR and CD45 down to undetectable levels compared to NCM.

Conclusion: The study found that ADSC-M negatively impacted mesenchymal and adipocyte-derived stem cell markers, especially in N-MYC- positive KELLY neuroblastoma cells, indicating poor prognosis.

Keywords: Neuroblastoma, adipocyte-derived stem cell media, stem cell markers, normal conditioned media

ÖZ

Amaç: Farklı kök hücre ortamlarının farklı kanserlerde çeşitli etkileri olduğu bildirilmiştir. Nöroblastom, çocukluk çağının en yaygın ekstrakraniyal tümörüdür. Hastalığın patofizyolojisinin anlaşılması, etkili tedavi için kritik öneme sahiptir. Bu çalışma, adiposit türevli kök hücre ortamının (ADSC-M) nöroblastom hücrelerindeki kök hücre belirteçleri üzerindeki etkinliğini belirlemeyi amaçlamıştır.

Yöntem: Çalışmada, SH-SY5Y/N-MYC(-) ve KELLY/N-MYC(+) hücreleri üretilmiş ve bu hücrelere ADSC-M uygulanmıştır. Hücrelerin kendi proliferatif ortamı (NCM) ve ADSC-M ile oluşan kök hücre belirteçlerindeki değişiklikler, akış sitometrisi kullanılarak değerlendirilmiştir.

Bulgular: ADSC-M uygulaması sonrasında KELLY hücrelerinde CD14, CD33, CD44, CD73 ekspresyonlarının kaybolduğu, SH-SY5Y nöroblastom hücrelerinde ise CD34 seviyelerinin NCM'ye kıyasla azaldığı görülmüştür. ADSC-M uygulandığında, CD90 ve CD106 ekspresyonları KELLY hücrelerinde artış, SH-SY5Y hücrelerinde ise azalış göstermiştir. HLA-ABC ekspresyon seviyesi, SH-SY5Y hücrelerinde ADSC-M uygulandığında NCM'ye kıyasla iki kat azalmıştır. Yine SH-SY5Y hücrelerinde, ADSC-M tedavisi, HLA-DR ve CD45 ekspresyonlarının NCM'ye kıyasla tespit edilemez hale gelmesine neden olmuştur.

Sonuç: Çalışma, adiposit türevli kök hücre ortamının, özellikle N-MYC pozitif KELLY nöroblastom hücrelerinde, mezenkimal ve adiposit türevli kök hücre belirteçlerini olumsuz etkilediğini ve bunun kötü prognozla ilişkili olabileceğini ortaya koymuştur.

Anahtar kelimeler: Nöroblastom, adiposit kök hücre ortamı, kök hücre belirteçleri, normal şartlandırılmış ortam

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INTRODUCTION

Neuroblastoma (NB) is the most common extracranial solid tumour in children, accounting for 8-10% of all pediatric cancers. It accounts for 15% of cancer deaths in children[1]. According to the National Pediatric Cancer Registry (Turkish Pediatric Oncology Group/ Turkish Pediatric Hematology Association Pediatric Cancer Registry) data, 1243 (7.9%) out of 15713 cases registered between 2009 and 2018, were diagnosed with sympathetic system tumours[1]. In modern protocols including induction chemotherapy, surgical resection, high-dose chemotherapy with autologous stem cell rescue, external beam radiotherapy and immunotherapy or treatment with different antitumoral agents, three-year survival rates currently exceed 60%, demonstrating achievement of better outcomes[2]. Cisplatin is the main chemotherapeutic agent used in the treatment of NB which greatly improves survival in pediatric patients with NB. However, it causes serious side effects such as nephrotoxicity, ototoxicity and peripheral neurotoxicity[3,4]. The biggest therapeutic challenge in the advanced disease stage of NB is the presence of minimal residual disease. Mesenchymal stem cells (MSCs) can be defined as non-hematopoietic multipotent stem cells capable of differentiating into mesodermal (adipocytes, osteocytes and chondrocytes), ectodermal (neurocytes) and endodermal lineages (hepatocytes). MSCs may be derived from many sources including adipose tissue, bone marrow, peripheral blood and neonatal tissues[5].

Human MSCs are defined by three main criteria: expression of CD73, CD90 and CD105 and lack of expression of hematopoietic markers such as CD11b, CD34, CD45, CD79, CD19 and human leukocyte antigen complex (HLA-DR), *in vitro* differentiation into osteoblasts, chondrocytes and adipocytes and plastic adhesion[6]. It is well established that the source of MSCs has a significant impact on their yield, the range of surface markers they express, and their cytokine profiles. The best source of adipose tissue-derived MSCs is adipose tissue, among other MSC sources[5].

Adipocyte-derived stem cells (ADSCs) are self-renewing and can differentiate along various mesenchymal tissue lineages, including adipocytes, osteoblasts, myocytes, chondrocytes, endothelial cells and cardiomyocytes[7,8]. ADSCs can differentiate into neuronal and vascular structures both *in vitro* and *in vivo* settings[9,10]. Therefore, ADSCs are considered promising agents for regenerating tissues and organs damaged by injury and disease. It has been currently revealed that the environment of stem cells has different effects in different diseases[11,12]. ADSC-derived media (ADSC-M) may be protective for neuronal tissue by causing a dose-dependent decrease in infarct volume both *in vitro* and *in vivo* cerebrovascular infarct models[11]. In another study, ADSC-M was found to increase proliferation, migration and invasion in melanoma and colorectal cancer cells[12]. However, direct injection of MSCs into the tumour in a subcutaneous animal NB tumor model has been shown to reduce tumor growth by inhibiting proliferation and causing apoptosis[13]. It was found that human ADSCs can transform into neuronal phenotype and can be positive for glial acidic fibrillary protein (GFAP), nestin and neuronal nuclei (NeuN)[10]. Olfactory ensheathing cells and B104 NB cells showed neuronal cell properties after treatment with ADSCs which expressed markers of both progenitor and mature neurons such as nestin, PGP 9.5 and MAP2[14]. In a recent study, the use of dedifferentiated adipose cells conditioned medium and PI3K inhibitors together has been shown to reduce proliferation and differentiation rates of NB cells[15]. Despite all these studies, the effect of ADSC environment on MSC markers in NB cells with different prognosis has not yet been investigated. ADSC-M alters the expression of stem cell markers in NB cells, with more pronounced changes in N-MYC-positive (KELLY) cells compared to N-MYC-negative (SH-SY5Y) cells, potentially indicatig a worse prognosis for N-MYC-positive NB. This hypothesis clearly states the expected effect of the ADSC-M on different NB cell lines and disease prognosis.

The aim of this study is to determine whether ADSC-M could alter the surface markers of these cells in the presence of N-MYC expression, a key prognostic factor for NB.

MATERIALS and METHODS

Cell Culture

Human SH-SY5Y (N-MYC-) (DSMZ) and KELLY (N-MYC+) (DSMZ) NB cell lines were grown in 5% CO_2 and 37 °C humid conditions. For human SH-SY5Y, KELLY cell lines, DMEM (Gibco), RPMI (Gibco), ADSC basal media (Gibco), 10% fetal bovine serum (FBS), 1% penicillin/streptomycin and 1% L-glutamine were added on the medium to be used for the evaluation of cell proliferation.

ADSC Conditioned Media Applications

ADSC basal medium (Gibco) prepared with stem cell quality FBS 10%, L-glutamine and pencillin/ streptomycin 1% was applied to KELLY and SH-SY5Y cells for 48 hours. The effects of cells on adipose-tissue

stem cell surface markers and whether they differed from normal conditioned medium (NCM) medium were evaluated.

Flow Cytometry Analysis of Stem Cell Markers

Stem cells were collected after 48 hours of incubation with adipose tissue-derived stem cell conditioned medium and also with their own specific medium. For this purpose, cells were removed from their flasks by trypsinization, counted and homogenized in phosphate buffered saline (PBS) solution to approximately $8x10^6$ cells.

The stem cells were then incubated for 45 minutes in the dark with fluorescein isothiocyanate- and phycoerythrin-conjugated monoclonal antibodies (BD™ Biosciences) (Table 1) specific for the identified cell surface markers and 10 µL of appropriate isotype controls. After incubation, PBS containing 0.1% sodium azide was added to the cell suspension, washed and resuspended. The prepared cell suspension was read at the relevant excitation and emission wavelengths in FACS Calibur flow cytometry device (BD). Flow cytometric analyses were performed using BD Cell Quest TM software program[16].

Statistical Analysis

Data were evaluated using the non-parametric Mann-Whitney U test. Mean values were evaluated by Student's t-test using SPSS 15.0 program. Stem cell surface protein expressions were evaluated as at least ≥2 fold increases/decreases compared to the control group. $p<0.05$ was considered statistically significant.

RESULTS

In this study, changes both in the number of both stem cell surface markers representing good and poor prognosis in NB cells and also in the number of stem cell surface markers in N-MYC expressing and non-expressing cells in the adipocyte-derived conditioned medium prepared from stem cells were revealed for the first time.

Flow Cytometry Results

CD29 MSC markers were expressed at rates of 48.20% with NCM and 57.30% with ADSC-M in KELLY cells. In SH-SY5Y cell line, the expression level of 78.40% with NCM decreased to 56% with ADSC-M (Figures 1 and 2).

CD44 MSC marker is involved in a wide range of cellular functions, including lymphocyte activation, circulation and targeting, hematopoiesis and tumor metastasis. In KELLY cells, they were expressed at a rate of 15.70% with NCM, whereas expression (0.90%) was abolished with ADSC-M. Again, in SH-SY5Y cell line, the expression level, which was 35.30% with NCM, decreased to 20.30% with ADSC-M (Figures 1 and 2).

CD73 lymphocyte differentiation marker is normally a positive marker in mesenchymal and adipocyte stem cells. In KELLY cells, it was expressed at 10.70% with NCM, whereas expression disappeared in ADS-CM medium (1.10%). In SH-SY5Y cell line, the expression level of NCM at 11.20% decreased to 8.50% in ADSC-M (Figures 1 and 2).

Table 1. Antibodies used in flow cytometry
CD10 (N-cadherin/common leukocyte lymphocytic leukemia antigen-CALLA; PE)
CD14 (monocyte differentiation antigen/LPS receptor; FITC)
CD29 (integrin b1 chain; PE)
CD33 (sialic acid-binding immunoglobulin-like lectin 3; SIGLEC3; a surface marker for very early bone marrow-derived hematopoietic stem cells; PE)
CD34 (hematopoietic progenitor cell antigen; PE)
CD44 (hyaluronate/lymphocyte homing-associated cell adhesionmolecule-HCAM; PE)
CD45 (protein tyrosine phosphatase, receptor type, C/PTPRC/leukocyte common antigen/cell marker of hematopoietic origin; FITC)
CD73 (5'-nucleotidase, ecto;NT5E/integrin b5; PE)
CD90 (Thy-1/Thy-1.1-FITC)
CD106 (vascular cell adhesion molecule, VCAM-1- FITC)
CD166 (activated leukocyte cell adhesion molecule; ALCAM/integrin a3;mesenchymal stem cell marker; PE)
HLA-DR (major histocompatibility complex, MHC class II, cell surface receptor; FITC)
HLA-ABC[Q3] (major histocompatibility class I antigen reseptor; PE)
Monoclonal antibodies and isotype controls used to identify cell surface markers. FITC: Fluorescein isothiocyanate, LPC: Lipopolysaccharid, PE: Phycoerythrin, MHC: Major histocompatibility complex

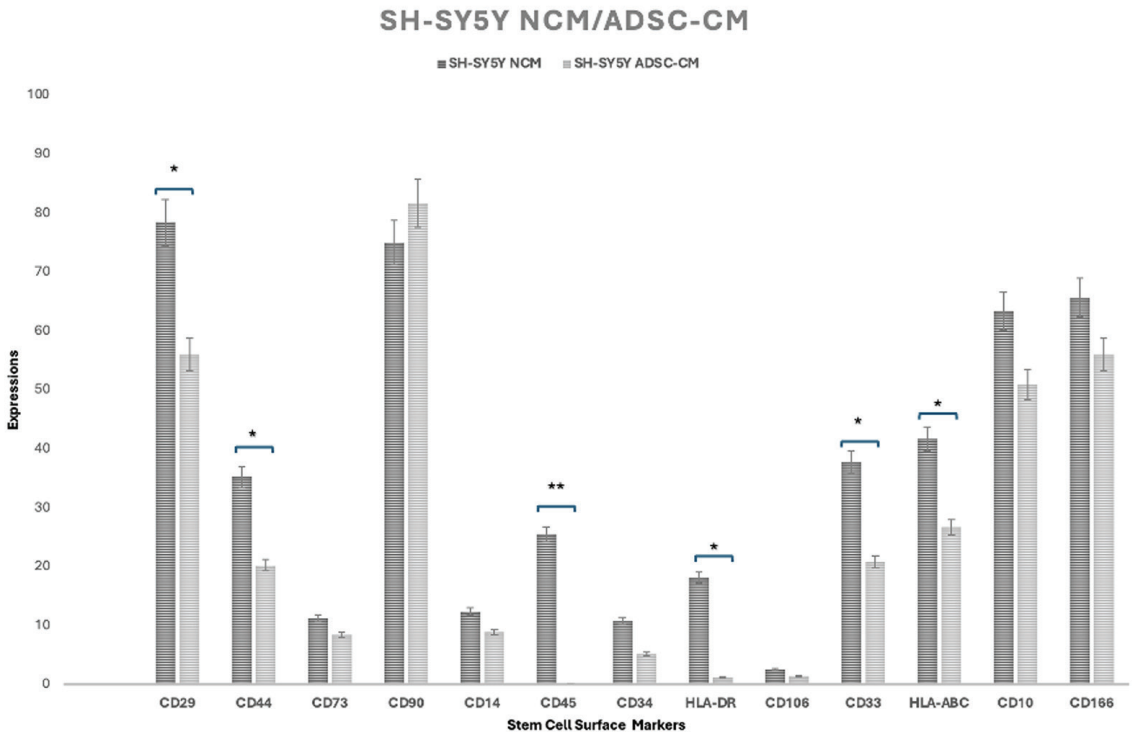

Figure 1. Evaluation of the expression levels of stem cell surface markers in SH-SY5Y cells compared to ADSC-M and NCM. Assessment of the expression levels of stem cell surface markers in SH-SY5Y cells following treatment with ADSC-M and NCM is shown

NCM: Normal conditioned medium, ADSC-M: Adipocyte-derived stem cell media

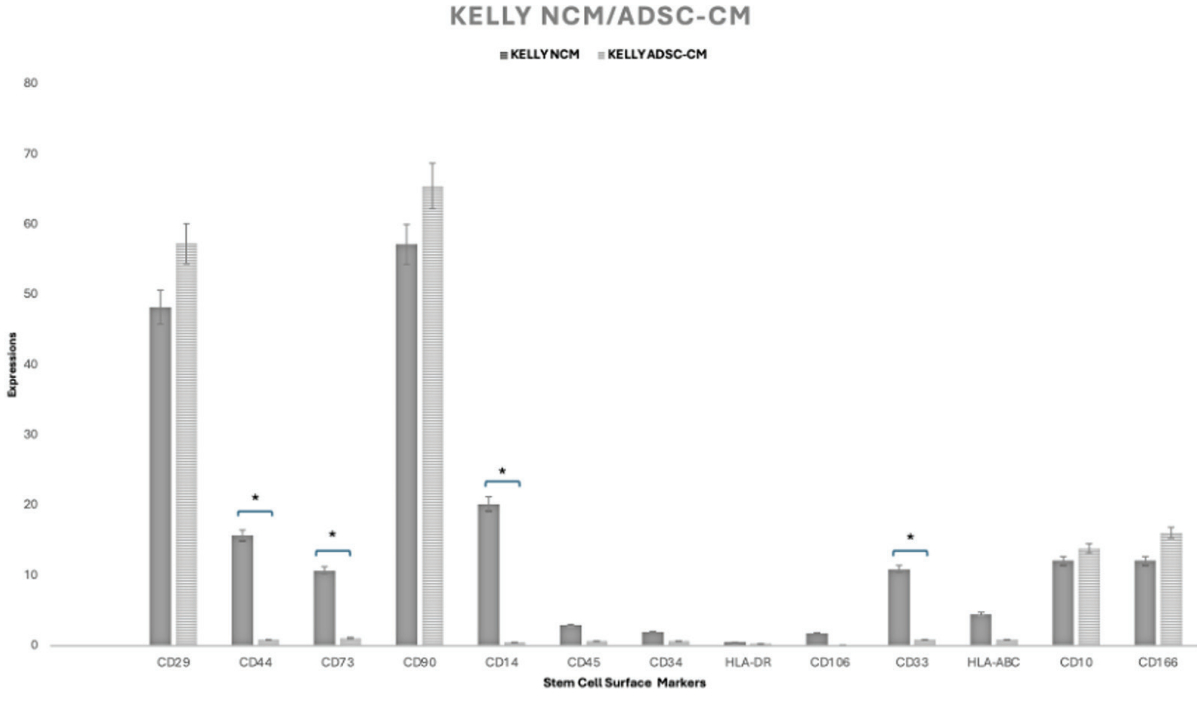

Figure 2. Evaluation of the expression levels of stem cell surface markers in KELLY cells compared to ADSC-M and NCM. Following treatment with ADSC-M and NCM, the expression levels of stem cell surface markers in KELLY cells are evaluated and shown

NCM: Normal conditioned medium, ADSC-M: Adipocyte-derived stem cell media

CD90 adipocyte-derived MSC marker showed an expression in the same direction in KELLY and SH-SY5Y cell lines. While the expression level was 57.20% with NCM in KELLY cells, the expression level increased to 65.50% in ADSC-M. In SH-SY5Y cell line, the expression level of NCM was 75.00%, while the expression level increased to 81.70% in ADSC-M (Figures 1 and 2).

CD14 monocyte differentiating marker is not normally expressed in MSCs and therefore shows negative expression in mesenchymal and adipose tissue. Accordingly, while NCM was expressed at 20.20% in KELLY cells, expression was lost with ADSC-M (0.50%). In SH-SY5Y cell line, the expression level of NCM at 12.40% decreased to 8.90% with ADSC-M (Figures 1 and 2).

CD45 leukocyte surface marker; not expressed in adipose cells. In KELLY cells, no expression was detected with NCM (2.90%) and 0.70% with ADSC-M. In SH-SY5Y cell line, the expression level of NCM was 25.50%, while the expression was abolished with ADSC-M (Figures 1 and 2).

CD34 hematopoietic stem cell marker is expressed on endothelial progenitor cells and endothelial cells. It is also an important adhesion molecule. No expression was detected in KELLY cells with NCM (2%) and ADSC-M (0.70%). In SH-SY5Y cell line, NCM expression level was 10.90% and decreased to 5.20% with ADSC-M (Figures 1 and 2).

HLA-DR is an MHC class II cell surface receptor, and one of the key cell surface molecules expressed on antigen presenting cells (monocytes, macrophages and dendritic cells). It is responsible for presentation of antigens to T cells and initiation of the inflammatory cascade during infection. While it is expressed in MSCs, but not in mesenchymal cells of adipocyte origin. In KELLY cells, there is no expression when incubated in either NCM (0.50%) or ADSC-M (0.30%). In SH-SY5Y cell line, NCM expression level was 18.20%, while its expression level in ADSC-M decreased (1.20%) (Figures 1 and 2).

CD106 MSC marker; also known as vascular cell adhesion molecule-1. It mediates adhesion of lymphocytes, monocytes, eosinophils and basophils to vascular endothelium. It was not expressed with NCM (1.80%; 2.60%) and ADSC-M (0.10%; 1.40%) in KELLY and SH-SY5Y cells (Figures 1 and 2).

CD33 myeloid leukemia (AML) marker is expressed on blast cells and represents a tumour-associated target antigen suitable for antibody-based therapies. Accordingly, in KELLY cells, it showed 10.90% expression with NCM, whereas expression was lost with ADSC-M (0.90%).

In SH-SY5Y cell line, the expression level was 37.80% with NCM and decreased to 20.80% with ADSC-M (Figures 1 and 2).

HLA-ABC, MHC I cell surface receptor: While NCM expression level was 4.50% in KELLY cells, no expression level (0.90%) was detected with ADSC-M application. In SH-SY5Y cell line, while its expression level was 41.70% with NCM, it decreased to 26.70% with ADSC-M (Figures 1 and 2).

CD10 acute lymphoblastic leukemia antigen is one of the endopeptidases which are widely expressed cell surface proteins. In KELLY cells, its expression levels with NCM and ADSC-M was 12.10% and 13.90%, respectively and did not change. In SH-SY5Y cell line. Its expression level decreased from 63.40% with NCM to 50.90% with ADSC-M (Figures 1 and 2).

CD166 leukocyte cell adhesion molecule is incorporated into neurite extensions by neurons through heterophilic and homophilic interactions. In KELLY cells, it was expressed at a rate of 12.10% with NCM, while its expression was slightly increased as 16.10% with ADSC-M. In SH-SY5Y cell line. Its expression level was 65.70% with NCM, but it decreased to 56% with ADSC-M (Figures 1 and 2). All numerical data of the flow cytometry results are shown in (Table 2).

According to these data, the expression levels of MSC markers CD14, CD33, CD34, CD44, CD45 and CD73 disappeared in KELLY NB cells with ADSC-M application, while expression levels of SH-SY5Y cells decreased almost by 50 percent. Expression level of CD90, an ADSC marker, showed an 10% increase with ADSC-M, although not significant in both cells. CD45 and HLA-DR, which were not expressed in adipocytes and mesenchymal-derived cells, were found to be expressed only in SH-SY5Y with NCM application, whereas they were eliminated with ADSCM-M. Again, expression levels of HLA-ABC decreased 2-fold in SH-SY5Y cells with ADSC-M, whereas it was eliminated in KELLY. CD106 did not show expression and change in both NB cells with both media. While CD10 and CD166 expression did not show a significant change in KELLY cells, their expression decreased in SH-SY5Y cells with ADSC-M compared to NCM. CD29 expression was slightly increased by ADSC-M in KELLY, but decreased in SH-SY5Y.

Table 2. Stem cell surface marker flow analyses of KELLY and SH-SY5Y cell lines

SH-SY5Y NCM													
Marker	**CD44**	**CD34**	**CD14**	**CD73**	**CD106**	**CD33**	**HLA-DR**	**HLA-ABC**	**CD90**	**CD10**	**CD45**	**CD29**	**CD166**
Percent	35.30%	10.90%	12.40%	11.20%	2.60%	37.80%	18.20%	41.70%	75.00%	63.40%	25.50%	78.40%	65.70%
SH-SY5Y ADSC-M													
Marker	**CD44**	**CD34**	**CD14**	**CD73**	**CD106**	**CD33**	**HLA-DR**	**HLA-ABC**	**CD90**	**CD10**	**CD45**	**CD29**	**CD166**
Percent	20.30%	5.20%	8.90%	8.50%	1.40%	20.80%	1.20%	26.70%	81.70%	50.90%	0.20%	56.00%	56.00%
KELLY NCM													
Marker	**CD44**	**CD34**	**CD14**	**CD73**	**CD106**	**CD33**	**HLA-DR**	**HLA-ABC**	**CD90**	**CD10**	**CD45**	**CD29**	**CD166**
Percent	15.70%	2.00%	20.20%	10.70%	1.80%	10.90%	0.50%	4.50%	57.20%	12.10%	2.90%	48.20%	12.10%
KELLY ADSC-M													
Marker	**CD44**	**CD34**	**CD14**	**CD73**	**CD106**	**CD33**	**HLA-DR**	**HLA-ABC**	**CD90**	**CD10**	**CD45**	**CD29**	**CD166**
Percent	0.90%	0.70%	0.50%	1.10%	0.10%	0.90%	0.30%	0.90%	65.50%	13.90%	0.70%	57.30%	16.10%

NCM: Normal conditioned medium, ADSC-M: Adipocyte-derived stem cell media

DISCUSSION

Adult adipose tissue is now recognised as a useful source of adult MSCs[17,18]. ADSCs can differentiate into neuronal and vascular structures both *in vitro* and *in vivo* settings[9,10].

It has been shown that transplantation of ADSCs into the brain could improve neurological problems in rats with ischemia[19]. Direct intratumoral injection of MSCs in NB animal tumor model decelerates tumour development by driving tumour cells to apoptosis[13]. Conventional cell growth media such as Dulbecco's Modified Eagle's Medium and RPMI, which are used for the *in vitro* cultivation of NB cells in a culture media provide the necessary ingredients for the continuous proliferation of these cancer cells. These media usually lack metabolites normally found in human fluids. However, substances such as glucose, glutamine or pyruvate are usually contained in higher concentrations in these media[20]. A liquid solution called "stem cell-derived conditioned medium" (CM) is made up of several bioactive substances that are released by stem cells. These substances, which are thought to be involved in tissue regeneration and repair, can include growth factors, cytokines, and extracellular matrix constituents[21,22]. It is possible to obtain the stem cells needed to create the conditioned media from adipose tissue, bone marrow, or umbilical cord blood, among other sources.

Since, they are cultivated in a controlled setting, these stem cells are able to release advantageous chemicals into the medium[22]. ADSC-M is a stem cell culture multiplication medium derived from human adipose tissue and is rich in growth factors such as epithelial growth factor, fibroblast growth factor, transforming growth factor-β1 and bioactive substances such as various cytokines, enzymes and extracellular matrix structural protein. Thus, these factors help to activate the cells by stimulating aging, damaged and inactive cells. A study has shown that ADSCs can secrete various biologically active molecules that affect the microenvironment through a specific paracrine mechanism[23]. To utilize the paracrine effects of ASCs without utilizing the cells themselves, conditioned media is frequently employed in wound healing, regenerative medicine, and other research fields.

The processes of anti-inflammation, tissue healing, angiogenesis, and immunomodulation are thought to be enhanced by the substances produced in the conditioned media[24]. The major goals of ASC's own medium also known as basal medium, are to maintain, develop, and multiply the stem cells produced from adipocytes in culture. It provides the cells with vital nutrition and environmental circumstances they need to survive and operate[25]. Bioactive substances, including growth factors and cytokines, are present in ADScs, whereas basal media comprise vital minerals, vitamins, salts, serum, and supplements. Although ASC's own medium is designed to sustain the cells and guarantee their appropriate development in culture, the conditioned medium is employed to capitalize on the chemicals the cells release, which may exert regenerative or therapeutic benefits[26]. In particular, it has been reported that the conditioned media from ADSC culture can be used as an antioxidant agent for skin to reduce the rate of collagen degradation and repair wounds in animal models[27].

Given its potential therapeutic implications, research on ADSC-M is essential when examining NB. ADSC-M has been shown to affect the behavior of NB cells by stimulating differentiation and preventing proliferation of these cells, which may result in the development of new therapeutic approaches. It has been demonstrated that ADSC-M, especially derived from dedifferentiated fat cells, induces neurite elongation and promotes the production of tubulin and neurofilament in NB cells, indicating its differentiation impact[15]. It has been also observed that the release of certain microRNAs, like miR-124, via exosomes produced from ADSCs might induce differentiation and depress the proliferation of NB cells[28]. Through controlling important biochemical pathways, such as the activity of GABBR1, ADSC-derived extracellular vesicles have been shown to suppress the proliferation of NB cells[29].

Phosphatidylinositol 3-kinase inhibitors in conjunction with ADSC-M greatly improve the inhibition of NB cell viability[15]. On the other hand, although ADSC-M exhibits promise, further research is necessary to completely comprehend its therapeutic efficiency in NB due to the intricacy of tumor microenvironments and possible diversity in responses. Tumor heterogeneity and resistance to therapy are greatly impacted by differences between NB cell lines in terms of stem cell markers. A poor prognosis and aggressive tumor behavior have been associated with linked to varied expression levels of stemness markers in NB[30]. Stem cell markers such as CD133 and CD15 exhibit variable expression levels in different NB cell lines. This variability affects the cancer stem cell (CSC)-like phenotype and identifies possible targets for the treatment of NB[31]. In another study, it was reported that stem cell markers such as CD133, KIT, HUMNF1ISO, GPRC5C and NOTCH1 were expressed at higher levels in malignant stem cells when compared to neuroblastic and substrate-adherent cells and potentially affected NB progression[32]. In a study by Vangipuram et al.[33], the authors demonstrated that in different NB cell lines, varying percentages of CD133+ stem-like cells affect resistance to chemotherapeutic agents. They reported that targeting CD133-expressing cells can improve the efficacy of NB treatment. In a research, they showed that NB cell lines expressed different versions of stem cell markers such CD133, NESTIN, and MSI1, suggesting the existence of a subpopulation that resembles CSCs that may have consequences in the clarification of tumor behavior[34]. In a flow cytometric study performed with MSCs, it has been demonstrated that ADSCs expressed CD90 [glycosylphosphatidylinositol-bound glycoprotein) and CD29 (integrin b1 chain), but not hematopoietic surface markers CD11b and CD45[35]. In this study, while CD90 expression was high in SH-SY5Y and KELLY NB cells, especially in cells treated with ADSC-M, CD45 was not expressed, which correlated with the literature data. In a study in gliomas, ADSC-M treatment led to a significant increase in migration in the wound model compared to the control (DMEM SF)[36]. CD44 is an adhesion protein that plays a role in tumor progression, metastasis and stemness in different cancers. In a study, high CD44 expression was shown to be associated with reduced survival in high-grade human NB, independent of MYCN amplification. It has also been reported that CD44 positive cells may lead to the development of more tumourigenic, metastatic and aggressive neuroblastic tumours with a high frequency after transplantation[37]. In this study, while CD44 expression was abolished by ADSC-M application in KELLY cell line, which can be considered as an indication of poor prognosis, it led to a decrease in its expression in SH-SY5Y cells, suggesting that ADSC-M application should be evaluated in terms of prolongation of survival in N-MYC-expressing NBs with poor prognosis. Stigliani et al. reported that high CD14 expression in primary tumors of high-risk NB patients was predictive of better survival.

They suggested that increased CD14 expression may affect the immune status of the tumor and the natural history of this pediatric cancer[38]. In another study, expression levels of genes representing tumour-associated macrophage infiltrates (CD33, CD16, etc.) were found to be significantly higher in metastatic tumours of young patients (<18 months) compared to tumors of patients aged ≥18 months. This suggests that the inflammatory response and tumor microenvironment may have important effects on the natural history and outcome of NB in certain patient groups[39]. In the present study, the disappearance of CD14 and CD33 expression in KELLY cells with ADSC-M treatment, while it remained at a similar level in SH-SY5Y cells, was consistent with the results of a study performed by Stiglani et al. in patients with NB. These changes in expression levels of CD14 and CD33 may be especially important in terms of their relationship with poor prognosis in NB. Again, the change in CD14 expression indicates that the potential effect of ADSC-M administration should be evaluated in terms of modulation of the immune microenvironment and contribution to prognosis in terms of NB cells with and without N-MYC expression. In a different study, the association of CD34 surface expression (in 92 patients) with NB stage/clinical outcomes was investigated and it

was shown that CD34 positivity in NB may be associated with advanced disease stage and poor prognosis in high-risk patients with N-MYC amplification[40]. In the present study, ADSC-M application eliminated CD34 expression in N-MYC positive cells and decreased it by half in N-MYC negative SH-SY5Y cells, indicating that ADSC-M application should be considered especially in terms of contribution to survival in poor prognostic NB. In a study by Jain et al. in a cohort of 87 NB patients, loss of protein expression of CD73 was associated with poor overall survival and relapse-free survival in high-risk, MYCN-amplified and high-risk non-MYCN-amplified subgroups. Furthermore, overexpression or silencing of CD73 was found to regulate classical cadherins (E-cadherin, N-cadherin, vimentin) during epithelial-mesenchymal transition (EMT), stemness maintenance (Sox2, Nanog, Oct3/4), self-renewal capacity (Notch) and inhibition of differentiation by leukemia inhibitory factor proteins. It has been suggested that loss of CD73 in NB may contribute to the maintenance of activated EMT and stemness and subsequently promote disease progression[41]. In this study, while CD73 expression was abolished in N-MYC positive KELLY cells with ADSC-M application, it decreased by half in SH-SY5Y cells in the absence of N-MYC, indicating that the mechanism of ADSC-M in NB in terms of contribution to the reduction in disease progression should be evaluated. AADSC-M is a good alternative source for stem cells compared to other media. ADSCs can firstly transform into neurospheres, and then into stem cell-like structures[42]. It was determined that these stem cell-like structures can effectively induce the differentiation of SH-SY5Y human NB cells and can also form myelin structure with neuronal neurites[42]. In another study, it was found that MSCs express brain natriuretic factor and β-neuronal growth factor specific to their subpopulations[43]. It was determined that BDNF expression levels of SH-SY5Y NB cell line and co-culture systems formed with MSCs supported development of neuritis by increasing NB cell viability[43]. It was found that human ADSCs can transform into neuronal phenotype and can be positive for GFAP, NeuN[44]. Recently, it was found that olfactory ensheathing cells and B104 NB cells showed neuronal cell, and both progenitor and mature neuronal properties such as nestin, PGP 9.5 and MAP2 after treatment with ADSCs[14].

ADSC environment around NB cells may stimulate the transformation of NB cells into neuronal mature cells by creating a special microenvironment and regulating the immune microenvironment[15,45].

Many signalling pathways are involved in especially embryonic development of NB. Among these, especially the Hippo signalling pathway plays a role in stem cells, CSCs and tumourigenesis[46]. It is suggested that ADSC-M may activate the Hippo pathway at certain stages, leading to inhibition of cell proliferation and promotion of apoptosis; however, the exact mechanisms underlying the interaction between ADSC-M and the Hippo pathway are still under investigation and may vary depending on the specific experimental conditions and cell types involved[47]. We believe that stem cells and associated media have the potential to be used in the treatment of some types of cancer, especially NB. The goal of conventional treatment strategies has been to lower the total tumor load. These techniques, however, frequently ignore the CSCs, a vital group of cells that are in charge of high recurrence rates and drug resistance. Further studies are needed to elucidate this issue.

Research on precision medicine that selectively targets CSCs has been sparked by the shortcomings of conventional therapy. Its scope covers the recognition and targeting of certain surface markers, signaling pathways, and the particular microenvironments in NB that sustain the development of CSCs[48]. High-risk NB patients who undergo stem cell transplantation now have better survival rates thanks to a variety of factors, including more effective treatment modalities, enhanced stem cell sources, tailored approaches, reduced toxicity, integration with targeted therapies, and better long-term care[48]. CSCs which are resistant to treatment with conventional medications, are important targets for novel therapeutic approaches[49]. In the field of regenerative medicine, there is growing awareness regarding capability of stem cell media to act as therapeutic agent solvents. According to the results of a research study by Ackermann et al.[1] on stem cell materials, their compositions, and methods of use a wide range of bioactive components found in stem cell-CM can improve the effectiveness of treatment According to our research, stem cells and similar bioactive material may one day be used for the treatment of various cancer types, particularly NB. Stem cell media may have the potential to be used as a solvent of therapeutic agents so that blastic cells can differentiate into mature cells and contribute to the recovery of the disease.

CONCLUSION

In this study, it has been shown for the first time that exposure to adipocyte-derived cell media leads to changes in stem cell markers that may results in the

improvement in prognosis and prolongation of survival, especially in N-MYC-expressing NB cells. In the light of the results of the study, it is necessary to evaluate more comprehensively whether ADSC environment can be a treatment option in terms of modulation of the immune microenvironment by revealing its favorable effects on molecular and functional changes.

Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Footnotes

Author Contributions

Surgical and Medical Practices: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Concept: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Design: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Data Collection or Processing: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Analysis or Interpretation: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Literature Search: S.K.Ö., Z.A., E.S., S.A., P.E., N.O., Writing: S.K.Ö., Z.A., E.S., S.A., P.E., N.O.

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REFERENCES

1. Ackermann T, Tardito S. Cell culture medium formulation and its implications in cancer metabolism. Trends Cancer. 2019;5(6):329-32. doi: 10.1016/j.trecan.2019.05.004
2. Anghileri E, Borsini F, Dehni G, Zennaro E, Rocci A, Bagnati R. Neuronal differentiation potential of human adipose-derived mesenchymal stem cells. Stem Cells Dev. 2008;17(5):909-16. doi: 10.1089/scd.2007.0197
3. Aravindan N, Salin M, Muthusamy V, Kantekure N, Abdulrahman A, et al. Significance of hematopoietic surface antigen CD34 in neuroblastoma prognosis and the genetic landscape of CD34-expressing neuroblastoma CSCs. Cell Biol Toxicol. 2021;37(3):461-478. doi: 10.1007/s10565-020-09557-x
4. Asgharzadeh S, Salo JA, Matthay KK, DeClerck YA, Gallager D, Muench M, et al. Clinical significance of tumor-associated inflammatory cells in metastatic neuroblastoma. J Clin Oncol. 2012;30(28):3525-32. doi: 10.1200/JCO.2011.40.9169
5. Bianchi G, Tasso R, Tedesco M, Renzulli L, Santi S, Bosticardo M, et al. Close interactions between mesenchymal stem cells and neuroblastoma cell lines lead to tumor growth inhibition. PLoS ONE. 2012;7(10):e48654. doi: 10.1371/journal.pone.0048654
6. Castresana J, Villanueva T, Bueren JA. Analysis of stemness gene expression and CD133 abnormal methylation in neuroblastoma cell lines. Oncol Rep. 2010;24(5):1355-62. doi: 10.3892/OR_00000993
7. Chien WY, Wang Y, Yao H, Chen Y, Lin Y, Hsu W, et al. Stem cell-derived conditioned medium for alopecia: a systematic review and meta-analysis. J Plast Reconstr Aesthetic Surg. 2023;88:182-92. doi: 10.1016/J.BJPS.2023.10.060
8. Chu Y, Zhang L, Zhang D, Zhou Y, Huang X, Luo Y, et al. Adipose-derived mesenchymal stem cells induced PAX8 promotes ovarian cancer cell growth by stabilizing TAZ protein. J Cell Mol Med. 2021;25(9):4434-43. doi: 10.1111/jcmm.16511
9. Chung C, Bansal N, Li X, Kim J, Krishnan A. Neuroblastoma. Pediatr Blood Cancer. 2021;68(S2). doi: 10.1002/pbc.28473
10. Crigler L, Elango R, Wren J, Grant J, Ge S, Woods D, et al. Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritogenesis. Exp Neurol. 2006;198(1):54-4. doi: 10.1016/j.expneurol.2005.10.029
11. Egashira Y, Imai R, Aoki S, Matsumoto Y, Matsumoto N, Murakami M, et al. The conditioned medium of murine and human adipose-derived stem cells exerts neuroprotective effects against experimental stroke model. Brain Res. 2012;1461:87-95. doi: 10.1016/j.brainres.2012.04.033
12. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circ Res. 2007;100(9):1249-60. doi: 10.1161/01.res.0000265074.83288.09
13. Gunes D, Arslan E, Kucuk HF, Dalkiran A. Evaluation of the effect of acetyl L-carnitine on experimental cisplatin ototoxicity and neurotoxicity. Chemotherapy. 2011;57(3):186-94. doi: 10.1159/000323621
14. Guo M, Wang H, Zhao Z, Wang M, Sun X, Zhang Y, et al. Adipose-derived stem cell-derived extracellular vesicles inhibit neuroblastoma growth by regulating GABBR1 activity through LINC00622-mediated transcription factor AR. J Leukoc Biol. 2021;111(1):19-32. doi: 10.1002/jlb.1mia0321-164r
15. Gutiérrez-Fernández M, Rodríguez-Frutos B, Cerezo-Guisado MI, Ortega-Gutiérrez S, Morales J, García-Yébenes I, et al. Effects of intravenous administration of allogenic bone marrow- and adipose tissue-derived mesenchymal stem cells on functional recovery and brain repair markers in experimental ischemic stroke. Stem Cell Res Ther. 2013;4(1):159. doi: 10.1186/scrt159
16. Hidaka A, Tanaka Y, Yoshimura M, Nakanishi M, Kinoshita M, Yoshida M, et al. Effects of dedifferentiated fat cells on neurogenic differentiation and cell proliferation in neuroblastoma cells. Pediatr Surg Int. 2022;39(1). doi: 10.1007/s00383-022-05304-x
17. Iser IC, Carpes MF, Kümpel M, Oliveira LA, Azevedo RP, Leal RB. Conditioned medium from adipose-derived stem cells (ADSCs) promotes epithelial-to-mesenchymal-like transition (EMT-Like) in glioma cells *in vitro*. Mol Neurobiol. 2016;53(10):7184-99. doi: 10.1007/s12035-015-9585-4
18. Jain D, Pandey S, Sharma A, Mishra R, Verma P. Prognostic significance of NT5E/CD73 in neuroblastoma and its function in CSC stemness maintenance. Cell Biol Toxicol. 2023;39(3):967-89. doi: 10.1007/s10565-021-09658-1
19. Kang SK, Shin JH, Yim SV, Cho JH, Kim H, Lee JY, et al. Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats. Exp Neurol. 2003;183(2):355-66. doi: 10.1016/s0014-4886(03)00089-x

20. Karaöz E, Erbaş G, Karanlık H, Yılmaz A. Isolation and *in vitro* characterization of dental pulp stem cells from natal teeth. Histochem Cell Biol. 2010;133(1):95-112. doi: 10.1007/s00418-009-0646-5

21. Ko JH, Lee S, Lee D, Lee WJ, Ko YS, Lee HJ, et al. Conditioned media from adipocytes promote proliferation, migration, and invasion in melanoma and colorectal cancer cells. J Cell Physiol. 2019;234(10):18249-61. doi: 10.1002/jcp.28456

22. Kum Özşengezer S, Altun Z, Olgun N. Neuroblastoma and Hippo signaling pathway. J Dr Behcet Uz Childrens Hosp. 2021;11(1):1-8. doi: 10.5222/buchd.2021.58826

23. Li L, Liu H, Wang W, Zhang W, Yang X. Conditioned medium from human adipose-derived mesenchymal stem cell culture prevents UVB-induced skin aging in human keratinocytes and dermal fibroblasts. Int J Mol Sci. 2020;21(1):49. doi: 10.3390/ijms21010049

24. Lo Furno D, Bella V, Sala A, Messina S, Bianchi G, Banfi G, et al. Differentiation of human adipose stem cells into neural phenotype by neuroblastoma- or olfactory ensheathing cells-conditioned medium. J Cell Physiol. 2013;2109-18. doi: 10.1002/jcp.24386

25. Walton JD, Moy MP, Spengler BA, Biedler JL, Gerald WL, Cheung N-KV, Ross RA. Microarray analyses reveals "what's up" in human neuroblastoma malignant stem cells. Cancer Res. 2024;66:1018-30. Available at: https://aacrjournals.org/cancerres/article/66/8_supplement/1018/530749

26. Mishra VK, Jain A, Shahid M, Kumar R, Singh P, Verma A, et al. Identifying the therapeutic significance of mesenchymal stem cells. Cells. 2020;9(5):1145. doi: 10.3390/cells9051145

27. Mohanvelu S, Hassan U, Subramanian K, Singh P, Sharma S, Joshi S, et al. Stem cell therapy for high-risk neuroblastoma: stem cell transplantation and targeting cancer stem cells. Reference Module in Biomedical Sciences. 2024. doi: 10.1016/b978-0-443-15717-2.00072-x

28. Ngan ESW. Heterogeneity of neuroblastoma. Oncoscience. 2020;7(9):223-34. doi: 10.18632/oncoscience.582

29. Olgun N, Ozdemir S, Erbayraktar Z, Aydin F, Demirkol D, Yılmaz T, et al. Mesenchymal stem cells in neuroblastoma. Mol Cell Pediatr. 2019;6(1):2. doi: 10.1186/s40348-019-0135-7

30. Ordóñez R, López-Pérez R, Ramos-Morales F, Pérez-Vázquez I, Sanchis-Gomar F, et al. Genome-wide microarray expression and genomic alterations by array-CGH analysis in neuroblastoma stem-like cells. PLoS One. 2014;9(11):113105. doi: 10.1371/journal.pone.0113105

31. Peng L, Jia Z, Yin X, Zhang X, Liu Y, Chen P, et al. Comparative analysis of mesenchymal stem cells from bone marrow, cartilage, and adipose tissue. Stem Cells Dev. 2008;17(4):761-73. doi: 10.1089/scd.2007.0217

32. Persson CU, Svensson P, Hagerling C, Lindh M, Stal O, Mörén T, et al. Neuroblastoma patient-derived xenograft cells cultured in stem-cell promoting medium retain tumorigenic and metastatic capacities but differentiate in serum. Sci Rep. 2017;7(1). doi: 10.1038/s41598-017-09662-8

33. Planat-Benard V, Menard C, Larghero J, Silvestre JS, Audat F, Peyrafitte M, et al. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation. 2004;109(5):656-63. doi: 10.1161/01.cır.0000114522.38265.61

34. Safford KM, Bartosh TJ, Ylostalo JH, Prockop DJ, Lewis M. Characterization of neuronal/glial differentiation of murine adipose-derived adult stromal cells. Exp Neurol. 2004;187(2):319-28. doi: 10.1016/j.expneurol.2004.01.027

35. Shariati Najafabadi S, Najafabadi AM, Imani H, Nematollahi-Mahani SN, Shahrzad S. Human adipose derived stem cell exosomes enhance the neural differentiation of PC12 cells. Mol Biol Rep. 2021;48(6):5033-43. doi: 10.1007/s11033-021-06497-5

36. Sharif S, Ghahremani MH, Soleimani M, Soleimani M. Differentiation induction and proliferation inhibition by a cell-free approach for delivery of exogenous miRNAs to neuroblastoma cells using mesenchymal stem cells. Cell. 2021;22(4):556-64. doi: 10.22074/cellj.2021.6928

37. Smolinská V, Boháč M, Danišovič Ľ, Škultétyová I. Current status of the applications of conditioned media derived from mesenchymal stem cells for regenerative medicine. Physiol Res. 2023;72(3):233-45. doi: 10.33549/physiolres.935186

38. Ghosh S, Singh S, Awasthi M, Choudhury A, Kumar M. Cancer stem cells. Int J Trends OncoSci. 2023;1(4):1-12. doi: 10.22376/ıjtos.2023.1.4.1-12

39. Andreevich SA, Ivanovna KA, Igorevich DA, Andreevich SA, Vladimirovich AN. Stem cell material, compositions, and methods of use. 2019. Available at: https://typeset.io/papers/stem-cell-material-compositions-and-methods-of-use-3rjefi82si

40. Stigliani S, Stabile H, Savoia A, Pignataro L, Caravita T, Vannini M. Expression of FOXP3, CD14, and ARG1 in neuroblastoma tumor tissue from high-risk patients predicts event-free and overall survival. Biomed Res Int. 2015. doi: 10.1155/2015/347867

41. Tholpady SS, Katz AJ, Ogle RC. Mesenchymal stem cells from rat visceral fat exhibit multipotential differentiation *in vitro*. Anat Rec A Discov Mol Cell Evol Biol. 2003;272(1):398-402. doi: 10.1002/ar.a.10039

42. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol. 2008:726-36. doi: 10.1038/nri2395

43. Vangipuram SD, Wang ZJ, Lyman WD. Resistance of stem-like cells from neuroblastoma cell lines to commonly used chemotherapeutic agents. Pediatr Blood Cancer. 2010;54(3):361-8. doi: 10.1002/PBC.22351

44. Vega FM, Colmenero-Repiso A, Gómez-Muñoz MA, Rodríguez-Prieto I, Aguilar-Morante D, Ramírez G, et al. CD44-high neural crest stem-like cells are associated with tumour aggressiveness and poor survival in neuroblastoma tumours. EBioMedicine. 2019;49:82-95. doi: 10.1016/j.ebiom.2019.10.041

45. Wei M, Yuan X. Cisplatin-induced ototoxicity in children with solid tumor. J Pediatr Hematol Oncol. 2018. Available at: www.jpho-online.com

46. Xu Y, Liu L, Li Y, Zhou C, Xiong F, Liu Z, et al. Myelin-forming ability of Schwann cell-like cells induced from rat adipose-derived stem cells *in vitro*. Brain Res. 2008;1239:49-55. doi: 10.1016/j.brainres.2008.08.088

47. Yano F, Takeda T, Kurokawa T, Tsubaki T, Chijimatsu R, Inoue K, et al. Effects of conditioned medium obtained from human adipose-derived stem cells on skin inflammation. Regener Ther. 2022;20:72. doi: 10.1016/j.reth.2022.03.009

48. Zhang B, Wu Y, Mori M, Yoshimura K. Adipose-derived stem cell conditioned medium and wound healing: a systematic review. Tissue Eng Part B Rev. 2022;28(4):830-47. doi: 10.1089/ten.teb.2021.0100

49. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7(2):211-228. doi: 10.1089/107632701300062859

50. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2002;13(12):4279-95. doi: 10.1091/mbc.e02-02-0105