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Research Article

Development of a Method of Isolating Exosomes from Cell Cultivations

Desyatova M[1], Antonova S[2], Ufimtseva M[3], Korotkov A[4] and Makeyev O[5]*

[1]Assistant, Department of Medical Biology and Genetics, Ural State Medical University, Yekaterinburg, 620028, Russia

[2]Associate Professor, Department of Dermatology and Life Safety Genetics, Ural State Medical University, Yekaterinburg, 620028, Russia

[3]Chief Physician, Sverdlovsk Regional Skin and Venereological Dispensary, Professor, Department of Dermatology and Life Safety, Ural State Medical University, Yekaterinburg, 620028, Russia

[4]Associate Professor, Department of Medical Biology and Genetics, Ural State, Medical University, Yekaterinburg, 620028, Russia

[5]Professor, Department of Medical Biology and Genetics, Ural State Medical University, Yekaterinburg, 620028, Russia

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***Corresponding author:** Makeyev O, Professor, Department of Medical Biology and Genetics, Ural State Medical University, Yekaterinburg, 620028, Russia,
E-mail: larim@mail.ru

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Abstract

The results of epidemiological studies conducted in divergent countries demonstrate the high prevalence of Atopic Dermatitis (AD) and the dynamics of the increase in the incidence of this pathology over the past decades. According to current research, AD is a multi-factorial disease, the pathogenesis of which is associated with both mutations in genes encoding epidermal structural proteins and epigenetic changes in gene expression. Generally accepted methods of treating skin damage in AD allow achieving remission, but not achieving a complete cure. It is shown that the composition of exosomes having a plasma origin can be supplemented with biologically active molecules introduced into the pericellular medium. The results obtained under the conditions of damage modeling in *in vitro* suggest that the use of exosomes for correcting the epigenetic landscape of cells under *in vitro* damage conditions is promising.

Introduction

Atopic Dermatitis (AD) is a multi-factorial, genetically determined inflammatory skin disease with a chronic recurrent course with age-related features. The prevalence of the disease among the population of the Russian Federation ranges from 11% to 21% [1-3]. It should be noted that AD is the most common chronic skin disease in children. The available research data indicate that Atopic Dermatitis (AD) has a polygenic pattern of inheritance, typically involving one or more dominant genes. These genes, along with a network of structural genes regulated by transcription factors, interact with environmental influences to contribute to disease onset and progression [1,2]. Currently, the genes associated with AD can be categorized into the following functional groups:

- Genes that affect the function of the epidermal barrier, encode the production of biologically active substances by keratinocytes (IL-25 and IL-33,TSLP) and affect the innate and adaptive immune response;
- Genes that regulate DNA methylation (KIF3A).

In addition, the association of AD with genes regulating the metabolism of ergo- and cholecalciferol and the synthesis of calcitriol receptors (CYPCYPA1, 27A1, CYP2R1, and VDR genes) was noted.

However, only 30% - 50% of patients with AD had the disease persist after puberty [3-5].

The data of genetic and epidemiological observation suggest that the mechanisms of development of this pathology are based

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on epigenetic mechanisms. Thus, a number of studies have shown that these mechanisms are the leading ones in various, allergic conditions similar to AD. For example, in the work of Nedoszytko, et al. [6], during the study of the relationship between DNA methylation and factors contributing to the development AD, 36 genes were identified, the expression of which significantly differed in the group of patients and healthy people due to methylation of their promoters and more than 490 promoters of other genes.

As far as miRNA research is concerned, two approaches can currently be distinguished – the study of the influence of known miRNAs in the development of AD and the search for new, previously unexplored miRNAs, which are found only among patients suffering from AD. Thus, using the first approach, the role of miR-124, miR-143, which is involved in the regulation of T cell proliferation, and miR-26, which activates hyaluronic synthase-3, was shown.

On the other hand, when searching for miRNAs differentially expressed in the blood serum of patients compared with healthy people, it was shown that the expression level of some miRNAs was significantly higher, for example, miR-144, miR151a, and miR-409, while the previously described miR-146a did not show any differences in the concentration in the blood of patients with AD and healthy people, despite the previously demonstrated role of this miRNA in the regulation of the immune system and signaling pathways of inflammatory responses.

However, differences in the level of gene expression in healthy people and patients suffering from AD, as well as the concentration of various types of small interfering RNAs, clearly indicate the role of epigenetic regulation in the development of AD.

Modern AD therapy is aimed at controlling the severity of inflammation and prolonging the time of remission. The choice of therapeutic tactics depends on the patient's age and clinical features of the course of AD [7,8].

Drugs used in clinical practice for local and systemic therapy of AD are not always effective and have a number of side effects that limit their use.

To date, there are no data on therapy with drugs that affect epigenetic processes in AD [9]. However, these data suggest that epigenetic factors may play a major role in the pathogenesis of atopic dermatitis.

The latter is supported by the facts of «spontaneous» cure, as well as by clinical differences in the course of the disease in children of different gender and age groups and the dependence of the severity of the disease on the action of environmental factors and on the nature of nutrition [10].

Summarizing all the above, as well as the fact that epigenetic changes are subject to external correction, the most promising approach is to influence epigenetic mechanisms.

Exosomes are extracellular vesicles with a diameter of up to 100 nanometers, which can serve as the main «horizontal» way of intercellular communication, since they contain many signaling molecules that allow cells to synchronize their functions [11,12].

Probably, the development of AD maybe based on a violation of cellular cooperation, when a «pathological» cell communicates its «pathological» response to environmental changes to neighboring cells. Then the use of exosomes secreted by normal cells can radically correct this situation. At the same time, we did not find any data on the implementation of this approach in the available literature. Thus, the use of exosomes of intact cells as a carrier of signaling molecules for the treatment of AD seems promising.

Materials and methods

Preparation of radiolabeled exosomes

MiRNA (18-20 pairs of nucleotides) was prepared by chemical synthesis on an ASM 800 synthesizer (Russia) under the conditions of replacing uridine with a radioactively labeled analog 14Curidine (Merck). Cell cultures obtained at the previous stage were cultured in the presence of a mixture of ^{14}C labeled protein hydrolysate at a dose of 37 kBq /ml for 180 minutes, or with ^{14}C- labeled miRNA. Then the cells were washed from the label that was not included on nitrocellulose filters with a pore diameter of 220 nm (Merck Pte. Ltd. Singapore).Subsequently, the cell lines were incubated for a day in RPMI-1640 medium with L-glutamine. The resulting «conditioned» medium was collected and stored at -85 °C in a freezer (Sanyo / Panasonic, Japan). ^{14}C labeled exosomes were subsequently used to evaluate transfer and insertion into the cell and by measuring the radioactivity of the latter in a simple alcohol-toluene scintillator on a Beta-2 counter. The results were expressed in Becquerel per Nano gram of protein.

Exosome isolation

In all cases, commercial Exo Quick-TC kits (System Biosciences, USA) were used to isolate exosomes according to the manufacturer's protocol.

Modeling of cell damage

Temperature stress was modeled using a TV-20-PZ-K thermostat (Russia) with the latter filled with a 5% CO_2 mixture$_2$ at sufficient humidity. At the preliminary stage, the temperature sensitivity of cultured skin cells (fetal fibroblasts) was studied in the temperature range from 38 to 43C in 0.25 °C increments in order to determine LD50 (the minimum temperature that causes 50% of cells to die within 24 hours). The found temperature was 42 oC. To simulate a temperature stress, fibroblasts were transplanted into Petri dishes and cultured at 37 °C, CO_2 concentration of 5%, and 95% humidity. After reaching 50% confluence in the swarm cell culture, the temperature in the cups was increased to 42 oC. After 24 hours, the total number of cells in the cultures was evaluated to determine the proportion of dead cells. It is known that the development of a number of diseases is caused by the

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formation of oxidative stress. Based on a number of literature data, we chose the effect of hydrogen peroxide as a model of oxidative stress.

Preliminary experiments made it possible to establish that for human fibroblasts under *in vitro* conditions *vitro*, the concentration of hydrogen peroxide, which has an absolute cytotoxic effect, is 30 nM/ml. In this regard, the range of 7.2 – 22.0 nM/ml was chosen as the working concentrations of hydrogen peroxide.

Methods for assessing epigenetic status. DNA was isolated from the cells by the sorbent method. The resulting DNA was transferred to clean centrifuge cryotubes (Eppendorf) and stored at-86C. To assess the levels of global DNA methylation, a quantitative method of analysis using 96 well plates was used. Specific antibodies were used in the study and quantitative colorimetry was performed. Total methylated DNA (Fully methylated DNA, Sigma Aldrich) and negative control/ blank, without DNA, were used as control samples. A Multiscan GO flatbed spectrophotometer (ThermoFisher Scientific) was used to record the signals. The optical density was detected at OD = 450 nm. Histone proteins were isolated using a mixture of guanidine thiocyanate and phenol in a single-phase solution, which allows efficient protein dissolution during cell lysis. The next step was the addition of 1-bromo-3-chloropropane and centrifugation for protein precipitation and subsequent isolation of the organic phase containing histones. To study the level of histone modification, a colorimetric evaluation system was used. An active nuclear extract was used as a positive control. The substrate was acetylated by the use of active histone acetyltransferase, which releases the free form of CoA, which serves as a coenzyme for the production of NADH. A soluble tetrazolium dye was added to the reaction product. The results were recorded spectrophotometrically (Multiscan Go, Thermo Fisher Scientific).

Statistical processing

Statistical processing of the obtained data was performed using statistical software packages for STATISTICA 6. The results were considered significantly different at $p < 0.05$.

Results and discussion

Selection of a cell line for studying the properties of exosomes (Table 1).

Thus, when isolating exosomes when using multipotent adipose mesenchymal stromal cells (MMSCT) as donor cells, the obtained data are characterized by an extreme variation, exceeding one order of magnitude—from 16.4 (Patient 3) to 180.9 (Patient 4) . This study was carried out on the basis of a decision of the LEK Ural State Medical University of the Ministry of Health of the Russian Federation and on the basis of the written informed consent of the patient. At the same time, the deviation for each patient is small, since the source of samples is the primary culture of each patient.

In turn, the variation can be explained by gender and age-related causes, as well as the presence of hidden (unspoken) chronic diseases in intact patients.

The obtained results made it necessary to standardize the source of exosomes, which is achieved by using standard cell lines as their source. In our case, we selected the HELF-3/81 cell line, obtained from the ENIIVI of the SSC VB Vector.

In the course of experiments with the HELF-3/81 cell line culture, the following results were obtained (Table 2). The comparative similarity of the number of exosomes obtained from cells of passage 4-6 is noteworthy. This may indicate that there is a certain synchronization of rapidly proliferating cells of embryonic origin. At the same time, the exosome isolation of HELF-3/81 cells is comparable to that of patients 1 and 4, which can be characterized as maximal.

The desire for reproducibility of the results of model experiments on exosome extraction leads to the transition of further studies to their implementation with exosomes obtained from HELF-3/81 line cells, especially, since the proportion of early progenitors in cultures is at least 20%, which exceeds that of adult adipose tissue stromal mesenchymal cells patients.

Evaluation of exosome transport. It is known, that exosomes are theoretically and practically capable of transferring a significant number of different molecules between cells, including signaling ones, nucleic acids, cytokines, etc. [13]. In an *in vitro* model experiment, to evaluate this characteristic of exosomes, we used the method of evaluating the inclusion of ^{14}C-labeled macromolecules, and proteins and miRNAs incorporated by exosomes.

The results of including the contents of radioactively labeled exosomes are presented in Table 3.

Table 1: Isolation of exosomes in ng/ ml of conditioned medium of patient cell cultures.

Patient Number	Sex	Exosome Yield (ng/mL) ± SD
1	Male	160.22 ± 1.23
2	Female	78.13 ± 2.12
3	Male	16.04 ± 0.50
4	Female	180.88 ± 2.11
5	Male	30.67 ± 1.01
6	Male	120.22 ± 2.07
7	Male	56.13 ± 1.11
Total (Mean ± SD)	M+/ - m	91.76 ± 8.85

Table 2: Isolation of exosomes in ng/ml of conditioned medium of HELF-3/81 cell line cultures.

Sample Number	Exosome Yield (ng/mL) ± SD
1	173.22 ± 1.17
2	178.13 ± 1.52
3	186.04 ± 1.58
4	180.88 ± 1.33
5	178.67 ± 1.71
6	162.22 ± 2.07
7	196.13 ± 1.91
Total (Mean ± SD)	179.33 ± 1.62

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The presented data indicate that exosomes actively accumulate labeled peptides and miRNA-like molecules. Although the object of comparison is not given in Table 3, it is nevertheless important that the three-hour cultivation of cells with the corresponding radionuclides is accompanied by the accumulation of the latter not only in cells, but also in exosomes released by cells into the culture medium. The latter allows us to consider exosomes as a means of targeted delivery of therapeutic factors.

Effects of exosomes on cell remodeling and damage. Despite the fact that the pathogenesis of atopic dermatitis remains largely poorly understood, based on the literature data, it can be concluded that as a result of the development of pathology, skin cells lose their resistance to the effects of adverse environmental factors. The latter allows us to count on the reproduction in test cultures of cells under local hyperthermia (+42 °C) and oxidative stress, x which underlie the pathogenic effect on the skin of many unfavorable environmental factors. Thus, the reduction of hyperthermia (+42 °C) is accompanied by a halving of the number of viable cells (Table 4). Despite the fact that the temperature of the «core» of the human body is close to 40 °C, however its deviation by 2 °C - 2.5 °C is already critical for storage of cellular vital activity. In the course of research, it was shown that the use of exosomes is accompanied by an increase in cell resistance to hyperthermia, which is expressed in increase in the proportion of viable cell forms (Table 4).

The latter suggests a therapeutic effect of exosomes in skin diseases accompanied by local hyperthermia. The following experiments have demonstrated the inability of exosomes to reduce cells due to the development of oxidative stress. The results obtained are presented in Table 5.

If we take into account that the main component of the development and progression of AD is epigenetic rearrangements and that the correction of the latter is possible by introducing exosomes of healthy cells, then under the conditions of our model experiments, the introduction of exosomes should inevitably be accompanied by epigenetic rearrangements.

Our studies have demonstrated that cell damage affects the level of DNA methylation and histone acetylation. So, in the course of preliminary studies, in cells of the HELF-3/81 line (n = 12), the level of DNA methylation was 0.64 cu, a histone acetylation-0.775cu ,while as a result of modeling oxidative stress, the level of methylation decreased by 21%, and acetylation increased by 17%. The use of exosomes allowed us to bring these indicators closer to the norm (Table 6).

Conclusion

Based on the obtained experimental data, we can conclude that:

- Exosome isolation may depend on many factors, including gender, age, and possible x diseases in intact patients, which is accompanied by significant variability in exosome output. The latter in model experiments gives grounds for preference for standard cell lines with similar potential to VT MMSCs in adult patients.
- The composition of exosomes of cytoplasmic origin can be purposefully supplemented with biologically active molecules introduced into the pericellular environment;
- It has been shown that cultured cells are able to accumulate both peptides and miRNA-like molecules, which subsequently enter the culture medium as part of exosomes. This allows us to hope for the prospects of using exosomes as a means of delivering biologically active substances to target cells;
- The use of exosomes of intact cells reduces damage to cultured cells under the influence of factors that determine the pathogenetic basis of inflammation in atopic dermatitis;

The results obtained in the modeling of hyperthermia and oxidative stress suggest that the use of exosomes for correcting the epigenetic landscape of cells is favorable and effective.

Table 3: Radioactivity of exosomes of cell cultures after treatment miRNA labeled with ^{14}C uridine or with^{14}C protein hydrolysate. Bk/ng of exosome protein

Labeled Molecules	Radioactivity (Bk/ng of Exosome Protein) ± SD
miRNA labeled with 14C Uridine	148.00 ± 23.68
14C Protein Hydrolysate	954.65 ± 152.44

Table 4: Percentage of dead cells as a function of exosome exposure under temperature stress (+42°C) Expressed as a percentage of the initial amount .

Group	Average Value	Minimum Value	Maximum Value
Hyperthermia	48.80	47.10	50.70
Hyperthermia in the Presence of Exosomes of Intact Cells	27.40*	15.00*	29.70*
Differences are significant at $p < 0.05$.			

Table 5: The proportion of surviving cells when the studied solutions were introduced into the culture in various dilutions (concentrations) under 24-hour chemical stress, modeled by the introduction of hydrogen peroxide into the culture medium.

Hydrogen peroxide concentration	7.2 nM/ ml			14.4 nM/ ml			21.6 nM/ ml		
Control	92.39625 +/- 3.078712								
Exposure to hydrogen peroxide	44,07 ± 1,11*	49.71 + 2,36*	51.22 + 2,22*	37,47 + 09,00*	32.44 + 1,10*	42.83 + 2,01*	30.01 + 09,0=	27.20 + 1,01=	33.45 + 2,2*
The result of using exosomes	81,11 + 2,74	77.20 + 1,07*	83.24 + 9,27	71,65 + 1,43*	68,83 + 2,55*	74,60 + 3,11	87,84 + 3,00	85,11 + 3,07	90,61 + 1,06
*Differences from the control $p < 0.05$.									

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Table 6: Dynamics of the epigenetic landscape of HELF 3/81 cells in normal conditions, when modeling damage and correcting damage using exosomes

Epigenetic Marker	Before Exposure (Oxidative Stress)	After Exposure	After Exosome Application
DNA Methylation Level	0.640667 ± 0.065828	0.141229 ± 0.019779*	0.592887 ± 0.065828
Histone Acetylation Level	0.775112 ± 0.0112333	0.9116233 ± 0.009222*	0.66510 ± 0.02020

Differences are significant at $p < 0.05$.

References

1. Leung DYM, Bieber T. Atopic dermatitis. Lancet. 2003;361(9352):151–160. Available from: https://doi.org/10.1016/s0140-6736(03)12193-9
2. Bieber T. Atopic dermatitis. N Engl J Med. 2008;358(14):1483–1494. Available from: https://doi.org/10.1056/nejmra074081
3. Zhou L, Leonard A, Pavel AB, Malik K, Raja A, Glickman J, et al. Age-specific changes in the molecular phenotype of patients with moderate-to-severe atopic dermatitis. J Allergy Clin Immunol. 2019;144–156. Available from: https://doi.org/10.1016/j.jaci.2019.01.015
4. Yang G, Seok JK, Kang HC, Cho YY, Lee HS, Lee JY, et al. Abnormalities and immune dysfunction in atopic dermatitis. Int J Mol Sci. 2020;21(8):2867. Available from: https://doi.org/10.3390/ijms21082867
5. Kantor R, Silverberg JI. Environmental risk factors and their role in the management of atopic dermatitis. Expert Rev Clin Immunol. 2017;13(1):15–26. Available from: https://doi.org/10.1080/1744666x.2016.1212660
6. Nedoszytko B, Reszka E, Gutowska-Owsiak D, Trzeciak M, Lange M, Jarczak J, et al. Genetic and epigenetic aspects of atopic dermatitis. Int J Mol Sci. 2020;21(18):6484. Available from: https://doi.org/10.3390/ijms21186484
7. Hofmann MA, Fluhr JW, Ruwwe-Glösenkamp C, Stevanovic K, Bergmann KC, Zuberbier TX. Role of IL-17 in atopy—A systematic review. Clin Transl Allergy. 2020;11(6):e12047. Available from: https://doi.org/10.1002/clt2.12047
8. Kiebert G, Sorensen SV, Revicki D, Fagan SC, Doyle JJ, Cohen J, et al. Atopic dermatitis is associated with a decrement in health-related quality of life. Int J Dermatol. 2002;41(3):151–158. Available from: https://doi.org/10.1046/j.1365-4362.2002.01436.x
9. Worm M, Francuzik W, Kraft M, Alexiou A. Modern therapies in atopic dermatitis: biologics and small molecule drugs. J Dtsch Dermatol Ges. 2020;18(10):1085–1092. Available from: https://doi.org/10.1111/ddg.14175
10. Rindler K, Krausgruber T, Thaler FM, Alkon N, Bangert C, et al. Spontaneously resolved atopic dermatitis shows melanocyte and immune cell activation distinct from healthy control skin. Front Immunol. 2021;12:397–420. Available from: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2021.630892/full
11. Dilsiz N. Hallmarks of exosomes. Future Sci OA. 2021;8(1):FSO764. Available from: https://doi.org/10.2144/fsoa-2021-0102
12. Prasanna P, Rathee S, Rahul V, Mandal D, Chandra Goud MS, Yadav P, et al. Microfluidic platforms to unravel mysteries of Alzheimer's disease: how far have we come? Life (Basel). 2021;11(10):1022. Available from: https://doi.org/10.3390/life11101022
13. Leblanc P, Arellano-Anaya ZE, Bernard E, Gallay L, Provansal M, Lehmann S, et al. Isolation of exosomes and microvesicles from cell culture systems to study prion transmission. Methods Mol Biol. 2017;1545:153–176. Available from: https://doi.org/10.1007/978-1-4939-6728-5_11

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