Frontiers in Bioscience Landmark

*Front. Biosci. (Landmark Ed)* **2025**; 30(4): 28255
https://doi.org/10.31083/FBL28255

*Original Research*

# Early and Late Responses of Cultured Human Mesenchymal Stem Cells (MSCs) to Cell-free DNA (cfDNA) in Patients With Acute Myocardial Infarction

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Academic Editor: Rajesh Katare
Submitted: 15 November 2024 Revised: 29 December 2024 Accepted: 25 February 2025 Published: 31 March 2025

**Abstract**

**Background**: Acute myocardial infarction (AMI) is accompanied by damage to heart tissues and some cell death. Stem cells are localized in the affected area and contribute to tissue repair. Studies have previously shown that the concentration of cell-free DNA (cfDNA) in the blood (ami-cfDNA) increases significantly in patients with AMI, and GC-rich and oxidized DNA fragments accumulate in the composition of ami-cfDNA. As a result, ami-cfDNA exhibits biological activity in vitro against various types of differentiated human cells. Potentially, ami-cfDNA can influence the functional activity and direction of stem cell differentiation. To verify this assumption, we investigated the effect of ami-cfDNA fragments isolated from the blood of patients with AMI on human adipose tissue mesenchymal stem cells (MSCs) *in vitro*. **Materials and Methods**: The MSC line was used and characterized by stem cell surface markers. Ami-cfDNA and control (hc-cfDNA) samples were isolated from the blood plasma of seven AMI patients and ten healthy donors. The early (0.5–3 hours) and late (1–3 weeks) responses of MSCs to cfDNA action were analyzed. The level of reactive oxygen species, the expression level of numerous genes (*NOX4*, *NRF2*, *BRCA1*, *BCL2*, *BAX*, *MYOD1*, *MYOG*, *MYF5*, *MRF4*, *RUNX2*, *SPP1*, *OCN*, *LPL*, *AP2*), the level of double-stranded DNA breaks in nuclei, and changes in the spatial organization of the chromatin in the nucleus were determined using the quantitative (real-time) polymerase chain reaction (qPCR), flow cytometry, fluorescence microscopy, fluorescent *in situ* hybridization (FISH) assays. **Results**: Introducing ami-cfDNA fragments into the cell culture medium stimulates rapid and transient induction of oxidative stress in MSCs (early response). Oxidative stress stimulates the spatial reorganization of chromatin to develop an adaptive response (AR). The adaptive response includes an antioxidant and anti-apoptotic response and activation of repair genes. The ami-cfDNA fragments, unlike hc-cfDNA, stimulate the myogenic differentiation of MSCs under prolonged exposure (late response). **Conclusions**:The ami-cfDNA increases the survival of MSCs in the model system by inducing a pronounced adaptive cellular response. Prolonged exposure to ami-cfDNA provokes myogenic differentiation of MSCs. Under acute stress conditions caused by AMI in the body, ami-cfDNA may positively affect the restoration of damaged heart muscle.

**Keywords:** acute myocardial infarction; cell-free DNA; human mesenchymal stem cells (MSCs)

## 1. Introduction

Acute myocardial infarction (AMI) is associated with massive death of heart cells and with increased levels of inflammation and oxidative stress in the patient's body. Oxidative stress induces the death of other body cells, primarily blood cells. Part of the DNA pool of dead cells circulates in peripheral blood plasma and is called circulating cell-free DNA (cfDNA) [1–3]. The number of cfDNA fragments in the blood of an AMI patient is significantly increased. The concentration of cfDNA in blood plasma is considered by many authors as a marker of the level of damage to the heart muscle. The mean concentration of cfDNA in patients with AMI was several folds higher during the onset of disease compared with healthy volunteers. Measuring cfDNA in patients with AMI offers an alternative approach to monitor the disease and has potential clinical applications to identify high-risk individuals [4–13]. The origin of part of the ami-cfDNA fragments from the cells of the damaged heart muscle was shown by the authors, who compared the methylation profile of ami-cfDNA and gDNA. Epigenetic changes in cfDNA are more specific to cardiac tissue compared to those in gDNA [14–16]. The characteristics of ami-cfDNA differ significantly from those of DNA, which is part of the blood cells of the same patients. Guanine-cytosine rich (GC-rich) chromatin fragments accumulate in cfDNA, for example, mitochondrial DNA [17,18] and fragments of the transcribed ribosomal repeat region [19,20]. GC-rich fragments of mitochondrial DNA (mtDNA) and ribosomal DNA (rDNA) contain motifs that are ligands for TLR9 receptors [20]. Stimulation of these receptors activates the transcription factor NF-$\kappa$B, which controls the synthesis of

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proinflammatory cytokines in cells. It is known that under conditions of oxidative stress in the body, cfDNA is significantly oxidized. Oxidized cfDNA binds to cells and induces a secondary explosion of ROS synthesis. An increase in the level of ROS in cells caused by model fragments of oxidized DNA causes the development of an adaptive response in the cell population [21,22]. Model fragments of oxidized DNA [23], unlike GC-rich fragments [24], block adipogenic differentiation of MSCs. The high content of GC-rich and/or oxidized fragments causes the appearance of biological activity in cfDNA against various types of cells. For example, it has recently been shown that cfDNA isolated from the blood plasma of patients with schizophrenia causes an adaptive response in cultured human skin fibroblasts [25]. There is no data in the literature on the biological activity of ami-cfDNA in relation to human stem cells. Stem cells are localized in the affected area of the heart muscle and contribute to tissue repair. The data obtained for the cfDNA model samples suggest that potentially ami-cfDNA can influence the functional activity and direction of differentiation of stem cells. To verify this assumption, we investigated *in vitro* the effect of ami-cfDNA fragments isolated from the blood of patients with AMI on human adipose tissue mesenchymal stem cells (MSCs).

## 2. Materials and Methods

### *2.1 Preparation of DNA Samples*

Serum of patients examined and treated at the Cardiological Unit of Central Clinical Hospital of Russian Academy of Sciences, were used in this study. All patients with AMI (4 men and 3 women of the average age 78 $\pm$ 7 years old) suffered from ischemic heart disease and arterial hypertension stage II and III. The criterion for inclusion of patients in the study was confirmed AMI, we accepted the criteria for AMI used in Clinical Hospital. These nevertheless all included standard electrocardiography (ECG) changes and cardiac enzymes (creatinine phosphokinase as minimum). The criterion for exclusion: patients did not have inflammatory or other previously diagnosed diseases, except for AMI. Peripheral blood serum was obtained within 3 h after the development of AMI.

Blood sampling from patients with acute myocardial infarction was performed immediately upon the first examination of the patient by a doctor in order to conduct a primary multicomponent blood test, before any manipulation or administration of medications.

The group of negative control contained patients of basically healthy donors (5 men and 5 women aged from 41 to 78 years old; the average age 48 $\pm$ 14 years old), had no history of arterial hypertension or AMI. At the time of the study, patients in the control group had no inflammatory or any other diseases, all indicators of general and biochemical blood analysis were normal.

The isolation of DNA from the blood serum of sick and healthy people and from leukocytes of the blood of healthy people and the determination of its concentration in solution was described in detail earlier [19]. A standard extraction method with organic solvents was used. The facial serum was pretreated with a lysing solution (0.25 M EDTA, 5% sodium laurylsarcosylate), RNase A (75 µg/mL, 1 hour, 37 °C) and proteinase K (200 µg/mL, 24 h, 37 °C). The blood cfDNA isolated from various samples were combined into one sample to obtain the required amount of sample for conducting *in vitro* experiments.

CfDNA concentration was determined by the standard method based on fluorescence of DNA bound dye, PicoGreen (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), using LS 55 (PerkinElmer, Waltham, MA, USA).

The determination of the DNA content in DNA samples was carried out according to a technique developed by us, which is described in detail in a previous publication [26]. The technique includes the use of the non-radioactive quantitative hybridization (NQH) method, which allows analyzing the content of rDNA in the composition of damaged cfDNA samples.

The content of 8-oxodG in DNA samples was carried out using the enzyme immunoassay method, which was described in detail earlier [27].

### *2.2 Cell Culture*

Mesenchymal stem cells were retrieved from the biospecimen collection maintained by the Research Centre for Medical Genetics. MSCs were obtained from adipose tissue of patients subjected to regular surgery [23,24]. Mesenchymal stem cells (MSCs) were obtained from adipose tissue of patients subjected to surgical operation. To obtain stromal cells, minced adipose tissue was digested with collagenase. The tissue samples were disrupted using a mechanical process in a medium called Dulbecco's Modified Eagle medium (DMEM), which was provided by Paneko from Moscow, Russia. The medium contained gentamycin, penicillin, and streptomycin, all at concentrations of 250 µg/mL, 60 U/mL, and 60 U/mL, respectively. The cells were separated by incubating them in DMEM with 10% fetal bovine serum (FBS) at 37 °C for 16 hours. The cells were then centrifuged at 200 $\times$g for 10 minutes and transferred to slide flasks.

The mesenchymal stem cells (MSCs) were cultivated in AmnioMax C-100 Basal Medium, which was provided by Gibco Products from Big Cabin, OK, USA. The medium contained AmnioMax Supplement C-100 and 20 mmol/L HEPES, which was provided by Paneko from Moscow, Russia. Before any treatments, the cells were split no more than four times.

The MSCs expressed major histocompatibility complex molecules (HLA-ABC+) and adhesion molecules (CD44+, CD54 (low), CD90+, CD106+, CD29+, CD49b (low), CD105 (low)). Nevertheless, these cells were negative for hematopoietic markers (CD34–, CD45–, HLA–

DR–) and the marker CD117. The cells were induced to differentiate into adipocytes using a kit for adipogenic differentiation (MesenCult Adipogenic Differentiation Kit, STEMCELL Technologies, Vancouver, BC, Canada) [24]. The mesenchymal stem cells were cultured in the presence of DNA samples (50 ng/mL) at 37 °C for 0.25–3 and 24 hours or for 14 days.

Cell line was validated by short tandem repeat (STR) profiling and tested negative for mycoplasma.

### *2.3 Ethical Approval for Operating with Cultured (MSCs) and Primary (Blood) Human Cells*

The research was carried out in accordance with the most recent version of the Helsinki Declaration and was approved by the Independent Interdisciplinary Ethics Committee for the Review of Clinical Trials (the approval number: 2021-4/6). Each participant provided informed consent for their involvement in the study and for the publication of anonymized results, after the procedures had been thoroughly explained.

### *2.4 ROS Assay*

The total reactive oxygen species (ROS) assay was performed to evaluate the mesenchymal stem cells (MSCs) in a 96-well plate. The MSCs were rinsed with phosphate-buffered saline (PBS) and incubated with 5 micromolar H2DCFH-DA (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) in the dark for 20 minutes at 37 °C. After rinsing with PBS, the cells were immediately analyzed using fluorescence-activated cell sorting (FACS). The fluorescence intensity was measured at an excitation wavelength of 488 nm and an emission wavelength of 528 nm using an EnSpire instrument (PerkinElmer, Turku, Finland). Each DNA sample and the control were analyzed eight times, with four replicates per measurement.

### *2.5 Flow Cytometry*

The cells were rinsed with Versene solution (Paneko, Moscow, Russia), then treated with 0.25% trypsin (Paneko, Moscow, Russia) under the supervision of a light microscope Axiovert 10 (Carl Zeiss, Oberkochen, Germany). The cells were transferred to Eppendorf tubes, washed with culture media, and then centrifuged and resuspended in PBS (Paneko, Moscow, Russia). The cells were stained with various antibodies as follows.

To fix the cells, paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) was added at a final concentration of 2% and incubated at 37 °C for 10 minutes. The cells were then washed three times with 0.5% BSA-PBS and permeabilized with 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 15 minutes or with 70% ethanol at 4 °C.

Cells (~50 $\times$ $10^3$) were washed three times with 0.5% BSA-PBS and stained with 1–2 µg/mL antibodies for 3 h at 4 °C, then again washed thrice with 0.5% BSA-PBS and stained with 1 µg/mL the appropriate antibodies listed below for 1 h at 4 °C. To measure the baseline fluorescence, we treated a subset of cells with only secondary FITC-labeled antibodies. The cells were then examined using CyFlow Space (Sysmex Partec, Görlitz, Germany).

### *2.6 Annexin V Binding Assays*

The cells were separated from the culture, rinsed with phosphate-buffered saline, and then subjected to treatment with annexin V-FITC (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) in a buffer solution containing 10 mM HEPES at pH 7.4, 140 mM NaCl, and 2.5 mM $CaCl_2$. The cells were incubated at 20 °C for 15 minutes, and their fluorescence was immediately analyzed using flow cytometry.

### *2.7 Fluorescence Microscopy*

The microscope AxioScope A1, manufactured by Carl Zeiss in Oberkochen, Germany, was used to capture the images of cells.

### *2.8 Immunocytochemistry*

The cells were preserved in 3% formaldehyde at 4 °C for 20 minutes, then rinsed with PBS and subsequently treated with 0.1% Triton X-100 in PBS for 15 minutes at room temperature. Afterward, they were subjected to a blocking process using 0.5% BSA in PBS for 1 hour, followed by incubation overnight at 4 °C with the appropriate antibody.

Following the incubation, the cells were rinsed with 0.01% Triton X-100 in PBS and then incubated for 2 hours at room temperature with FITC goat anti-mouse IgG (1:200, ab6785, Abcam Inc., Waltham, MA, USA). Afterward, they were rinsed again with PBS and stained with DAPI.

The following antibodies were used: dyLight488-$\gamma$H2AX (pSer139) (1:100, nb100-78356G NovusBio, Saint Louis, MO, USA); CY5.5-NADPH oxidase 4 (1:200, bs-1091r-cy5-5, Bioss Inc., Woburn, MA, USA); MYF5 (1:100, NBP1-19565 NovusBio, Saint Louis, MO, USA); MYF6 (1:100, NBP1-55582 NovusBio, Saint Louis, MO, USA); MYOG (1:100, NBP1-95760 NovusBio, Saint Louis, MO, USA); BCL2 (1:200, Sc-783, Santa Cruz, Dallas, TX, USA); A350-BCL2 (1:200, bs-15533r-a350; Bioss Antibodies Inc. Woburn, MA, USA).

### *2.9 Fluorescent in Situ Hybridization*

Prior to the process of hybridization, the slides were subjected to treatment with RNase A, 100 µg/mL (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). The hybridization procedure and the solutions were sourced from Abbott Laboratories (Abbott Laboratories, Abbott Park, IL, USA). The hybridization was conducted in the ThermoBrite thermostat (StatSpin, Norwood, MA, USA) at a temperature of 42 °C. The MSCs were stained with propidium iodide (PI). The f-SatIII FISH probe was a 1.77-kilobase fragment of human satellite DNA that had been

cloned using EcoRI [28]. The plasmid pUC1.77 was labeled using nick translation with the CGH Nick Translation Kit (Abbott Molecular, Des Plaines, IL, USA), following the manufacturer's instructions.

The statistical analysis of changes in the spatial organization of chromatin based on FISH data was described in detail earlier [29].

### 2.10 Quantification of mRNA Levels

The total mRNA was extracted using the RNeasy Mini kit (Qiagen, Hilden, Germany). The quantity of RNA was determined using the Quant-iT RiboGreen RNA dye (MoBiTec, Göttingen, Germany) on a tablet reader (EnSpire Equipment, PerkinElmer, Turku, Finland) at $\lambda$em = 487 nm, $\lambda$fl = 524 nm. After treating the RNA with DNase I, it was reverse transcribed using the Reverse Transcriptase kit (Sileks, Moscow, Russia). The reverse transcription process was followed by PCR, which was conducted using specific primers and the SYBR-Green intercalating dye on a StepOnePlus device (Applied Biosystems, Foster City, CA, USA). The primers were designed and synthesized by Evrogen (Moscow, Russia). The internal standard was the *TBP* gene.

The PCR reaction mixture, in a volume of 2.5 µL PCR buffer (700 mmol/L Tris-HCl, pH 8.6); 166 mmol/L ammonium sulfate, 35 mmol/L $MgCl_2$, 2 µL 1.5 mmol/L dNTP solution; and 1 µL 30 pmol/L primer solution. The PCR conditions were tailored for each primer pair. The reaction began with a denaturation phase of 4 minutes at 95 °C, followed by 40 amplification cycles. Each cycle consisted of 20 seconds at 94 °C, 30 seconds at 56–62 °C, 30 seconds at 72 °C, and a final extension phase of 5 minutes at 72 °C. The data were analyzed using a calibration plot, with an error margin of 2%.

The following primers were used (Sintol, Moscow, Russia):

*BRCA1* (breast canser gene type 1) (F: 5′-TGTGAGGCACCTGTGGTGA-3′; R: 5′-CAGCTCCTGGCACTGGTAGAG-3′);

*NRF2* (NF-E2-related factor 2) (F: 5′-TCCAGTCAGAAACCAGTGGAT-3′; R: 5′-GAATGTCTGCGCCAAAAGCTG-3′);

*BCL2* (B-Cell Lymphoma 2) (F: 5′-TTTGGAAATCCGACCACTAA-3′; R: 5′-AAAGAAATGCAAGTGAATGA-3′);

*BAX* (BCL2-associated X protein) (F: 5′-CCCGAGAGGTCTTTTTCCGAG-3′; R: 5′-CCAGCCCATGATGGTTCTGAT-3′);

*NOX4* (NADPH oxidase 4) (F: 5′-TTGGGGCTAGGATTGTGTCTA-3′; R: 5′-GAGTGTTCGGCACATGGGTA-3′);

*LPL* (Lipoprotein lipase) (F: 5′-ACAAGAGAGAACCAGACTCCAA-3′; R: 5′-GGTAGTTAAACTCCTCCTCC-3′);

*MYOD1* (myogenic differentiation gene type 1) (F: 5′-GGTCCCTCGCGCCCAAAAGAT-3′; R: 5′-GTTCTCCCGCCTCTCCTAC-3′);

*MYOG* (Myogenin) (F: 5′-AGTGCACTGGAGTTCAGCG-3′; R: 5′-TTCATCTGGGAAGGCCACAGA-3′);

*MYF5* (Myogenic factor 5) (F: 5′-CTGCCAGTTCTCACCTTCTGA-3′; R: 5′-CGTCCCCAAATTCACCCTCG-3′);

*MRF4* (myogenic regulatory factor 4) (F: 5′-AATCTTGAGGGTGCGGATTTC-3′; R: 5′-CTCCTCCTTCCTTAGCCGTTA-3′);

*RUNX2* (runt-related transcription factor 2) (F: 5′-CCGTCTTCACAAATCCTCCCC-3′; R: 5′-CCCGAGGTCCATCTACTGTAAC-3′);

*SPP1* (secreted phosphoprotein 1) (F: 5′-CTCCATTGACTCGAACGACTC-3′; R: 5′-GGTCTGCGAAACTTCTTAGAT-3′);

*OCN* (osteocalcin) (F: 5′-CCCTCACACTCCTCGCCCTATT-3′; R: 5′-AAGCCGATGTGGTCAGCCAACTCGT-3′);

*AP2* (Activating Protein 2) (F: 5′-TGTGCAGAAATGGGATGGAAA-3′; R: 5′-CAACGTCCCTTGGCTTATGCT-3′);

and *TBP* (TATA-box binding protein, reference gene) (F: 5′-GCCCGAAACGCCGAATAT-3′; R: 5′-CCGTGGTTCGTGGCTCTCT-3′).

### 2.11 Statistical Analysis

All the results presented here were replicated at least twice as separate biological replicates. The significance of the observed disparities was assessed using the non-parametric Mann-Whitney U-test ($p$) and Kolmogorov-Smirnov statistics (D and $\alpha$). The analysis was conducted using StatPlus 2007 professional software (AnalystSoft Inc., Vancouver, BC, Canada) and Statistica (TIBCO Software Inc. (2018), version 13, Palo Alto, CA, USA). All $p$-values were calculated with two-tailed tests and were considered statistically significant when $p < 0.01$.

## 3. Results

### 3.1 Description of the Experiment

DNA samples were isolated from the blood plasma of 7 patients with acute myocardial infarction and 10 healthy people. To analyze the biological effect of cfDNA on stem cells, all samples were combined into one ami-cfDNA sample and hc-cfDNA sample, respectively. In addition, a sample of gDNA isolated from leukocytes from 10 healthy donors was used for control. The content of the GC-rich fragments (rDNA) marker in the cfDNA samples, the level of oxidation (the content of the 8-oxodG marker) and the approximate size of the fragments are presented in Table 1.

The cfDNA of patients contains increased amounts of GC-rich rDNA fragments and is much more strongly oxidized than the cfDNA of healthy controls and gDNA. The effect of three DNA samples on mesenchymal stem cells

**Table 1. Characteristics of the DNA probes.**

| DNA Probe | Origin of the DNA Sample | rDNA CN | 8-oxodG/$10^6$ N | Length, kb |
|---|---|---|---|---|
| gDNA (genomic DNA) | HC (health control) leukocytes (n = 10) | 429 ± 78 | 0.45 ± 0.1 | 10–15 |
| ami-cfDNA (cell-free DNA isolated from the blood of patients with acute myocardial infarction) | AMI plasma (plasma of patients with acute myocardial infarction) (n = 7) | 1412 ± 115 | 128 ± 45 | 5–11* |
| hc-cfDNA (cell-free DNA isolated from the blood of healthy patients) | HC (health control) plasma (n = 10) | 843 ± 56 | 29 ± 7 | 8–10* |

*Samples contain low-molecular-weight DNA fragments. CN, rDNA copy number.

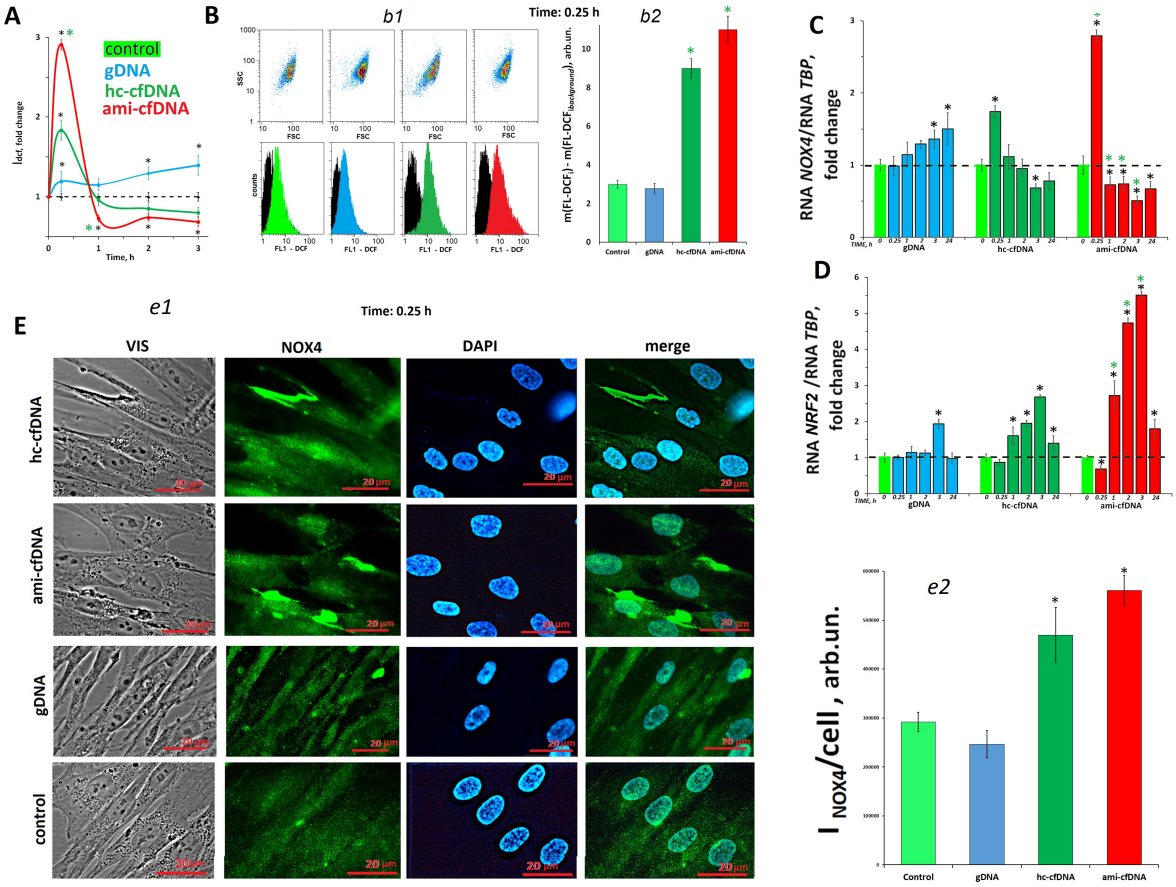


**Fig. 1. Ami-cfDNA induces a short-term increase in the ROS level in the MSCs.** (A) The production of reactive oxygen species (ROS) in mesenchymal stem cells (MSCs) after incubation for 0.5 to 3 hours in the presence of DNA samples at a concentration of 50 ng/mL. The results of the quantification of fluorescence in cells treated with H2DCFH-DA using a plate reader. (B) b1—the most representative examples of DCF (dichlorodihydrofluorescein) analysis using FCA (flow cytometry analysis); vertically SSC (side scatter)—the indicator of lateral light scattering, horizontally FSC (forward scatter)—the indicator of direct light scattering. b2—the values of the medians of FL-DCFi (fluorescence intensity of DCF). (C) The change in the expression of RNA *NOX4* (NADPH oxidase 4) in MSCs in the presence of DNA samples. (D) Change of the RNA *NRF2* (NF-E2-related factor 2) in MSCs in the presence of DNA samples. (E) e1—analysis of the NOX4 location in MSCs in the presence of DNA samples, the scale bars = 20 µm. e2—the intensity of fluorescence, reflecting the protein content in one cell. The average values for 20 randomly selected cells are given. The cultivation time and the DNA samples are indicated on the graph. Average values for three measurements and standard deviation are given. (*) and (* green) present the differences with the control or hc-cfDNA are significant ($p < 0.01$, nonparametric U test). *TBP* (TATA-box binding protein)—reference gene.

obtained from breast adipose tissue was studied. DNA fragments at a concentration of 50 ng/mL were added to the culture medium. The cells were cultured for 0.5–3 hours (early response) and 2 weeks (late response).

### *3.2 CfDNA Fragments Induce an Adaptive Response in MSCs (Early Response)*

It was previously shown that oxidized model DNA fragments in the cell culture medium induce a transient

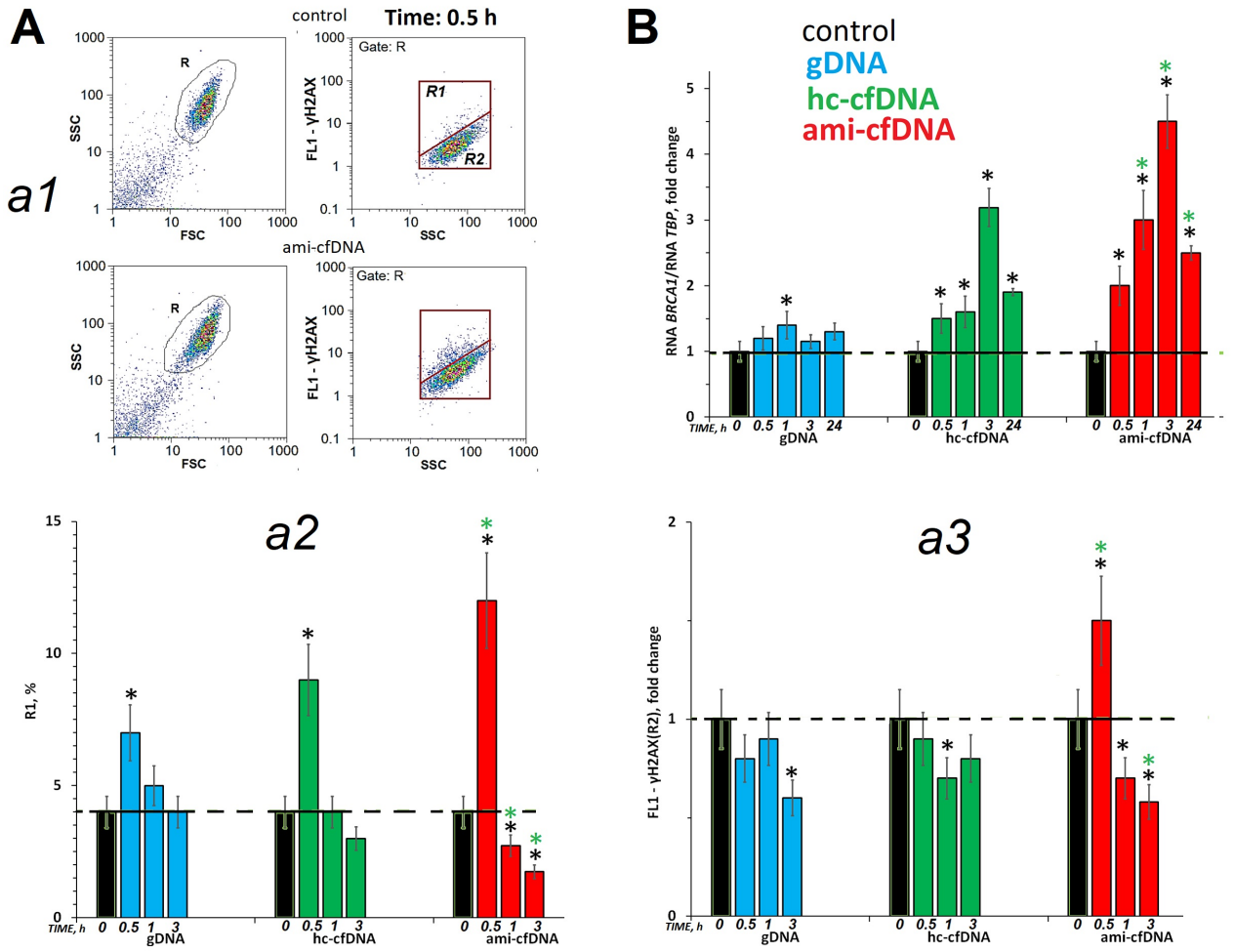


**Fig. 2. Ami-cfDNA induces a transient increase in double-stranded DNA breaks in MSCs.** (A) a1—the most typical examples of the γH2AX analysis with FCA; a2—changes in the content of the R1 (γH2AX) fraction in MSCs; a3—changes in the median FL-γH2AX for R2 fraction in MSCs. (B) Changes in the level of *BRCA1* RNA in MSCs that were cultured in the presence of DNA samples. The cultivation time and the DNA samples are indicated on the graph. Average values for three measurements and standard deviation are given. (*) and (* green) presents the differences with the control or hc-cfDNA are significant ($p < 0.01$, nonparametric U test).

increase in ROS levels in cells of various types [24]. A short-term increase in ROS levels causes an adaptive response (AR) in cells, which includes activation of antioxidant genes, repair genes and genes regulating apoptosis. AR is accompanied by a change in the structure of the chromatin of the nucleus. The observed response to the action of oxidized cfDNA is similar in a number of ways to the adaptive response of cells induced by the action of low doses of ionizing radiation [21].

### *3.3 Changes in the ROS Level*

Changes in the ROS level in the presence of cfDNA samples were analyzed using the H2DCFH-DA reagent (Fig. 1).

Using a tablet reader, the kinetics of changes in the total ROS level in cells and in the MSCs culture medium was determined (Fig. 1A). Already 15 minutes after incubation of cells with cfDNA samples (50 ng/mL of medium), we observed a sharp increase in the amount of ROS in cells. The ami-cfDNA sample showed the maximum effect, while the gDNA sample had the minimum effect. After 1 hour, there was a sharp decrease in the level of ROS in the presence of ami-cfDNA and hc-cfDNA samples. Moreover, the ami-cfDNA sample induced a decrease in the level of ROS below the control one. The gDNA sample, on the contrary, stimulated an increase in ROS levels during the first three hours of cultivation.

The FCA method allows us to analyze changes in the level of ROS directly in cells (Fig. 1B). Compared with the control, two subpopulations were detected in cells treated with ami-cfDNA and hc-cfDNA for 15 minutes—with an average level of ROS (about 85% of cells) and with a very high level of ROS (about 15% of cells) (Fig. 1B,b2). The gDNA sample does not induce the appearance of cells with abnormally high ROS levels in the MSCs population.

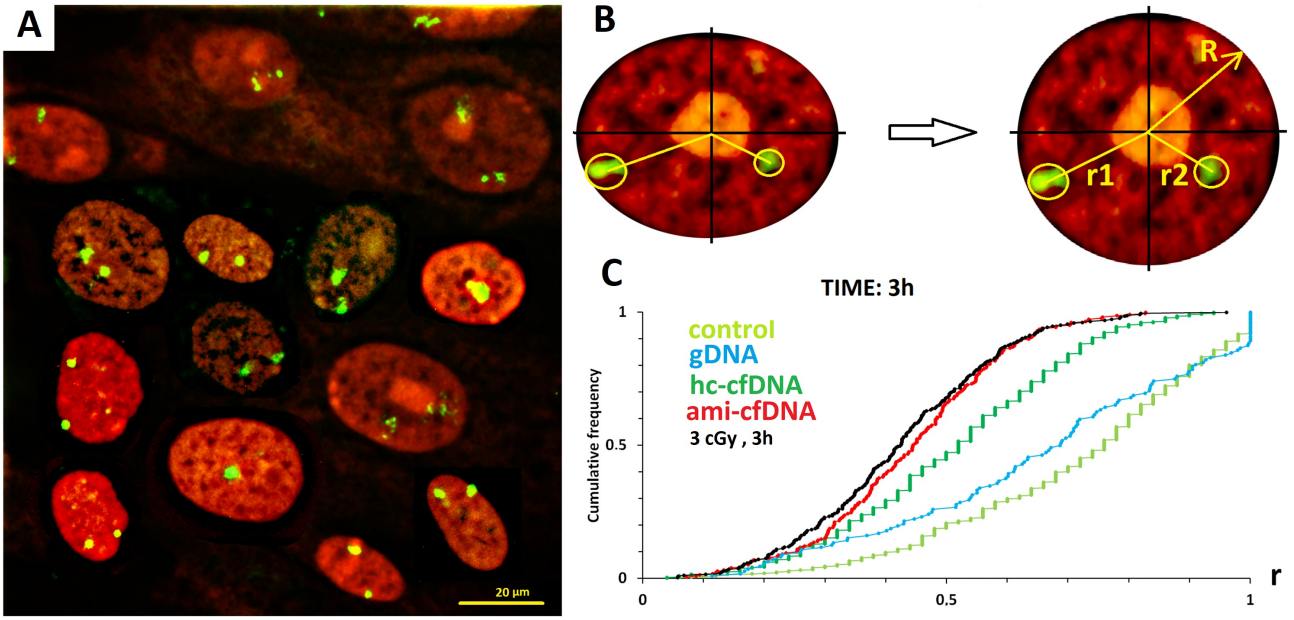


**Fig. 3. Ami-cfDNA induces a change in the architecture of the core in the MSCs associated with the development of an adaptive response.** (A) The FISH result for the control cells. The 1q12 loci in the nucleus are shown in red (PI) with two green fluorescent signals. The gallery of cells is composed of multiple photographs. The scale bars are 20 µm. (B) An illustration of the process of analyzing images of nuclei. Image processing involves calculating the center of gravity of the FISH signal, as well as the radius vectors of the signals (r1 and r2) and the radius R of the nucleus. The radius vector r is scaled to the value of the nucleus radius R and ranges from 0 (the center of the nucleus) to 1 (the surface of the nucleus). (C) The cumulative histograms of the frequency distribution of FISH signals by the radius vector r (0 being the center of the nucleus) for intact, cfDNA-treated, and irradiated (3 cGy) MSCs. The distributions of r values for ami-cfDNA-treated cells differ significantly from the control: D = 0.60, $\alpha$ <10–40 (Kolmogorov-Smirnov).

It was previously shown that a short-term increase in ROS levels in the presence of model DNA fragments is caused by an increase in the expression of the prooxidant gene *NOX4* [25] and blocking of antioxidant activity regulated by the Nuclear Factor, Erythroid 2-Like (*NFEL2L*) gene [25]. Ami-cfDNA and hc-cfDNA samples stimulate an increase in the expression of the *NOX4* gene in MSCs immediately after addition to the culture medium (Fig. 1C). Over time, the level of *NOX4* RNA in the cellular RNA pool significantly decreases. The gDNA sample induces a gradual increase in the level of *NOX4* RNA within 24 hours after addition to the culture medium. An increase in the transcription level of the *NOX4* gene in cells in the presence of ami-cfDNA and hc-cfDNA samples correlates with an increase in the amount of NOX4 protein (Fig. 1E). The amount of protein increases in all cells of the MSCs population. At the same time, approximately 10% of cells express abnormally high amounts of NOX4 on the surface. Thus, it can be assumed that the NOX4 enzyme is one of the main producers of ROS under the action of cfDNA on MSCs.

Along with a sharp increase in the activity of the *NOX4* gene in the first half hour of culturing cells with ami-cfDNA, we observed a decrease in the activity of the *NFEL2L* gene, which controls the antioxidant response (Fig. 1D). Longer cultivation is accompanied by an increase in the transcription activity of the *NFEL2L* gene against the background of a decrease in the activity of the pro-oxidative *NOX4* gene. We recorded the maximum differences with the control for the ami-cfDNA sample.

### *3.4 Changes in the Number of Double-Stranded DNA Breaks*

The explosion of ROS in the first half hour after the introduction of DNA samples into the culture medium of MSCs can cause oxidative modifications of DNA, provoking DNA damage. The level of the phosphorylated form of histone H2AX correlates with the number of double-strand breaks in DNA [24]. FCA was used to analyze the level of $\gamma$ H2AX in cells (Fig. 2).

The MSCs population contains cells with low levels of H2AX and a small number of cells (4%) with a high histone content (Fig. 2A,a1). The explosion of ROS in the first half hour of the action of cfDNA is accompanied by an increase in the number of cells with high levels of $\gamma$H2AX. The protein level in the main fraction of cells also increases in the presence of ami-cfDNA (Fig. 2A,a2,a3). Further cultivation of MSCs in the presence of DNA samples is accompanied by a decrease in the content of histone $\gamma$H2AX, which may indicate the activation of repair processes. Indeed, ami-cfDNA and hc-cfDNA samples stimulate an increase in the expression of the *BRCA1* gene, which is involved in

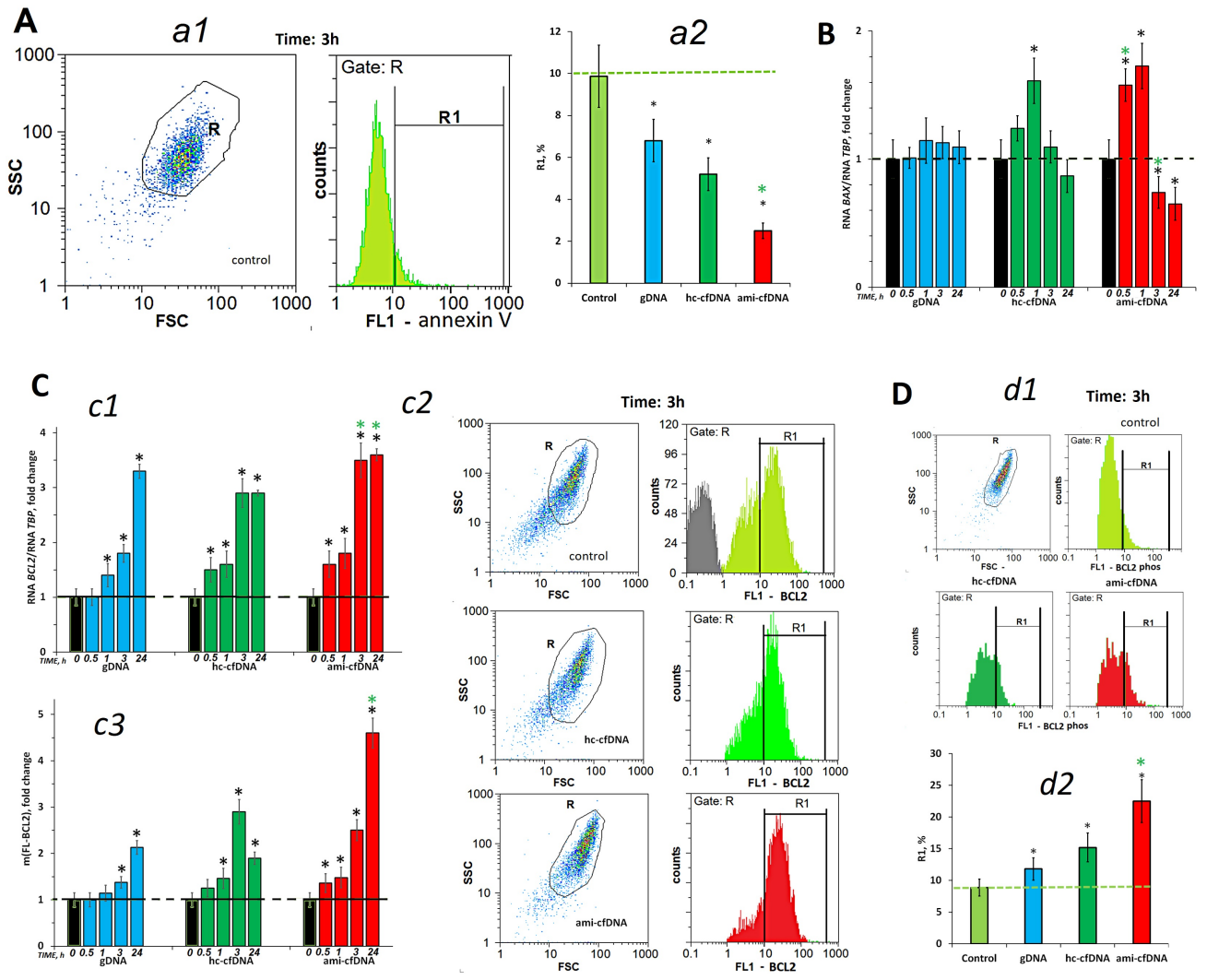


**Fig. 4. Ami-cfDNA induces an anti-apoptotic response in MSCs.** (A) The level of apoptosis was assessed using the protein annexin 5, a marker of early apoptosis. The concentration of the added fragments is 50 ng/mL, the exposure time is 3 hours. a1—the distribution of cells by anexin fluorescence, the fraction of R1 cells with signs of apoptosis is noted. a2 — the cell content of the R1 fraction. (B) Changes in the level of *BAX* (BCL2-associated X protein) RNA in MSCs that were cultured in the presence of DNA samples. (C) Changes in the level of the phosphorylated form of BCL2 (B-Cell Lymphoma 2) in MSCs that were cultured in the presence of DNA samples. c1—changes in the level of *BCL2* RNA in MSCs; c2—the most typical examples of the BCL2 analysis with FCA, changes in the content of the R1 fraction in MSCs; c3—changes in the median FL-BCL2 for MSCs. (D) Changes in the level of the phosphorylated form of BCL2 in MSCs that were cultured in the presence of DNA samples. d1—the most typical examples of the BCL2 analysis with FCA; d2—changes in the content of the R1 fraction in MSCs. The cultivation time and the DNA samples are indicated on the graph. Average values for three measurements and standard deviation are given. (*) and (* green) present the differences with the control or hc-cfDNA are significant ($p < 0.01$, nonparametric U test).

the repair of double-stranded DNA breaks (Fig. 2B). The gDNA sample does not cause significant changes in the *BRCA1* RNA level in cells.

### *3.5 Changes in the Spatial Organization of Chromatin Nuclei*

One of the conditions for the effective development of an adaptive response in cells under the action of oxidative stress inducers is a change in the spatial structure of chromatin, which is necessary to change the expression profile of the genome [22]. The location of the homologues of the first chromosome (region 1q12), which is analyzed by the FISH method, we consider as one of the markers of changes in the chromatin structure in response to stress. In unstimulated confluent cells, 1q12 sites are located near the surface of the nuclei. The action of agents stimulating an increase in the level of ROS, for example, ionizing radiation [22,29], induces the movement of these sites from the surface to the center of the nuclei (Fig. 3).

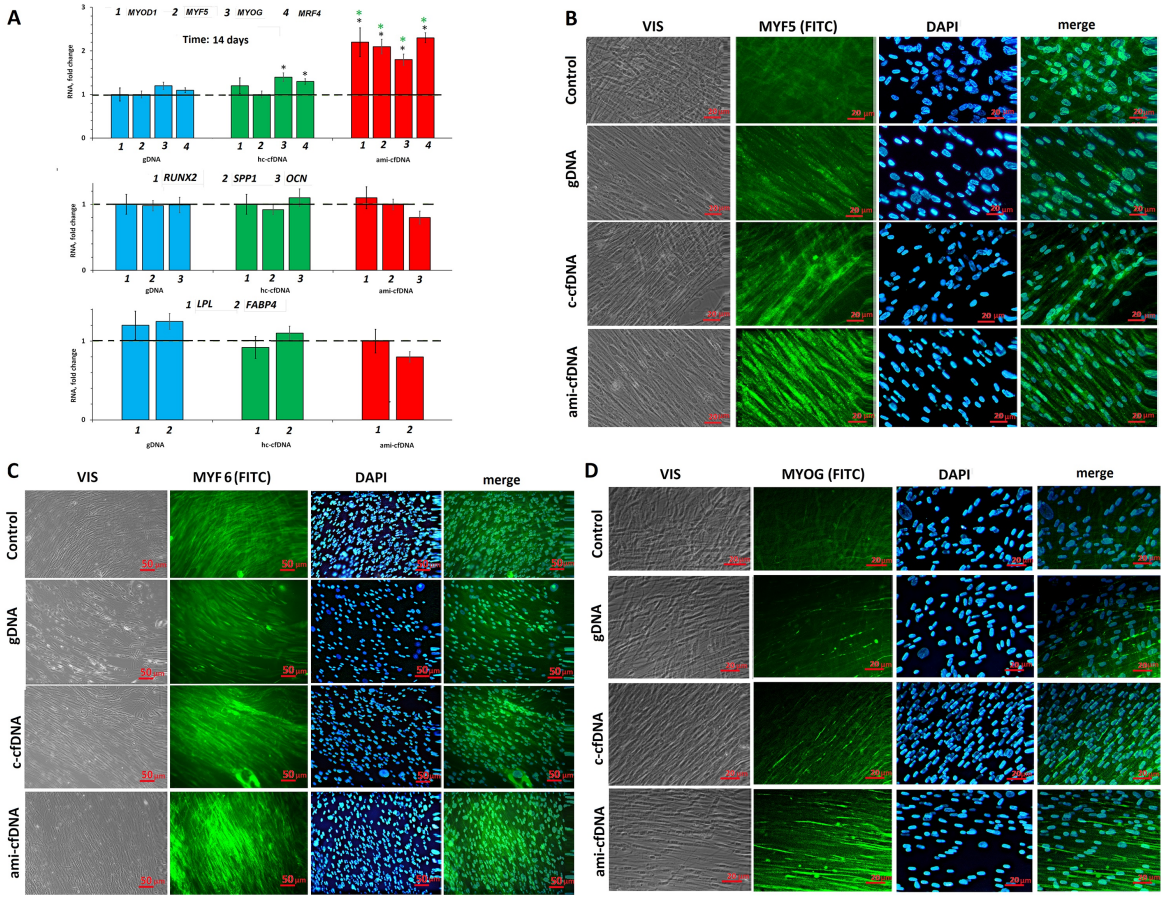


**Fig. 5. Ami-cfDNA induces myogenic differentiation in MSCs.** (A) The degree of expression of genes involved in myogenic, osteogenic, and adipogenic differentiation processes in mesenchymal stem cells (MSCs) after 14 days of exposure to fragments of cell-free DNA (cfDNA). (B–D) Expression and distribution of the transcription factors MYF5 (B), MYF6 (C) and MYOG (D) of myogenic differentiation 14 days after exposure to cfDNA probes (100 ng/mL). The scale bars are 20 and 50 µm. Fluorescence microscopy, (B,D) (40×) and (C) (20×). The cultivation time and the DNA samples are indicated on the graph. Average values for three measurements and standard deviation are given. (*) and (* green) present the differences with the control or hc-cfDNA are significant ($p < 0.01$, nonparametric U test).

The absence of such movement is associated with blocking the adaptive response to stress. The effect of the ami-cfDNA sample on the MSCs after 3 hours is accompanied by a change in the distribution of the normalized radius vector of the labels, which is comparable in magnitude to the effect of a dose of 3 cGy ionizing radiation (Fig. 3C). Hc-cfDNA and gDNA samples induce the movement of chromatin 1q12 sites in a smaller number of cells.

### *3.6 Changes in the Level of Cell Apoptosis*

DNA samples after 3 hours of cell culture reduce the number of cells with early signs of cell membrane disruption (marker: annexin 5) (Fig. 4).

The R1 fraction (Fig. 4A) decreases in the presence of all cfDNA samples, the maximum effect was observed in the case of ami-cfDNA. CfDNA samples stimulated a gradual increase in the expression of the anti-apoptotic *BCL2* gene, both at the transcription level and at the level of the BCL2 protein itself (Fig. 4C). The control cells contain two fractions of cells with different levels of BCL2 protein (Fig. 4C,c2). The amount of R1 fraction with a high protein content in the MSCs population in the presence of ami-cfDNA increases from 55% to 80%. The total level of BCL2 in cells increases 4–5 times when cultured with an ami-cfDNA sample for 3 or 24 hours. An increase in the amount of antiapoptotic protein was accompanied by an increase in the amount of its active (phosphorylated) form (Fig. 4D). The RNA level of the proapoptotic *BAX* gene, on the contrary, decreased after 3 hours of cultivation after a slight increase in the earlier period (Fig. 4B). The maximum effects were observed for the ami-cfDNA sample.

### *3.7 Differentiation of MSCs (Late Response)*

Musculoskeletal regulatory factors have been described in MSCs: MyoD, Myf5, Myf6 (Mrf4), myogenin [30], each of which is capable of activating transcription of

skeletal muscle genes. These transcription factors reveal an 80% identity of the amino acid sequence in the bHLH motif, which mediates DNA dimerization and binding. bHLH transcription factors form heterodimers with ubiquitin proteins of the E family, in the form of heterodimers they activate transcription of muscle-specific genes when binding to the CANNTG sequence (E-boxes) in promoters and enhancers of skeletal muscle genes. With prolonged cultivation of MSCs (14 days) in the presence of ami-cfDNA, cells are determined towards myogenic differentiation (Fig. 5, (*) and (* green) presents the differences with the control or hc-cfDNA are significant).

The level of expression of genes of transcription factors determining osteogenic differentiation increases 2–3 times: *MYOD1*, *MYOG*, *MYF5*, *MRF4* and the level of expression of genes of factors determining osteogenic differentiation is reduced or does not change: *RUNX2*, *SPP1*, *OCN* and adipogenic: *LPL* and *AP2* (*FABP4*) (Fig. 5A).

## 4. Discussion

In this study, we have shown that ami-cfDNA has a pronounced biological activity against MSCs of human adipose tissue. This activity appears to be a consequence of an increased GC composition and an increased level of cfDNA oxidation. A rDNA fragment was used as a marker of an increase in GC composition in cfDNA. An increase in the rDNA content in the cfDNA composition correlates with an overall increase in the content of GC pairs. The ribosomal repeat differs from other GC-rich DNA in the presence of a large number of Gn ($n > 2$) motifs [20]. In the composition of these motifs, guanosine has the lowest oxidation potential [31]. Thus, an increase in the rDNA content in cfDNA is associated with an increase in the level of DNA oxidation.

Fragments of ami-cfDNA *in vitro* induce an adaptive response in MSCs, which is similar to the response previously observed by us for model GC-rich or oxidized fragments of hcDNA. In the first minutes after the appearance of ami-cfDNA fragments in the culture medium, ROS synthesis is activated in cells, which is explosive. The source of ROS, apparently, is the activity of the enzyme NOX4, which catalyzes the formation of hydrogen peroxide at the sites of contact with cfDNA on the cell membrane [32]. Fragments of ami-cfDNA induce an increase in the activity of the *NOX4* gene in MSCs.

A sharp increase in ROS in cells stimulates the development of an adaptive response. The adaptive response in MSCs in the presence of ami-cfDNA is very similar in a number of ways to the adaptive response of MSCs induced by the action of low doses of ionizing radiation, which we described earlier [22,29,33]. ROS cause damage to DNA nuclei. In response to the formation of double-stranded DNA breaks that are dangerous for the genome, processes are activated in the cell aimed at repairing these breaks and reducing the level of ROS.

To change the genome expression profile in response to damage in order to develop an adaptive response, the spatial organization of the chromatin of the cell nucleus changes [29]. We tested changes in the chromatin architecture by changing the localization of heterochromatin sites of the first chromosome 1q12. The movement of sites 1q12 from the nuclear membrane to the center of the nucleus is associated with the activity of repair processes. These changes confirm the development of an adaptive response in the MSCs. The adaptive response involves three processes: antioxidant response, activation of repair systems and anti-apoptotic response.

We characterized the antioxidant response by changes in the activity of the *NFEL2L* gene, which encodes the NRF2 transcription factor. It is interesting to note that in the first hour of the action of ami-cfDNA, the amount of *NFEL2L* RNA in cells decreases. It is possible that temporary blocking of the activity of the antioxidant transcription factor against the background of activation of the prooxidant gene *NOX4* is necessary to maintain the necessary level of ROS in order to develop a full-fledged adaptive response in the cell population. The activity of the *NFEL2L* gene reaches its maximum after 3 hours of cell culture in the presence of ami-cfDNA.

We assessed the activation of repair processes by changes in the RNA level of the BRCA1 protein, which is part of the complex that repairs double-stranded DNA breaks and by a decrease in the level of the $\gamma$H2AX protein. An increase in the amount of RNA of the *BRCA1* gene positively correlates with a decrease in the level of histone $\gamma$H2AX in cells cultured with ami-cfDNA fragments. The antiapoptotic response of MSCs to the action of ami-cfDNA is confirmed by a decrease in the number of cells with signs of early apoptosis after 3 hours against the background of an increase in the activity of the antiapoptotic *BCL2* gene and a decrease in the activity of the proapoptotic *BAX* gene.

In this study, we showed for the first time that high-rDNA and high-oxidation (ami-cfDNA) cfDNA fragments can stimulate myogenic differentiation of stem cells. In the presence of ami-cfDNA, after 14 days, the expression level of genes of transcription factors determining osteogenic differentiation increases: *MYOD1*, *MYOG*, *MYF5*, *MRF4* and the expression level of genes of factors determining osteogenic differentiation is reduced or unchanged: *RUNX2*, *SPP1*, *OCN* and adipogenic: *LPL* and *AP2* (*FABP4*). Non-oxidized model DNA fragments previously induced adipogenic differentiation of MSCs [24]. Apparently, the direction of MSCs differentiation in the presence of cfDNA fragments largely depends on the level of cfDNA oxidation, and therefore on the level of oxidative stress in the patient's body.

Other DNA samples we studied (gDNA and hc-cfDNA) showed similar biological activity to ami-cfDNA, but the observed effects were less pronounced.

In acute myocardial infarction and after invasive treatment during surgical treatment of myocardial infarction, the level of cardiomyocyte cfDNA in circulating blood plasma increases [14]. Patients with sepsis have high concentrations of cardiac cfDNA, which accurately predict mortality, indicating an important role of cardiomyocyte death in mortality from sepsis [14]. In our studies, we also identified the stimulation of the development of oxidative stress in the first hours after exposure to cfDNA ami on MSCs. As in the case of an acute heart attack in myocardial and septic conditions, cell free DNA elimination options using plasmapheresis or different options for binding cfDNA using columns with adhesive materials specifically binding cfDNA in blood plasma can show good results in treatment early after the onset of the disease. Preclinical and clinical research data suggest that human mesenchymal stromal cells (HUC-MSCs) derived from the umbilical cord may be useful in treatment of heart failure and acute myocardial infarction (MI) [34]. Nevertheless, the effect of stem cell therapy on patients with heart failure remains the subject of ongoing debate, and the safety and efficacy of HUC-MSCs therapy has not yet been proven [34]. The pretreatment of MSCs with fragments of oxidized cfDNA can promote the stimulation of MSCs differentiation in the myogenic direction, as shown in this work, however, it is necessary to use samples of cfDNA with well-defined parameters as MSCs stimulation properties. In addition, these studies are in the initial stages of research and it is necessary to conduct a large number of additional tests to confirm the results obtained. The study was conducted on cells in a laboratory setting, and further experiments on animals are needed to confirm the results. In a study summarizing the progress of cardiorehabilitation in the treatment of patients with AMI, innovative solutions in the field of cardiorehabilitation were studied in detail and the positive correlation between the frequency of physical exercise in cardiological rehabilitation and an increase in the skeletal muscle index (SMI), as well as an improvement in cardiorespiratory endurance was especially emphasized [35]. In part, this also indicates that the significance of the data we have received. It is known that during physical exertion, the content of oxidized cfDNA in the blood plasma increases, which can contribute to the differentiation of stem cells moving to the site of heart damage in the myogenic direction.

There is no clear answer in the literature on the role of ami-cfDNA during AMI. The rapid development of an adaptive response and myogenic differentiation of stem cells in the presence of ami-cfDNA may indicate a beneficial effect of ami-cfDNA on the course of the pathological process. The adaptive response in MSCs makes it possible to preserve stem cells for a long time under conditions of chronic stress, which can differentiate into muscle cells in the lesion.

However, ami-cfDNA can negatively affect other blood cells and the cardiovascular system. For example, it was shown that ami-cfDNA samples isolated from the blood plasma of AMI patients in an *in vitro* experiment caused a significant decrease in the frequency of contractions in rat cardiomyocytes [36]. The cfDNA of AMI patients suppressed the synthesis of nitric oxide by endothelial cells [37]. In experiments on mice, it was shown that the simultaneous action of cfDNA and HMGB1 protein significantly worsens the manifestations of experimental AMI [38].

Thus, the question of whether ami-cfDNA should be the object of therapy requires further study.

## 5. Conclusions

In this study, we showed that oxidized ami-cfDNA fragments isolated from the blood plasma of AMI patients induce a pronounced adaptive response *in vitro* cultured MSCs, which increases the survival of MSCs in a model system. We described the effect of ami-cfDNA on the antioxidant and anti-apoptotic response of stem cells and activation of repair genes. In addition, we found that long-term exposure to ami-cfDNA induces myogenic differentiation of MSCs, which is important for future studies in the field of stem cell biology and the identification of factors for the rehabilitation of AMI patients.

## Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon request.

## Author Contributions

Conceptualization, SVK, NNV; methodology, SVK, EMM, and NNV; validation, ESE; investigation, EMM, ESE, MSK, LVK, SVK, NNV; resources, SVK; data curation, SVK, EMM and NNV; writing—preparation of the original project, NNV, EMM; writing—reviewing and editing, SVK, EMM and NNV; visualization, NNV; supervision, SVK, NNV. All authors have read and agreed with the published version of the manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. All authors contributed to editorial changes in the manuscript.

## Ethics Approval and Consent to Participate

The research was carried out in accordance with the most recent version of the Helsinki Declaration and was approved by the Independent Interdisciplinary Ethics Committee for the Review of Clinical Trials (the approval number: 2021-4/6). Each participant provided informed consent for their involvement in the study and for the publication of anonymized results, after the procedures had been thoroughly explained.

## Acknowledgment

Not applicable.

## Funding

This research received funding within the frameworks of a State Assignment of the Ministry of Education and Science of the Russian Federation.

## Conflict of Interest

The authors declare no conflict of interest.

## References

[1] Bronkhorst AJ, Holdenrieder S. Cell-Free Nucleic Acids: Physico-Chemical Properties, Analytical Considerations, and Clinical Applications. Diagnostics. 2023; 13: 2312. https://doi.org/10.3390/diagnostics13132312.

[2] Grabuschnig S, Bronkhorst AJ, Holdenrieder S, Rosales Rodriguez I, Schliep KP, Schwendenwein D, *et al*. Putative Origins of Cell-Free DNA in Humans: A Review of Active and Passive Nucleic Acid Release Mechanisms. International Journal of Molecular Sciences. 2020; 21: 8062. https://doi.org/10.3390/ijms21218062.

[3] Aucamp J, Bronkhorst AJ, Badenhorst CPS, Pretorius PJ. The diverse origins of circulating cell-free DNA in the human body: a critical re-evaluation of the literature. Biological Reviews of the Cambridge Philosophical Society. 2018; 93: 1649–1683. https://doi.org/10.1111/brv.12413.

[4] Chang CPY, Chia RH, Wu TL, Tsao KC, Sun CF, Wu JT. Elevated cell-free serum DNA detected in patients with myocardial infarction. Clinica Chimica Acta. 2003; 327: 95–101. https://doi.org/10.1016/s0009-8981(02)00337-6.

[5] Antonatos D, Patsilinakos S, Spanodimos S, Korkonikitas P, Tsigas D. Cell-free DNA levels as a prognostic marker in acute myocardial infarction. Annals of the New York Academy of Sciences. 2006; 1075: 278–281. https://doi.org/10.1196/annals.1368.037.

[6] Destouni A, Vrettou C, Antonatos D, Chouliaras G, Traeger-Synodinos J, Patsilinakos S, *et al*. Cell-free DNA levels in acute myocardial infarction patients during hospitalization. Acta Cardiologica. 2009; 64: 51–57. https://doi.org/10.2143/AC.64.1.2034362.

[7] Jing RR, Wang HM, Cui M, Fang MK, Qiu XJ, Wu XH, *et al*. A sensitive method to quantify human cell-free circulating DNA in blood: relevance to myocardial infarction screening. Clinical Biochemistry. 2011; 44: 1074–1079. https://doi.org/10.1016/j.clinbiochem.2011.06.083.

[8] Xu Y, Liu B, Zhao Z, Wang G, Zhu X, Pan S, *et al*. Clinical significance of peripheral blood circular DNA level measurements in patients with acute myocardial infarction. Chinese Journal of Medical Instrumentation. 2012; 36: 456–458. (In Chinese)

[9] Cui M, Fan M, Jing R, Wang H, Qin J, Sheng H, *et al*. Cell-Free circulating DNA: a new biomarker for the acute coronary syndrome. Cardiology. 2013; 124: 76–84. https://doi.org/10.1159/000345855.

[10] Polina IA, Ilatovskaya DV, DeLeon-Pennell KY. Cell free DNA as a diagnostic and prognostic marker for cardiovascular diseases. Clinica Chimica Acta. 2020; 503: 145–150. https://doi.org/10.1016/j.cca.2020.01.013.

[11] Tan E, Liu D, Perry L, Zhu J, Cid-Serra X, Deane A, *et al*. Cell-free DNA as a potential biomarker for acute myocardial infarction: A systematic review and meta-analysis. International Journal of Cardiology. Heart & Vasculature. 2023; 47: 101246. https://doi.org/10.1016/j.ijcha.2023.101246.

[12] Zhang Q, He X, Ling J, Xiang Q, Li M, Zhao H, *et al*. Association Between Circulating Cell-Free DNA Level at Admission and the Risk of Heart Failure Incidence in Acute Myocardial Infarction Patients. DNA and Cell Biology. 2022; 41: 742–749. https://doi.org/10.1089/dna.2022.0238.

[13] Artner T, Sharma S, Lang IM. Nucleic acid liquid biopsies in cardiovascular disease: Cell-free DNA liquid biopsies in cardiovascular disease. Atherosclerosis. 2024; 398: 118583. https://doi.org/10.1016/j.atherosclerosis.2024.118583.

[14] Zemmour H, Planer D, Magenheim J, Moss J, Neiman D, Gilon D, *et al*. Non-invasive detection of human cardiomyocyte death using methylation patterns of circulating DNA. Nature Communications. 2018; 9: 1443. https://doi.org/10.1038/s41467-018-03961-y.

[15] Cuadrat RRC, Kratzer A, Arnal HG, Rathgeber AC, Wreczycka K, Blume A, *et al*. Cardiovascular disease biomarkers derived from circulating cell-free DNA methylation. NAR Genomics and Bioinformatics. 2023; 5: lqad061. https://doi.org/10.1093/nargab/lqad061.

[16] Dash M, Mahajan B, Shah S, Dar GM, Sahu P, Sharma AK, *et al*. Distinct methylome profile of cfDNA in AMI patients reveals significant alteration in cAMP signaling pathway genes regulating cardiac muscle contraction. Clinical Epigenetics. 2024; 16: 144. https://doi.org/10.1186/s13148-024-01755-2.

[17] Barbalata T, Scarlatescu AI, Sanda GM, Toma L, Stancu CS, Dorobantu M, *et al*. Mitochondrial DNA Together with miR-142-3p in Plasma Can Predict Unfavorable Outcomes in Patients after Acute Myocardial Infarction. International Journal of Molecular Sciences. 2022; 23: 9947. https://doi.org/10.3390/ijms23179947.

[18] Cosentino N, Campodonico J, Moltrasio M, Lucci C, Milazzo V, Rubino M, *et al*. Mitochondrial Biomarkers in Patients with ST-Elevation Myocardial Infarction and Their Potential Prognostic Implications: A Prospective Observational Study. Journal of Clinical Medicine. 2021; 10: 275. https://doi.org/10.3390/jcm10020275.

[19] Veĭko NN, Bulycheva NV, Roginko OA, Veĭko RV, Ershova ES, Kozdoba OA, *et al*. Ribosomal repeat in the cell free DNA as a marker for cell death. Biomeditsinskaia Khimiia. 2008; 54: 78–93.

[20] Ershova ES, Konkova MS, Malinovskaya EM, Kutsev SI, Veiko NN, Kostyuk SV. Noncanonical Functions of the Human Ribosomal Repeat. Russian Journal of Genetics. 2020; 56: 30–40. https://doi.org/10.1134/S1022795420010044.

[21] Kostyuk SV, Ermakov AV, Alekseeva AY, Smirnova TD, Glebova KV, Efremova LV, *et al*. Role of extracellular DNA oxidative modification in radiation induced bystander effects in human endotheliocytes. Mutation Research. 2012; 729: 52–60. https://doi.org/10.1016/j.mrfmmm.2011.09.005.

[22] Ermakov AV, Konkova MS, Kostyuk SV, Izevskaya VL, Baranova A, Veiko NN. Oxidized extracellular DNA as a stress signal in human cells. Oxidative Medicine and Cellular Longevity. 2013; 2013: 649747. https://doi.org/10.1155/2013/649747.

[23] Loseva P, Kostyuk S, Malinovskaya E, Clement N, Dechesne CA, Dani C, *et al*. Extracellular DNA oxidation stimulates activation of NRF2 and reduces the production of ROS in human mesenchymal stem cells. Expert Opinion on Biological Therapy. 2012; 12: S85–S97. https://doi.org/10.1517/14712598.2012.688948.

[24] Kostyuk S, Smirnova T, Kameneva L, Porokhovnik L, Speranskij A, Ershova E, *et al*. GC-Rich Extracellular DNA Induces Oxidative Stress, Double-Strand DNA Breaks, and DNA Damage Response in Human Adipose-Derived Mesenchymal Stem Cells. Oxidative Medicine and Cellular Longevity. 2015; 2015: 782123. https://doi.org/10.1155/2015/782123.

[25] Kostyuk SV, Ershova ES, Martynov AV, Artyushin AV,

Porokhovnik LN, Malinovskaya EM, *et al*. In Vitro Analysis of Biological Activity of Circulating Cell-Free DNA Isolated from Blood Plasma of Schizophrenic Patients and Healthy Controls-Part 2: Adaptive Response. Genes. 2022; 13: 2283. https://doi.org/10.3390/genes13122283.

[26] Chestkov IV, Jestkova EM, Ershova ES, Golimbet VE, Lezheiko TV, Kolesina NY, *et al*. Abundance of ribosomal RNA gene copies in the genomes of schizophrenia patients. Schizophrenia Research. 2018; 197: 305–314. https://doi.org/10.1016/j.schres.2018.01.001.

[27] Ershova ES, Jestkova EM, Chestkov IV, Porokhovnik LN, Izevskaya VL, Kutsev SI, *et al*. Quantification of cell-free DNA in blood plasma and DNA damage degree in lymphocytes to evaluate dysregulation of apoptosis in schizophrenia patients. Journal of Psychiatric Research. 2017; 87: 15–22. https://doi.org/10.1016/j.jpsychires.2016.12.006.

[28] Cooke HJ, Hindley J. Cloning of human satellite III DNA: different components are on different chromosomes. Nucleic Acids Research. 1979; 6: 3177–3197. https://doi.org/10.1093/nar/6.10.3177.

[29] Konkova MS, Ershova ES, Savinova EA, Malinovskaya EM, Shmarina GV, Martynov AV, *et al*. 1Q12 Loci Movement in the Interphase Nucleus Under the Action of ROS Is an Important Component of the Mechanism That Determines Copy Number Variation of Satellite III (1q12) in Health and Schizophrenia. Frontiers in Cell and Developmental Biology. 2020; 8: 386. https://doi.org/10.3389/fcell.2020.00386.

[30] Olson EN, Klein WH. bHLH factors in muscle development: dead lines and commitments, what to leave in and what to leave out. Genes & Development. 1994; 8: 1–8. https://doi.org/10.1101/gad.8.1.1.

[31] Chernikov AV, Gudkov SV, Usacheva AM, Bruskov VI. Exogenous 8-Oxo-7,8-dihydro-2'-deoxyguanosine: Biomedical Properties, Mechanisms of Action, and Therapeutic Potential. Biochemistry. Biokhimiia. 2017; 82: 1686–1701. https://doi.org/10.1134/S0006297917130089.

[32] Martyn KD, Frederick LM, von Loehneysen K, Dinauer MC, Knaus UG. Functional analysis of Nox4 reveals unique characteristics compared to other NADPH oxidases. Cellular Signalling. 2006; 18: 69–82. https://doi.org/10.1016/j.cellsig.2005.03.023.

[33] Konkova M, Abramova M, Kalianov A, Ershova E, Dolgikh O, Umriukhin P, *et al*. Mesenchymal Stem Cells Early Response to Low-Dose Ionizing Radiation. Frontiers in Cell and Developmental Biology. 2020; 8: 584497. https://doi.org/10.3389/fcell.2020.584497.

[34] Abouzid MR, Ali K, Kamel I, Esteghamati S, Saleh A, Ghanim M. The Safety and Efficacy of Human Umbilical Cord-Derived Mesenchymal Stem Cells in Patients With Heart Failure and Myocardial Infarction: A Meta-Analysis of Clinical Trials. Cureus. 2023; 15: e49645. https://doi.org/10.7759/cureus.49645.

[35] Zhang S, Lin Y. Advancements, challenges, and innovative strategies in cardiac rehabilitation for patients with acute myocardial infarction: A systematic review. Current Problems in Cardiology. 2025; 50: 102934. https://doi.org/10.1016/j.cpcardiol.2024.102934.

[36] Bulicheva N, Fidelina O, Mkrtumova N, Neverova M, Bogush A, Bogush M, *et al*. Effect of cell-free DNA of patients with cardiomyopathy and rDNA on the frequency of contraction of electrically paced neonatal rat ventricular myocytes in culture. Annals of the New York Academy of Sciences. 2008; 1137: 273–277. https://doi.org/10.1196/annals.1448.023.

[37] Efremova LV, Alekseeva AY, Konkova MS, Kostyuk SV, Ershova ES, Smirnova TD, *et al*. Extracellular DNA affects NO content in human endothelial cells. Bulletin of Experimental Biology and Medicine. 2010; 149: 196–200. https://doi.org/10.1007/s10517-010-0906-3.

[38] Tian Y, Charles EJ, Yan Z, Wu D, French BA, Kron IL, *et al*. The myocardial infarct-exacerbating effect of cell-free DNA is mediated by the high-mobility group box 1-receptor for advanced glycation end products-Toll-like receptor 9 pathway. The Journal of Thoracic and Cardiovascular Surgery. 2019; 157: 2256–2269.e3. https://doi.org/10.1016/j.jtcvs.2018.09.043.