Gene Ther Mol Biol Vol 11, 305-314, 2007

Exogenous DNA can be captured by stem cells and be involved in their rescue from death after lethal-dose γ-radiation

Research Article

Anastasia S. Likhacheva[1], Valeriy P. Nikolin[1], Nelly A. Popova[1], Vladimir A. Rogachev[1], Maria A. Prokhorovich[1], Tamara E. Sebeleva[1], Sergei S. Bogachev[1,2,*], Mikhail A. Shurdov[2]

[1]Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, 10 Lavrentieva ave, Novosibirsk, 630090, Russia;
[2]LLC Panagen, 29 Choros-Gurkina street, Gorno-Altaisk, 649000, Russia

***Correspondence**: Sergei S. Bogachev, 10 Lavrentieva ave, 630090, Novosibirsk, Russia; Tel: +7-383-330-45-65; Fax: +7-383-333-12-78; e-mail: gorbi@bionet.nsc.ru

Key words: gene therapy, radioprotection, stem cells, exogenous DNA, DNA uptake
Abbreviations: double-strand break, (DSB); blood stem cell, (BSC); stem cell, (SC); embryonic stem cell, (ESC)

Received: 22 August 2007; Revised: 22 November 2007
Accepted: 4 December 2007; electronically published: December 2007

Summary

Here we demonstrate that stem cells capture exogenous DNA internalized into the nuclear space. Injection of fragmented exogenous DNA to lethally radiated mice affords a very strong radioprotective effect: up to 90% of treated mice survived long after exposure to γ-rays. We also demonstrate that the high survival of mice after lethal-dose radiation is due to blood stem cell rescue whose offspring give rise to spleen colonies participating in recovery of the damaged immune system. It is suggested that the DNA radioprotective effect may result from involvement of exogenous DNA as substrate in homologous recombination during repair of double-strand break sites induced by high-dose radiation.

I. Introduction

Collisions of ionizing particles with living tissue generate a cascade of extremely reactive free radicals that spontaneously react with any nearby molecule, including DNA. The broad spectrum of damage incurred to DNA molecule includes the appearance of thymidin dimers, disruption of sugar and phosphate bonds, base integrity loss. DNA double-strand breaks (DSBs) giving rise to two open broken DNA ends entail the most dramatic consequences (Bonner, 2003). Some DNA fragments between close DSBs can be lost during attempts to repair such damage. In their natural strive to recover chromosomal integrity, the eukaryotic cells join the broken DNA ends. This repair can be error-prone in situations where DNA strands lying in close vicinity, yet belonging to distinct chromosomes or chromosome regions, are joined. This faulty repair stops synthetic cellular processes and the cells then undergo apoptosis, or mutation fixed chromosomal aberrations can arise. The aberrations can increase the risk of pathological consequences, such as neoplastic transformation and cancer development.

Control mechanisms deal with the DSBs arisen in the mammalian cells. Their triggering causes assembly and activation of repair-recombination complexes whose major function is posttranscriptional histone modification (Peterson and Cote, 2004). Autocatalytic phosphorylation of the entire pool of ATM/ATR kinases leads to prompt phosphorylation of histone H2AX in a stretch covering megabases at either side of a DSB that may quite reasonably be the event highlighting the DSB emergence (Rogakou et al, 1999; Stiff et al, 2004).

There are two different, cell cycle dependent pathways for repair of DSBs arisen in human and mammalian cells (Takata et al, 2000, 2001; Rodrigue et al, 2006). In cells damaged in the G1 phase of the cell cycle, joining of the processed ends of the damaged DNA molecule is the major pathway for DSB repair. Heterodimer Ku70/Ku80, DNA-PK, a number of accessory factors that process the ends to be ligated, and DNA ligase IV are all involved in this repair pathway

(Scott and Pandita, 2006). As to cells lesioned in the S or G2 phase, homologous recombination serves as the major pathway for repair of DSBs. Factors responsible for homologous recombination in the cell were detected at the repair site. These included the RAD 51 paralogs and accessory proteins – specific nucleases and helicase, which remove secondary structure from the single-strand tail formed by specific exonuclease (Symington, 2005; Rodrigue et al, 2006). Repair with the involvement of the molecular machinery for homologous recombination can occur with homologous region exchange or without crossing over. Whatever the case, a sister chromatid or a homologous chromosome is used as substrate for homologous pairing of the filament formed by RAD 51 and its paralogs (Symington, 2005).

We have previously hypothesized the existence of a natural mechanism that may affect the genetic constituent of multicellular organisms by taking advantage of genomic DNA from biological fluid as external genomic standard (Yakubov et al, 2002, 2003). We have proposed that the cellular surface receptors that bind DNA deliver the genomic DNA fragments resulting from natural apoptosis from the external environment (blood plasma, intertissue fluid, lymph) into the nucleus and that the turnover is continuous. Once internalized within the nuclear space, the DNA fragments may participate in all the repair processes for whose enfoldment the presence of undamaged homologous sequence appears to be imperative (Rogachev et al, 2006).

Our subsequent study has demonstrated that integration of heterologous DNA into the adult mouse genome was feasible by using exogenous DNA as substrate for homologous recombination in interstrand cross-link repair and by correct timing of exogenous DNA injection (Likhacheva et al, 2007). Delivery of exogenous fragments into the cell during excision repair when a DSB and a single-strand region arose in the close proximity to the cross-link site was crucial.

Immunodetection of the foci formed by the modified γ-H2AX histones and recombination factors provided evidence indicating that the time DSBs appeared after radiation and chemotherapy was incomparable. Foci promptly appeared in response to high-dose radiation. Foci were undetectable 6 hrs after treatment with a cytostatic (mytomycin C, for example) and their peak was at 12 hrs after it (Rogakou et al, 1999; Niedernhofer et al, 2004).

The above concept and literary data are consistent with our idea. If the blood bed of totally radiated subjects contained DNA fragments, these fragments would be used as substrate in homologous recombination for repair of radiation-induced damage. Exogenous DNA fragments must be delivered at the same time as the foci of phosphorylated histones, the markers of arisen DSBs, appear.

The classical experiments with injection of a suspension of bone red marrow cells or a blood stem cell (BSC) enriched fraction to lethally radiated mice have demonstrated that colonies, each of which was offspring of a single BSCs delivered by injection, appeared in their spleens (Afanasiev et al, 2004). We proceeded on the following assumption. If BSCs were capable of capturing exogenous DNA, their injection to radiated mice followed by its use as substrate for homologous recombination in BSCs with incurred multiple DSBs would rescue a part of BSCs from programmed cell death, and they would form spleen colonies to give rise to differentiated offspring, and the restored immune system would beneficially affect the viability of the treated mice.

Here, we demonstrate that internalization of exogenous DNA in stem cell (SC) nuclei is feasible, providing the human embryonic SC as an example. We also demonstrate the radioprotective effect of exogenous DNA. Survival of lethally radiated mice that had received therapeutic DNA fragments from different sources reached 70-90% (after 30 days) in some of the replicates, and numerous colonies formed in their spleens. Both observations evidence that BSCs are rescued in amounts sufficient for recovery of the immune system of the treated mice.

II. Materials and methods

A. Cell culture

Colonies of a human embryonic stem cell (ESC) strain hSSMO1r were cultivated on gelatin covered 35 mm Petri dishes (Costar) in KODMEM medium (Invitrogen) containing 20% of serum substitute (Invitrogen), 0.1mM β-methapoethanol (Sigma), 1mM glutamine (Hyclone), 1x mixture of essential amino acids (Gibco), 4 ng/ml bFGF (Chemicon), and antibiotics under 6.5% CO_2 using a feeder of mitotically inactivated mouse embryonic fibroblasts.

B. Preparation of DNA and precursor

Twenty mkg of human placental DNA fragmented to 200-2000 bp were labeled by nick translation in the presence of Klenow fragment, 3 unlabeled and 1 P^{32}-labeled dNTPs. To remove the unlabeled precursor DNA was isopropanol precipitated two times according to Glover's procedure (1985). The yield of labeled DNA after purification was 15-18 mkg. DNA was diluted in an appropriate volume of distilled water and added to each 35 mm Petri dish in a volume not greater than 20 μl. Each Petri dish contained 1-3 ml of medium. An aliquot of labeled probe (1 μl) was taken for radioactivity counts.

An αdNTP* aliquot was diluted in appropriate H_2O volume; 1 μl of diluted triphosphate was taken for determination of radioactivity. About the same amount of α related to DNA with respect to cpm and volume was added at each experimental point.

Estimates for DNA (mkg, cpm) and αdNTP* (cpm) are given in **Table 1**.

Restriction DNA fragments of Carnegie 20-λ1.4(×8) plasmid DNA (Baricheva et al, 1996) were labeled in the presence of Klenow fragment, 3 unlabeled dNTPs and P^{32}-labeled dATP at the cohesive end resulting from SalGI digestion. After filling of the cohesive ends with unlabeled dATP and formation of the blunt ends, DNA was freed from the unlabeled precursors by double precipitation from 0.3 M NaAc with a 0.6 volume of isopropanol.

C. Treatment of fragmented DNA cells, preparation of cell extracts, analysis of cell compartment DNA

Labeled DNA treated as described above was added to culture medium in Petri dishes. Cell number per Petri dish was about $5x10^5$ on average. Cells were incubated with human DNA under 6.5% CO_2 for the required time at 37°C. The chosen

incubation time was 0 (P^{32}-labeled DNA added to medium kept on ice in Petri dish, medium promptly removed, Triton X-100 added); 1 h; 2 h; 3 h; 6 h; 12 h with variations from one experiment to another. Incubation time was 12 h for αdATP*. The DNA amounts in incubation medium per point are given in **Table 1**.

Petri dishes were placed on ice after incubation completion, medium was removed, Triton X-100 in buffer A was promptly added (buffer A containing 2 mM $CaCl_2$, 0.5% Triton X-100); 1 ml of lysis buffer was added to each Petri dish (Roberts, 1986). Cells were kept in lysis buffer on ice for 10 min. Lysed cells were resuspended several times and fractionated by 10% sucrose gradient centrifugation in buffer A. Cells were centrifuged 20 min at 600 g (2000 rpm) in 25 ml conical tubes using centrifuge K23 (bucket-rotor R-15). Supernatant, a cytoplasmic fraction, was transferred to a separate tube and isopropanol precipitated. Nuclear residue was washed two times in 250 ml of lysis buffer (buffer A containing 0.5% Triton X-100). After each washing, cell preparation was centrifuged 10 min at 600 g (2000 rpm) in a bucket-rotor.

In experiments with total fragmented human DNA as substrate nuclear fraction was separated to chromatin and interchromosomal fraction. Nuclei were resuspended in 500 μl of buffer A, examined cytologically, and transferred to tubes. Up to 2 M of NaCl and 1% SDS were added to nuclear suspension. Nuclear lysate was incubated for 30 min without shaking at 65°C until reaction mixture clarified, then it was centrifuged at 52,000 g (21,000 rpm) in Beckman J2-21 centrifuge (rotor JA-21) for 30 min at 30°C. Supernatant (interchromosomal material), was pipetted off and transferred to another tube; a 1:10 NaAc volume was added to supernatant, and it was precipitated with a 0.6 volume of isopropanol. 100 μl of water was added to transparent lentil-shaped residue, a chromosomal fraction; the mixture was left to swell for 60 min and immediately subjected to electrophoresis. All the procedures were performed at 0°C. After treatment, all samples were immediately isopropanol precipitated from 0.3 M NaAc into 25 ml conical tubes and centrifuged at 4000g (4 500 rpm) using centrifuge K23 (bucket-rotor R-15) for 20 min. Precipitate was dissolved in 100 μl of water. Amounts of labeled material were determined by the standard method using a 1209 RacBetta counter (Finland). Samples were separated on 0.7% agarose gels. Chromosomal fraction DNA was not dissolved to completion; to minimize DNA degradation, it was left to swell for 60 min. The jellyfish-like undissolved, yet swollen, chromatin residue was immersed into the agarose block. Chromosomal DNA was fixed at the start during DNA electrophoresis.

In case of SalG1 digested Carnegie 20-λ1.4(×8) DNA hybrid plasmid we have used the following procedure. Nuclear samples embedded in blocks of low-melting agarose, 80 μl in volume, were kept in an excessive amount of 20 mM EDTA for several days and the entire exogenous DNA, if retained in a sample as contaminant, inevitably diffused into storage buffer. There were about $2.5x10^5$ nuclei per agarose block. Before electrophoresis, agarose-fixed nuclei were treated with 1% SDS, 20 mM EDTA, and 200 mkg/ml ProtK for 2 h at 37°C and washed three times, 10 min each, in TE buffer. Then DNA was fractionated in 1.0% agarose gel.

After electrophoresis, agarose block was dried under a stream of hot air. Gels were exposed to X-ray Kodak films overnight or for a time dependent on the amounts of labeled material.

D. Quantitation of experimental results was based on the following values

Cell number in human ESC culture at each experimental point, $5x10^5$ DNA content per cell, 6 pg.

Total DNA content in cells at each experimental point, 3 mkg.

Amount of labeled DNA added at experimental point indicated in **Table 1**. Radioactivity counts at a point for added DNA expressed as cpm in **Table 1**.

Radioactivity counts at a point for αdNTP* expressed as cpm in **Table 1**.

Human cell haploid genome, $3.3x10^9$ bp

Size of the labeled genomic DNA fragments added to medium initially was 200-2000 bp.

We analyzed the qualitative and quantitative parameters for the behavior of extracellular DNA during its cell entry.

E. Radioprotection experiments

Three-months old CBA/Lac females maintained and bred at the animal facility of this Institute were used in the experiments; 9-10 mice were kept in separate plastic cages. Water and food were freely available. Their chow was standard granulated PK120-1 (Laboratorsnab, Moscow). They were radiated with a γ-radiation unit IGUR-1, Russia (Cs^{137}). Four experiments were performed.

In the first three experiments, different doses of nucleic acids and DNA of different origin were tested. DNA from CBA organs or human placenta DNA was administered i.p. to mice before / after radiation according to the schemes shown in **Table 2**. Radiation dose was lethal.

In experiment 4, the effect of human placenta DNA on RBSCs was examined in mice exposed to a sub-lethal dose radiation. These received i.p. human placenta DNA before radiation (**Table 2**). Mice were sacrificed on day 10, and the number of spleen homopoietic colonies was determined. Bone marrow is a radiosensitive tissue and, consequently, γ-ray exposed BSCs died. It was assumed that, by having gained access to the cells and becoming involved in repair, exogenous DNA allowed BSCs to survive. The rescued BSCs migrated to colonize lymphoid organs, such as lymph nodes, spleen, thymus, among others. This promoted further hemopoiesis recovery and mouse survival. It was also assumed that high homology between the mouse and human genomes would make feasible homologous recombination repair for healing of DSBs induced by ionizing radiation.

Table 1. Amounts of DNA* and αdATP* used in experiments.

Amount per experimental point (added to medium)	**mkg**	**cpm**
DNA*	2.7	$4.53x10^6$
αdATP*		$1.05x10^7$

Table 2. Time before / after radiation and amount of DNA administered to CBA mice.

Experiment number		1				2		3				4	
Dose		9.6 Gy (1.4 Gy/min)				9.5 Gy (1.3 Gy/min)						8.21 Gy (1.3 Gy/min)	
Preparation		Saline	CBA mouse DNA		Human DNA	Saline	CBA mouse DNA	Saline	CBA mouse DNA		Human DNA	Saline	Human DNA
Group		1	2	3	4	1	2	1	2	3	4	1	2
Treatment scheme	Before radiation		1 mg 30 min before			0.2 ml 20 min before	1-2 mg 20 min before	0.2 ml 10 min before	1 mg 20 min before		1 mg 20 min before	0.2 ml 30 min before	1 mg 30 min before
	After radiation	0.2 ml 30 min after and next 2 days		1 mg 30 min after and 0.5 mg next 2 days	1 mg 30 min after and 0.5 mg next 2 days	0.2 ml 30 min after and next 2 days	0.5 mg 30 min after and next 2 days	0.2 ml 30 min after and next 2 days		0.5 mg 30 min after and next 2 days	0.5 mg 30 min after and next 2 days	0.2 ml 30 min after and next 2 days	0.5 mg 30 min after, 0.25 mg on day 2, 0.17 mg on day 3

III. Results

A. Capture of exogenous DNA by SCs and their internalization in nuclear space

We analyzed the ability of SCs to capture exogenous DNA of different origin. For this purpose, we chose hSSMO1r human ESCs. To detect DNA internalization into intracellular space, fragmented human DNA was used. The fragments were as long as DNA apoptotic fragments present in blood plasma and multiples of DNA length forming a nucleosome (**Figure 1**, right blocks, 7-4*) and the SalG1 digested Carnegie 20-λ1.4(×8) DNA hybrid plasmid (**Figure 2**, right blocks, Carnegie 20-λ1.4(×8)SalG1) (Baricheva et al, 1996; Rogachev et al, 2006) with 1.4 kb and 10.8 kb marker fragments. Both substrates were labeled with P^{32}.

In the past decade, it has been established that there are several ways for DNA penetration into the intracellular space. One is by capture of DNA-containing apoptotic bodies. This is actually either the task of professional phagocytes or simply of tissue cells in the immediate vicinity to the apoptotic bodies (Lawen, 2003; Savill et al, 1993). The other way is receptor-mediated, and it provides penetration of extracellular DNA material into the intracellular compartments of the eukaryotic cell. Two major types of receptors on the cytoplasmic membrane provide this way of entry. Their molecular mass is 33 (30) and 79 (80) kDa (Benett et al, 1985; Loke et al, 1989; Zamecnik et al, 1994). Once bound to the receptor, the ligand becomes internalized within the acidic cellular compartment via pinocytosis and is delivered to the nucleus (Shestova et al, 1999; Lin et al, 1985). A variety of other proteins differing by molecular mass are involved in binding and uptake of nucleic acids by the cell (for reference, see Chelobanov et al, 2006). These are mainly different membranes proteins, also nuclear proteins and blood plasma proteins. Relevant findings were membrane channels formed by transmembrane proteins through which nucleic acids are directly transported. It is noteworthy that DNA has access to the cell in a short time (just a few sec or min) (for references, see Shestova et al, 1999; Ledoux, 1965). There is ample evidence indicating that the process is unrelated to the degradation of DNA and its subsequent resynthesis in the cell.

As reported for MCF-7 mammary adenocarcinoma cell culture (Rogachev et al, 2006), human ESCs captured exogenous DNA from the culture medium and, using the natural cellular mechanism, internalized exogenous DNA fragments into intracellular space. The compartments we analyzed were cytoplasm, interchromosomal fraction, and chromatin. Human ESCs efficiently captured exogenous DNA of different origin delivered to nuclear space (**Figures 1, 2**). Once within the nucleus, exogenous DNA became degraded at 0 point. Nevertheless, using the digested plasmid as substrate, we detected labeled fragments of size commensurate with that of substrate initial fragments in the interchromosomal space. Delivered DNA fragments were stored in the nucleus and not digested by nucleases.

The procedure for isolation of fragmented human DNA fraction internalized into nuclear compartment was essentially the same as previously described (Rogachev et al, 2006). Nuclei were lysed in suspension and promptly fractionated into chromatin and interchromosomal fraction. Exogenous fragments were internalized slower than in the case of MCF-7 cells. The DNA fragments delivered to the interchromosomal space are end joined, forming concatomers (**Figure 1B**, right block, interchromosomal fraction, 2, 6, 12). The time that joined DNA fragments were internalized into the nuclear space and of that of concatomers' formation differed greatly from the reported for MCF-7 mammary adenocarcinoma cells (Rogachev et al, 2006). As follows from the results, after 1 h exposure of cells with substrate, the DNA fragments within the nucleus retained their native mobility with no concatomeric joining. From 2 up to 12 h, a part of the fragments were joined, forming a fragment longer than 10 kb. Some of the fragments were in the migration zone of 200-300 kb, a possible evidence of continuous inflow of exogenous labeled material from culture medium to nuclear space (**Figure 1B**, right block, interchromosomal fraction, 0, 1). Labeled material was consistently found in the chromatin fraction (**Figure 1B**, right block, chromatin, 1, 2, 6, 12). In the cytoplasmic fraction, cell samples exposed to labeled substrate for different times showed no visible changes in amounts or in size of DNA.

In another experiment we used Carnegie 20-λ1.4(×8) clone plasmid DNA as substrate. Its SalG1 digestion liberated fragments of 1.4 and 10.8 kb. Copy number for 1.4 kb fragment was 8 monomers per plasmid. After incubation of ESCs with plasmid DNA cell nuclei were purified by gradient centrifugation, thoroughly washed,

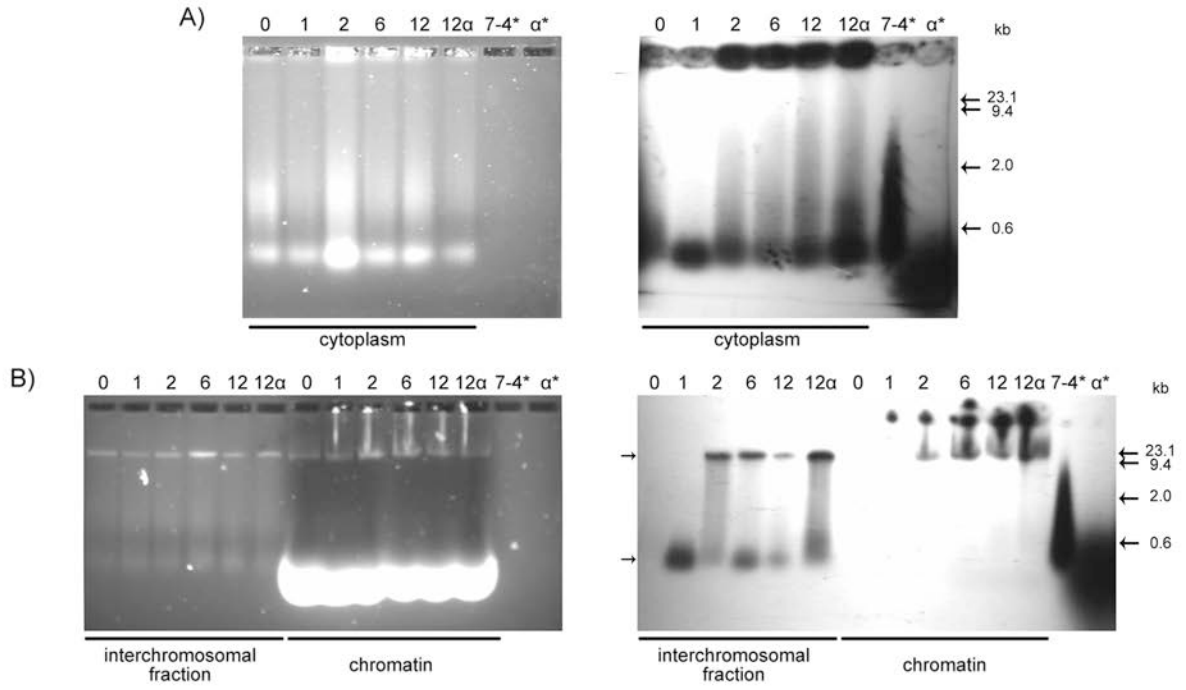

Figure 1. Analysis of the distribution among cellular compartments of hSSMO1r embryonic stem cell culture labeled fragmented human DNA, depending on the time of its presence in culture medium. **A)** cytoplasmic fraction; **B)** interchromosomal fraction and chromatin. Left, ethidium bromide stained blocks of agarose. Right, X-ray pattern of these blocks after drying. Numbers above blocks indicate incubation time (hrs) of cell culture with α-P^{32} labeled human extracellular DNA and αdATP* (12α). 7-4*, fragmented human DNA used for incubation with cell culture; α*, initial precursor αdATP*. Arrows indicate fragments of initial and processed DNA in interchromosomal space.

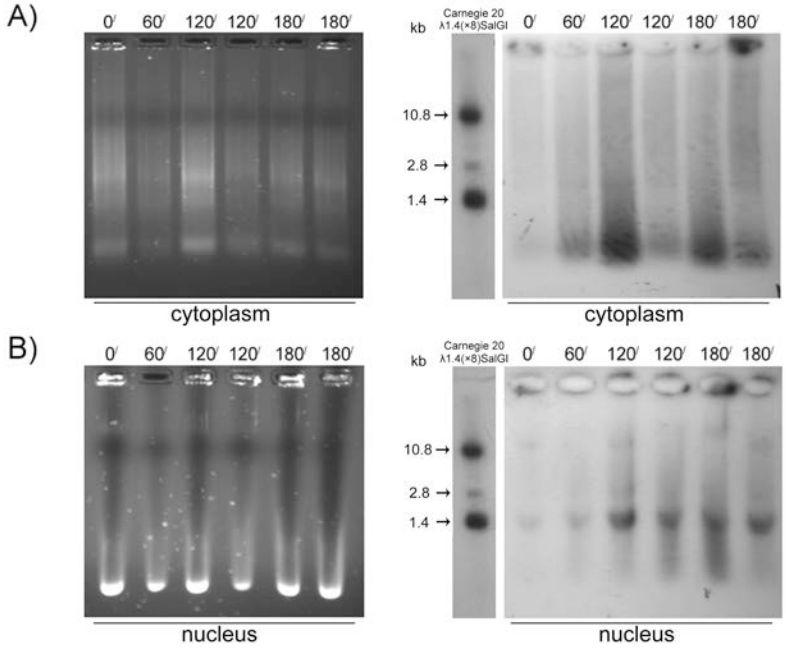

Figure 2. Analysis of the distribution among cellular compartments of hSSMO1r embryonic stem cell culture of two labeled individual plasmid DNA fragments (Carnegie 20-λ1.4(×8)SalGI) of 10.8 kb and 1.4 kb, depending on the time of its presence in culture medium. **A)** cytoplasmic fraction; **B)** nuclear fraction. Nuclei were fixed in low-melting agarose blocks and treated with PrK in lysis buffer. Freed nuclear material was separated in 1.0% agarose. Left, ethidium bromide stained agarose blocks. Right, X-ray pattern of these blocks after drying. Numbers above blocks indicate incubation time (min) of cell culture with α-P^{32} labeled fragments. Electrophoregram shows that heterologous plasmid and Drosophila DNA penetrates into the internal nuclear compartments, as observed for allogenic human DNA.

and embedded into agarose blocks. The embedded nuclei were lysed, PrK digested, and DNA was fractionated in 1.0% agarose gel. It was suggested that the whole cellular chromatin was intact, and it would not migrate in gels because of linear size; exogenous DNA fraction internalized into the nuclear space would migrate in gel according to molecular weight. As the results showed, plasmid DNA was already seen unaltered at the 0 experimental point in the nuclear space, 1.4 and 10.8 kb fragments being both present. Labeled material seen in the cytoplasmic fraction was a presumably degraded DNA fragments added to culture medium. Fragments of 1.4 and 10.8 kb and multimeric copies of concatomerized 1.4 kb fragment were also seen at time points 60, 120, and 180 min in the nuclear fraction (**Figure 2**). Once again, the results provided that the ESCs, like MCF-7 cancer cells, can capture exogenous DNA in culture medium and internalize the fragments into intracellular space. The following observations are noteworthy. In the case of MCF-7 cells, 1.4 and 10.8 kb fragments were seen intact in the intracellular space in the presence of salmon sperm competitor DNA only, which presumably blocked nuclease effect in culture medium, and at the point 0 only. In the case of hSSMO1r ESCs, undegraded DNA fragments were detected in the nuclear fraction at all the 0, 60, 120, and 180 min points; this can be explained by the very low nuclease concentration in medium or by the fact that the initial undegraded DNA fragments first delivered to nuclear space filled up interchromosomal volume and the other DNA molecules simply could not enter nucleus until those already there were all utilized.

We estimated the amounts of labeled material delivered to cellular compartments using genomic DNA fragmented to 200-2000 bp as substrate (**Table 3**). Exogenous DNA can reside as fragments making up 0.05% of the cell genome, or ~ 1700 kb, in the interchromosomal space of hSSMO1r cells. Taking into account the 200-2000 bp size of exogenous DNA fragments, about 8000-800 fragments were consistently present in the interchromosomal space of this cell type. This amount of internalized DNA was of 1.5 orders of magnitude less than for MCF-7 human adenocarcinoma cells, the difference may be ascribed to the biological features of these cells.

In control samples, we analyzed the ability of mitotically inactivated mouse embryonic fibroblasts (MEF) to deliver exogenous DNA to intracellular space. It was found (data not shown) that the analyzed compartments contained no labeled material, and this was evidence that MEF had no adequate system for DNA delivery to these cells.

It follows that ESCs can capture exogenous DNA and internalize it into nuclear space. This suggested that internalized DNA fragment could be used, in certain conditions, by the cell repair system as substrate for homologous recombination in repair of induced DSBs. We have reported that human DNA indeed integrated into the adult mouse genome, when treated with the cross-linking cytostatic cyclophosphan in combination with fragmented human DNA (Likhacheva et al, 2007). In the current study, the inducer of DSBs was high-dose radiation.

B. Radioprotective action of fragmented exogenous DNA

Death of BSCs that leads to irrecoverable leuko-erythropenia and ultimately death is the most serious consequence of ionizing radiation. In the current experiments, lethally radiated mice received injections of exogenous DNA (**Figure 3**). Of the 7 performed experiments, 4 proved to be successful. The correct timing of exogenous DNA administration for radioprotection was at first unknown. This might have been why some of the replicates failed. A more powerful unit allowed us to choose the lethal-dose within 5-10 min and injection of DNA preparations within 30 min after radiation provided a stable radioprotective effect. This was taken to mean that cell utilized during repair the delivered substrate of fragmented DNA. Doses of nucleic acids and DNA of different origin (isolated from mouse organs, human placenta) were tested in 3 experiments. In addition, fragmented DNA was administered either before or after radiation (**Table 2**). Mouse longevity was registered in each group. Survival of mice injected with exogenous DNA reached 70-90% (after 30 days), varying depending on the human or mouse origin of DNA substrate. **Figure 4** presents data on the effect of mouse and human DNAs on the survival of mice exposed to lethal-dose radiation. The grey-haired survivors lived to the end of the observation period, 1.5-2 years (**Figure 4D**).

Table 3. Quantitative characterization of labeled material in cell compartments depending on incubation time of cells with labeled substrate.

Incubation time, h	**0**	**1**	**3**	**6**	**12**
Cytoplasmic fraction (absolute count, cpm)	7176	6724	13344	15987	19431
% of amount added to medium	0.16%	0.15%	0.29%	0.35%	0.43%
Amount in mkg calculated from %	0.004	0.004	0.008	0.0094	0.012
Interchromosomal fraction (absolute count, cpm)	429	2318	3029	2812	1777
% of amount added to medium	0.009%	0.05%	0.06%	0.06%	0.039%
Amount in mkg calculated from %	0.0002	0.0013	0.0016	0.0016	0.001
% of the genome	0.007%	0.04%	0.05%	0.05%	0.03%
Chromatin (absolute count, cpm)	217	928	2064	5463	6308
% of amount added to medium	0.005%	0.02%	0.06%	0.12%	0.14%

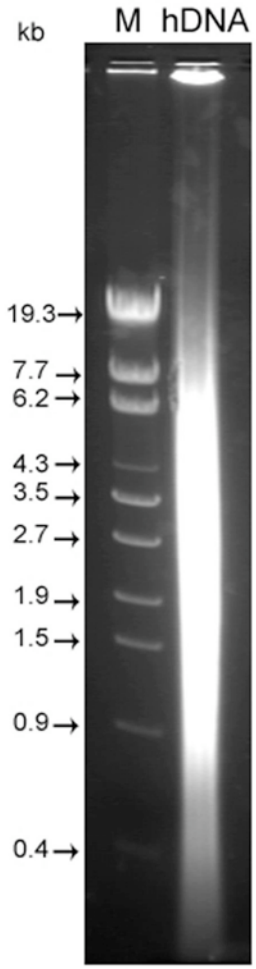

Figure 3. Electrophoretic characterization of exogenous therapeutic placental human DNA used for injection into treated mice. M, λDNA BssT1I digest molecular weight marker.

C. Formation of spleen colonies in lethally radiated mice under the effect of exogenous DNA

Here, we consider the radioprotective effect of exogenous DNA on BSCs in mice exposed to radiation at sub-lethal doses. Because bone marrow is a radiosensitive organ, γ-rays are deadly for BSCs. On our assumption, by gaining access to the cells and becoming involved in repair process, exogenous DNA gives BSCs chances to survive. The survived BSCs would migrate and colonize lymphoid organs such as lymph nodes, spleen, and thymus. This would further promote hematopoiesis and increase longevity. It was expected that differentiated BSC offspring that survived treatment with exogenous DNA would form spleen colonies distinctly seen and whose presence would be the hallmark features of BSC rescue from apoptotic death induced by high-dose radiation.

Consequently, the radioprotective effect of exogenous DNA was due to rescue of BSCs whose offspring gave rise to individual colonies in spleens of radiated mice (**Figure 5, Table 4**). The average number of spleen colonies increased by 18 times after i.p. DNA administration as compared with the control. The effect of administered DNA was specific in that it either occurred or not in a particular individual. It is encouraging that 50% of the treated mice responded favorably to treatment. Despite small sample size and the wide variability in spleen colony number in the treated group, the differences between the samples were significant at $p > 0.95$.

IV. Conclusions

SCs can capture exogenous extracellular DNA from the cell environment. The amount of exogenous DNA fragments internalized into the intracellular space was 0.05% of the eukaryotic cell genome, i.e. 1700 kb, or ~ 800-8000 fragments of 2.0-0.2 kb. When administered to lethally γ-radiated mice, exogenous intracellular DNA afforded a very strong radioprotective action. These radiated mice were rescued by BSCs whose survival was due to the beneficial effect of DNA preparation that gave rise to SC colony formation in spleen. Variations in individual sensitivity in the mouse experiments depended on availability of therapeutic DNA to BSCs at the time DSBs were induced. DNAs of different origin exerted a radioprotective action. This was presumably because repair was of the gene conversion type whereby a Holliday junction formed with crossover or without formation of the junction; in the latter case, repair synthesis on homologous substrate caused an elongation of the RAD 51 filament of the 3'-processed double-strand end following by joining of the homologous region at the other end of DSB (synthesis-dependent strand annealing) (Bartsch et al, 2000; Symington, 2005; for reference see also Abaji et al, 2005); homologous repair of this type does not require extensive homology between the processed end of the DSB site and molecule substrate. In the current experiments, numerous homologous regions in the mouse and human genomes allowed the SC repair machinery to utilize human DNA substrate for repair of radiation induced DSBs.

SC capacity to capture exogenous DNA and to deliver it to the nuclear space implies the involvement of exogenous DNA fragments as substrate for homologous recombination repair of DSBs in progenitor cells induced by high-dose γ-radiation exposure.

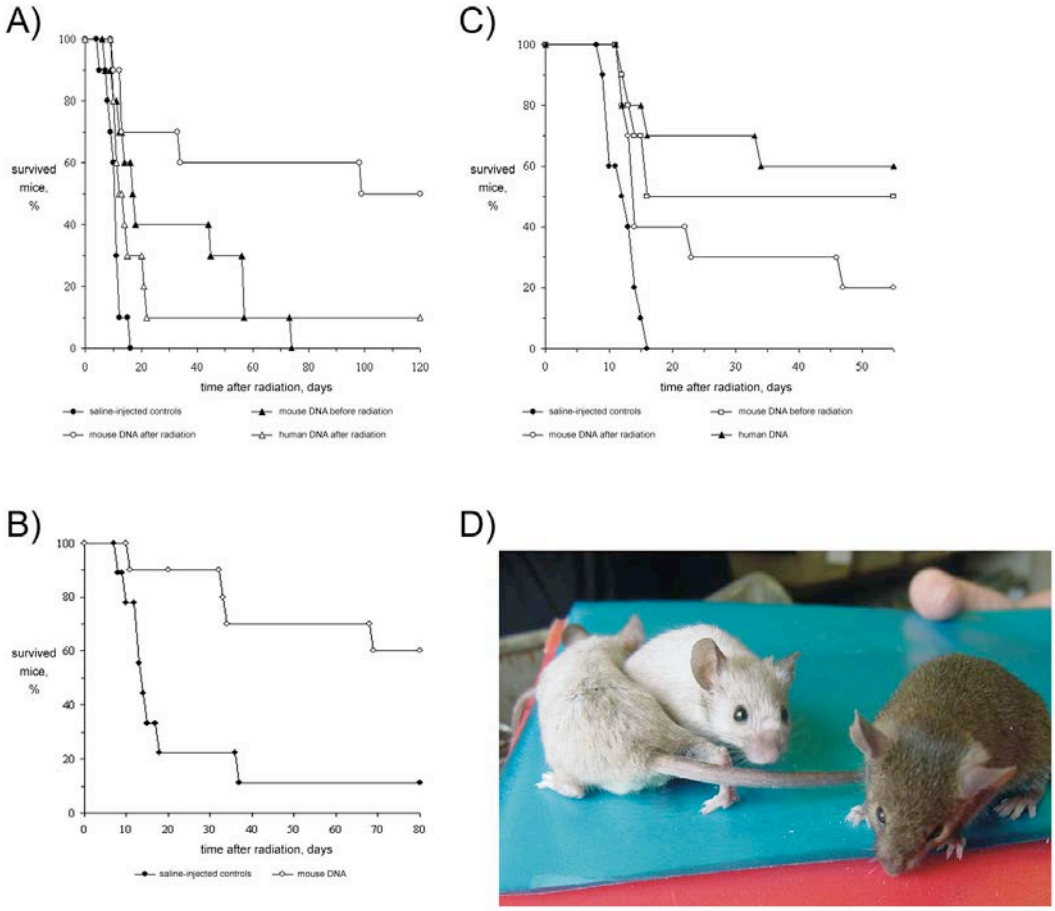

Figure 4. Effect of mouse and human DNA i.p. administered to mice before and after lethal-dose radiation on mouse survival. Experiments: **A)** № 1 (Yakubov et al, 2003), **B)** № 2, **C)** № 3; **D)** mice grown grey after exposure to lethal-dose radiation and treatment with allogenic DNA (compared with coat color of untreated CBA mice).

Table 4. Number of spleen colonies formed in mice under the effect of exposure to sub-lethal dose radiation combined with exogenous DNA therapy as compared with the untreated controls.

Control mice	0	0	0	0	0	1	1	1	1	2	**0,6**±0,22
Treated mice	0	0	1	3	4	5	9	24	30	33	**10,9**±4,09

Figure 5. Spleens from mice exposed to sub-lethal dose radiation treated with exogenous DNA **(A)** and untreated **(B)**. Arrows indicate spleen colonies appearing after treatment of radiated mice with extracellular DNA from human placenta.

Acknowledgements

The authors are grateful to Maria Lagarkova for providing them with cell culture hSSMO1r. They are also grateful to Anna Fadeeva for translating the manuscript from Russian to English.

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