Mechanism of Targeted Migration of Mesenchymal Stem Cells

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Experiments performed on mice C57Bl/6 with the use of GFP technology showed that activation of apoptosis is a potent physiological stimulus for targeted migration of mesenchymal stem cell from the blood to tissues. The effect of apoptosis-induced targeted migration of stem cells can be used in cell therapy of damaged organs and tissues in various pathological states.

Key Words: *apoptosis; cell therapy*

A direct relationship was found between the intensity of apoptosis and the rate of cell renewal in tissues [4]. Apoptosis, in contrast to necrosis, triggers a cascade of reactions stimulating proliferation of cells of different degree of maturity. The existence of the relationship between apoptosis intensity and targeted migration of progenitor SC suggests that artificially induced programmed cell death can trigger enhanced migration of mesenchymal SC from the blood into tissues exposed to apoptosis-inducing influences.

MATERIALS AND METHODS

Experiments were carried out on fibroblast culture and C57Bl/6 mice. In experimental series I, cultured fibroblasts were exposed to laser in doses inducing cell apoptosis and necrosis [3]. Apoptosis was evaluated as described elsewhere [1]. We also evaluated DNA fragmentation [5] and generation of reactive oxygen species [6]. BM cells were isolated from the femoral and tibial bones of C57Bl/6 mice expressing GFP protein [2]. In experimental series II, the skin of one ear was exposed to laser and the other ear served as the control. Nembutal-narcotized mice received a single injection of GFP-positive cell suspension into the jugular vein. The animals were decapitated under ether narcosis 1 day after GFP-positive cell injection. The preparations were examined under an LSM5 PASCAL microscope (Carl Zeiss). The results were processed using Student *t* test.

RESULTS

It was found that laser exposure in doses of 4 and 6 J/cm^2 (alexandrite) triggered DNA destruction in cells, but not in DNA solution. The concentration of reactive oxygen species in cells did not decrease. Irradiation in doses of 8 and 10 J/cm^2 increased the number of DNA breaks in solution and fibroblasts by 2 and 3 times, respectively. Exposure to a dose of 10 J/cm^2 induced oxidative burst in cells. Irradiation of fibroblast culture with a dye laser produced a pronounced destructive effect on DNA in cells and solution and activated lipid peroxidation processes in culture. Irradiation with alexandrite and dye lasers in doses of 6 and 4 J/cm^2 produced maximum apoptotic effect on fibroblasts (Table 1). The relative content of cells with signs of apoptosis was increased by 4 and 5 times, respectively. Increasing the irradiation dose was associated with a decrease in the number of apoptotic cells and accumulation of fibroblasts with signs of necrosis. In the control ear, a solitary GFP label was detected (Fig. 1). Single exposure to alexandrite laser in a dose of 6 J/cm^2 induced massive migration of GFP-labeled cells into the skin (Fig. 2); increasing the dose to 8 J/cm^2 led to tissue destruction and a decrease in the number of GFP-labeled cells (Fig. 3). Exposure

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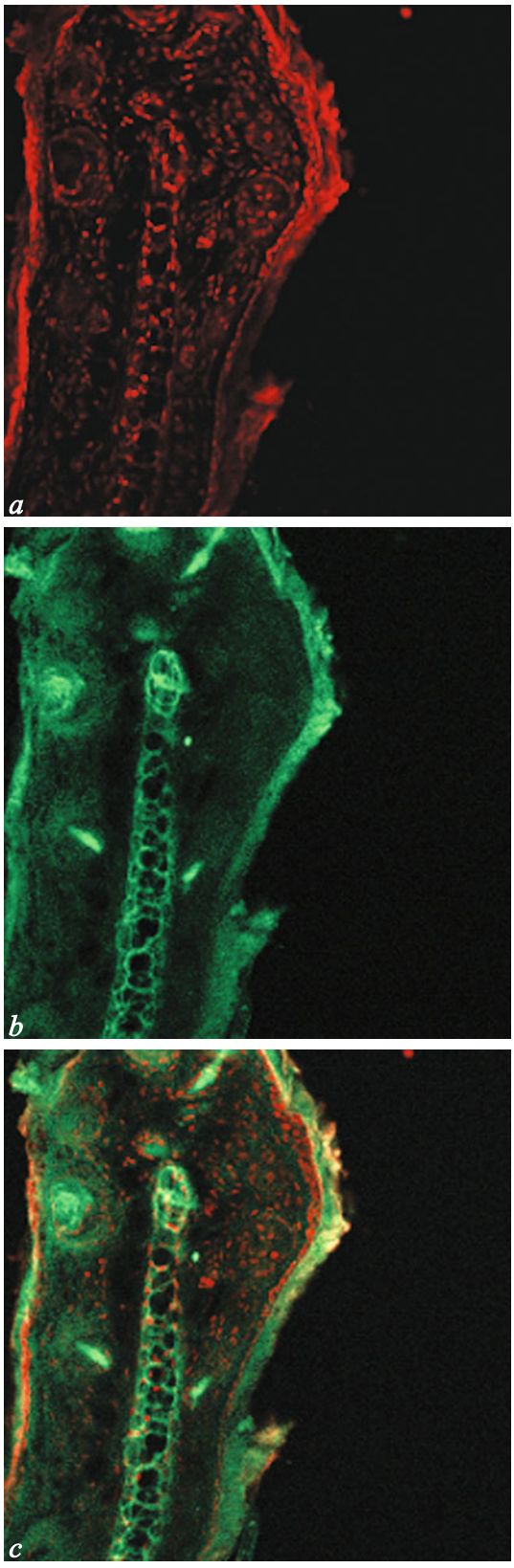

Fig. 1. Expression of GFP in the skin of control ear in a mouse: nuclei are post-stained with propidium iodide (objective ×10). Here and in Figs. 2, 3: *a*) propidium iodide staining of nuclei; *b*) natural GFP fluorescence; *c*) superposition of images.

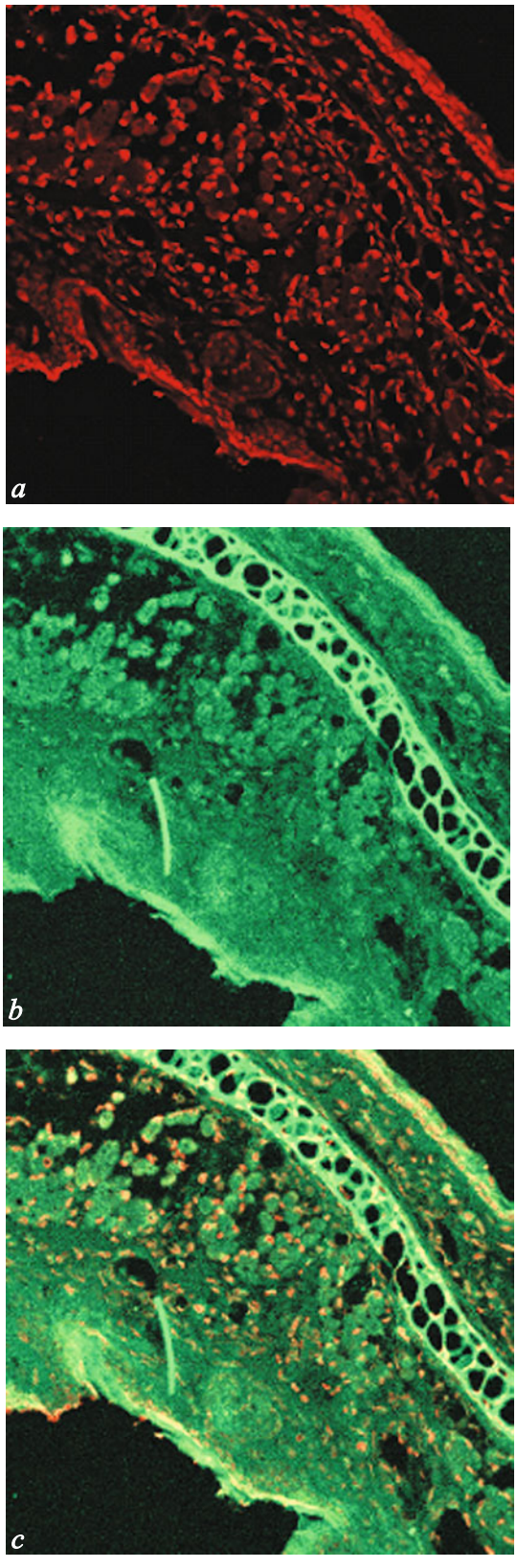

Fig. 2. Expression of GFP in mouse ear exposed to alexandrite laser (6 J, 1 pulse, objective ×10).

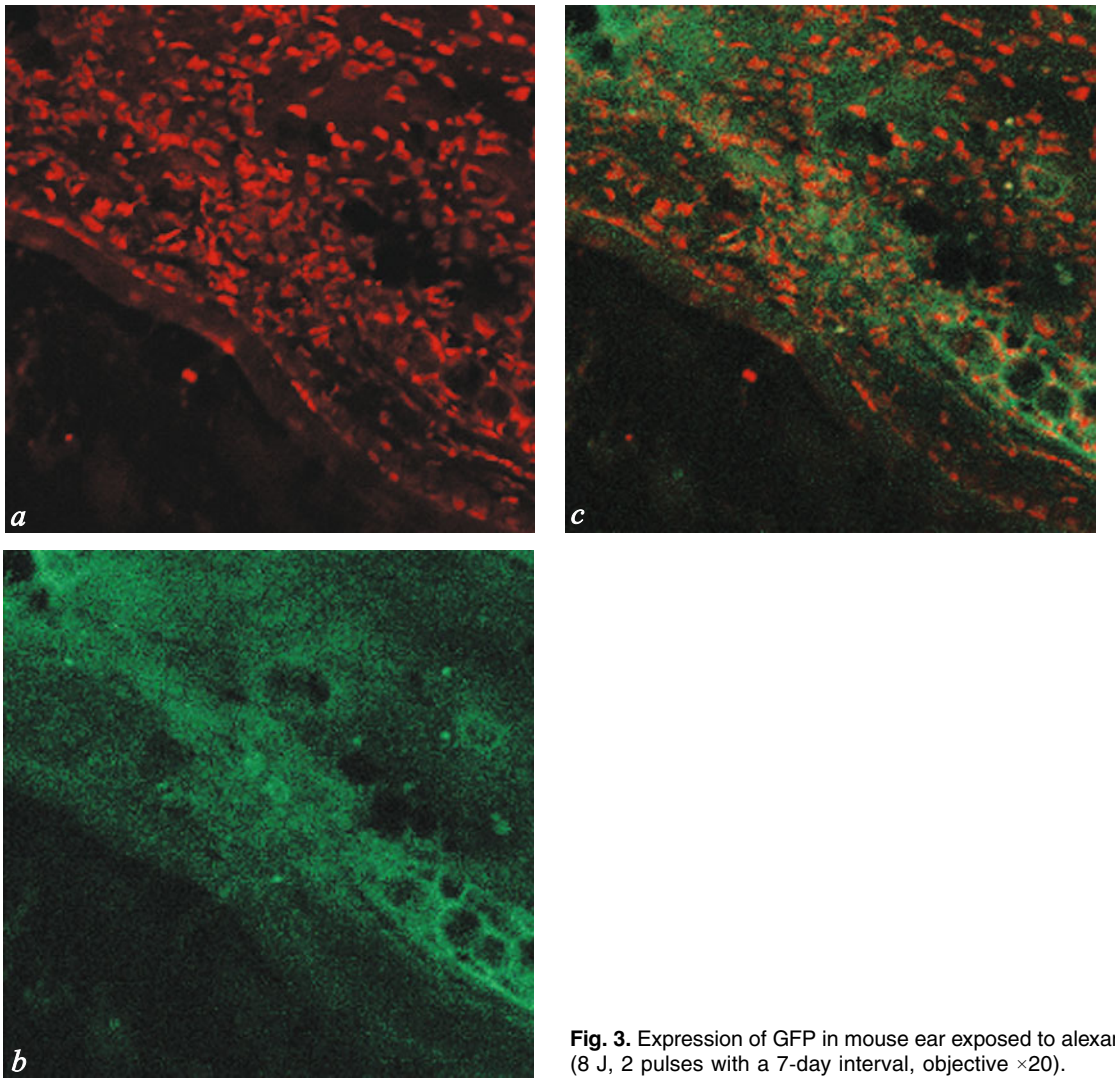

Fig. 3. Expression of GFP in mouse ear exposed to alexandrite laser (8 J, 2 pulses with a 7-day interval, objective ×20).

TABLE 1. Proportion between Apoptosis and Necrosis of Fibroblasts before and after Irradiation (n=10)

Laser; pulse dose, J/cm²		Apoptosis, %		Necrosis, %	
		before irradiation	experiment	before irradiation	experiment
Alexandrite (λ=755 nm)	6.0	6.8±1.2	28±2.0**	3.1±0.5	4.5±0.5
	8.0	6.8±1.2	14.7±1.0**	3.1+0.5	3.6±0.5
	10.0	6.8±1.2	13±2.0*	3.1±0.5	5.6±1.0*
Dye (λ=595 nm)	4.0	6.8±1.2	37±3.0**	3.1±0.5	4.0±0.5
	4.5	6.8±1.2	25±2.0**	3.1±0.5	4.0±0.5
	5.0	6.8±1.2	16±2.0**	3.1±0.5	7.0±1.5**

Note. *p<0.05, **p<0.01, in comparison with values before irradiation.

to dye laser in doses of 4 and 5 J/cm² resulted in focal cartilage destruction and tissue exfoliation in the ear. Solitary GFP-positive cells were detected only after single exposure to 4 J/cm².

Thus, exposure to alexandrite laser in a dose inducing apoptosis in fibroblast culture stimulated migration of mesenchymal cells from the blood into the skin. The destructive (necrotic) changes in the skin

block this process. These findings suggest that initiation of apoptosis in tissues can be considered as a physiological mechanism of targeted attraction of SC from the circulation into target tissue of the body. The signal molecules attracting mesenchymal SC into tissue appear as a result of programmed cell death. The effect of apoptosis activation as a "decoy" for mesenchymal SC is a universal mechanism and works also in other organs and tissues.

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