Effects of ^{60}Co Whole-Body γ-Irradiation in Different Doses on the Distribution of ^{188}Re-Labeled Autologous Mesenchymal Stem Cells in Wistar Rats after Intravenous (Systemic) Transplantation during Different Periods after Exposure

A. G. Konoplyannikov, V. M. Petriev, O. A. Konoplyannikova, S. Sh. Kal'sina, L. A. Lepechina, O. A. Smorizanova, I. V. Semenkova, and E. V. Agaeva

Translated from *Kletochnye Tehnologii v Biologii i Meditsine*, No. 2, pp. 90-96, April, 2008
Original article submitted December 19, 2007

The effects of whole-body γ-irradiation in different doses on the distribution of ^{188}Re-labeled mesenchymal stem cells obtained by culturing of the rat bone marrow cells were studied in different organs and tissues of animals after intravenous (systemic) injection. Irradiation stimulated homing of ^{188}Re-labeled mesenchymal stem cells in organs and tissues of animals in comparison with this process in intact non-irradiated rats. The intensity of homing increased with increasing the irradiation dose and decreased with prolongation of the period between irradiation and systemic transplantation. It was hypothesized that increased migration of transplanted mesenchymal stem cells into irradiated organs and tissues can be caused by developing cell death processes. It seems that systemic transplantation of mesenchymal stem cells shortly after irradiation can be used for stimulation of reparative processes in damaged tissues.

Key Words: *mesenchymal stem cells; rats; ^{60}Co γ-irradiation; ^{188}Re; stem cell homing; systemic cell transplantation*

Autologous and allogenic mesenchymal stem cells (MSC) are regarded as the most perspective agents for cell therapy of various diseases [5,13,19,22-24]. Intravenous (systemic) injection of MSC is considered to be a convenient and physiological method of their transplantation to recipients. This assumption is based on the hypothesis on selective accumulation of stem cells (SC) transplanted by this method in the foci of lesions, in other words, on the possibility of specific homing of MSC in various organs and tissues after injury [7,17,21], including the radiation injury [16,20]. The effects of whole-body and local irradiation in very high doses on the distribution of intravenously injected human MSC in organs and tissues were previously studied on NOD/SCID mice (animals with severe combined immunodeficiency and diabetes) [16,20]. However, these data are scanty and the total picture of radiation effects on MSC homing is little studied.

We studied this process in Wistar rats injected with ^{188}Re-labeled cultured bone marrow cells of these rats. These data are essential not only for evaluation of radiation effects on homing of syngeneic and allogenic MSC after systemic transplantation, but also for comparative study of the distri-

Medical of Radiologic Research Center, Obninsk, Russia. ***Address for correspondence:*** konopl@mrrc.obninsk.ru. A. G. Konoplyannikov

0007-4888/08/1454-0520

bution of intravenously injected MSC in organs and tissues after exposure to other factors, damaging the tissues (for example, injuries, tumor growth, *etc.*), as in future, no doubt, clinical studies on the use of systemic transplantation of MSC in the treatment of various diseases and injuries will be carried out on a wide scale. Radiolabeling of SC before their systemic transplantation has some advantages in comparison with other labeling methods (such as the use of GFP labeled cells, bromodeoxyuridine label, labeling with various dyes or magnetic particles, *etc.*), due to the possibility of quantitative evaluation of labeled cells in various organs and tissues during certain periods after their systemic transplantation, while its drawbacks are explained by the need of taking into consideration the stability of radioactive label binding to initial cells, short half-life period for the majority of isotopes used in these studies, and lack of data on spatial distribution of labeled cells, which can be provided by histological studies of various tissues (except the autoradiography methods). However, quantitative characterization of labeled cell entry in organs and tissues is now the least studied parameter of homing of transplanted MSC [25,28], which can later serve as an important factor for quantitative characterization of the paracrine mechanisms of realization of the regeneratory processes triggered by MSC homed in various organs and tissues after their systemic injection [11,27].

MATERIALS AND METHODS

Mesenchymal stem cell cultures were prepared by a previously described method for culturing of MSC from the bone marrow of Wistar rats [5]. The bone marrow was collected from the femoral bone of Wistar rats sacrificed under Nembutal narcosis (60 mg/kg). The material (~10^7 cells) was placed into tubes with heparin (100 U/ml punctate) and the erythrocytes were precipitated for 1-2 h at ambient temperature. The supernatant was then sucked off with a Pasteur pipette, isolated cells were washed in medium 199 (media, reagents, and glassware from Sigma were used for this and next procedures), centrifuged at 1000 rpm for 10 min, and the precipitate was resuspended in growth medium: RPMI-1640 with penicillin (100 U/ml), amphotericin (100 ng/ml), L-glutamine (2 mM) and 20% FCS. The cells were cultured in Carrel's plastic flasks with 25 cm^2 bottom area (5×10^6-10^7 bone marrow cells in 8 ml growth medium). The flasks were aerated with gas mixture containing 5% CO_2 and placed into a common thermostat (37°C). The flasks were aerated with this gaseous mixture during replacement of the medium or reinoculation of cells into new culture flasks. After formation of a confluent monolayer, the cells were reinoculated into new flasks using 0.25% trypsin, first with the same bottom area (25 cm^2) and then, as cell mass increased, into larger culture flasks with bottom area of 175 cm^2. Due to this method a population of rat MSC of $(1\text{-}2)\times10^8$ cells was obtained by the end of week 5. The cells were frozen in special 1-ml plastic tubes with caps (10^7 MSC in medium with 10% DMSO and 20% FCS) according to the standard procedure [3]. After freezing, the samples were stored in liquid nitrogen in a specially created MSC culture bank. Frozen MSC cultures after defrosting were used for repeated culturing and production of the needed quantity of MSC for our experiments (6 passages before use). Cytofluorometry showed that the resultant MSC had the $CD10^{low}$ $CD34^-$ $CD45^-$ $CD105^+$c-kit^- phenotype, characteristic of cells of mesenchymal origin [15].

Stem cells were labeled with ^{188}Re (sodium perrhenate ($Na^{188}ReO_4$) obtained from $^{188}W/^{188}Re$ generator by saline elution). Volume activity of ^{188}Re eluate was 14.0 MBq/ml. Suspension of MSC washed from initial medium and suspended in saline (1 ml; 10^7 cell/ml) was placed into a sterile 10-ml flask and 1.5 ml solution of ^{188}Re-labeled hydroxyethylidenediphosphonic acid monopotassium salt (^{188}Re-HOEDP) was added. After 15-min mixing, the reaction was carried out on an ice bath. The efficiency of ^{188}Re binding to cells was evaluated by paper chromatography (Filtrak-17). A mixture of acetone with 2 M sodium chloride (8:2) and 25% mixture of NH_4OH—EtOH—H_2O (1:2:5) served as the mobile phase. Elution with system 1 showed 80% binding of ^{188}Re to rat SC.

Changes in the distribution of ^{188}Re-labeled MSC in Wistar rat tissues and organs were studied in two series of experiments, depending on ^{60}Co γ-irradiation dose (2, 6, or 12 Gy) and period between γ-irradiation in a dose of 6 Gy and MSC injection (1, 7, and 14 days). The animals were irradiated on a Luch γ-device (irradiation dose ~50 sGy/min). Experiments were carried out on 60 male Wistar rats aged 2.5 months, which were injected with 2×10^6 ^{188}Re-labeled MSC into the tail vein after irradiation (experimental group) or without irradiation (intact control). The animals were decapitated (5 rats per term) for radiometry of organ and tissue specimens 1 and 24 h after MSC injection (experimental series I) or only 24 h after cell injection (experimental series II). Specimens of organs and tissues were collected, placed into plastic tubes, weighed on Sartorius electron scales, and radiometry was carried out by the photopeak of

^{188}Re γ-radiation at 155 keV on an NZ-138 well detector with NC-308 conversion device and NK-350/A high voltage block (Gamma). The content of labeled preparation per gram tissue and for the whole organ was calculated from radiometry data and expressed in percent of injected dose.

The differences between the means in different groups were evaluated using Student's *t* test [4].

RESULTS

The effect of whole-body γ-irradiation of Wistar rats in doses of 2, 6, and 12 Gy 24 before systemic transplantation of ^{188}Re-labeled rat MSC on the distribution of these cells in organs and tissues of animals was studied in experimental series I. Irradiation in a dose of 2 Gy is not lethal, though reactions to the hemopoietic system impairment manifest in animals during the acute period after exposure. Exposure to 6 Gy leads to the development of pronounced "bone marrow", and to 12 Gy of the "enteric" syndrome [6], which cause significant death of SC in the corresponding cell regeneration systems in an adult organism — hemopoietic SC and intestinal epithelial SC [1]. The highest level of labeled cells in intact animals 1 h after intravenous injection of ^{188}Re-labeled MSC was recorded in the lungs, kidneys, liver, spleen, and blood (by the concentration per unit of tissue weight), while by the absolute count per organ the highest values were recorded in the liver, blood, lungs, kidneys, and intestine (Tables 1, 2). The lowest percent and absolute counts of labeled cells were recorded in the heart and brain, though the radioactivity of tissues from these organs was statistically higher than the basal values. Twenty-four hours after cell transplantation, the percent and absolute content of radioactive label decreased significantly in the majority of intact animal tissues (all values of the label injected with MSC are given with consideration for ^{188}Re radioactive degradation, because measurements of tissue radioactivity were carried out in comparison with the control sample containing the same number of labeled cells injected intravenously to each animal). The percentage and absolute content of radioactive label during this period were maximum in the kidneys, liver, and lungs. Low level of the label, observed in the brain 1 h after

TABLE 1. Distribution of ^{188}Re-Labeled MSC in Organs and Tissues of Intact Wistar Rats Pre-Exposed to γ-Radiation in a Dose of 2 Gy

Organ, tissue		Time after injection of ^{188}Re-labeled MSC			
		% per gram tissue		% per organ	
		1 h	24 h	1 h	24 h
Intact control					
	blood	0.549±0.092	0.064±0.013	1.153±0.193	0.135±0.024
	lungs	2.153±0.559	0.188±0.041	0.551±0.053	0.039±0.009
	liver	0.829±0.126	0.235±0.061	1.244±0.190	0.367±0.046
	kidneys	1.320±0.030	0.362±0.084	0.546±0.050	0.141±0.024
	heart	0.121±0.033	0.037±0.014	0.020±0.003	0.004±0.001
	spleen	0.755±0.128	0.091±0.013	0.070±0.009	0.026±0.005
	small intestine	0.289±0.061	0.029±0.005	0.265±0.052	0.037±0.008
	brain	0.023±0.005	0.012±0.002	0.010±0.002	0.005±0.001
Exposure to 2 Gy					
	blood	0.561±0.122	0.021±0.002*	1.021±0.223	0.038±0.004*
	lungs	4.573±0.671*	0.300±0.049*	1.037±0.185*	0.055±0.008
	liver	1.163±0.146	0.432±0.050*	1.511±0.189	0.469±0.075
	kidneys	2.615±0.431*	0.671±0.061*	0.926±0.161*	0.211±0.019*
	heart	0.160±0.029	0.058±0.012	0.019±0.003	0.006±0.001
	spleen	1.033±0.202	0.646±0.132*	0.070±0.009	0.031±0.004
	small intestine	0.506±0.122*	0.083±0.016*	0.395±0.095	0.065±0.013*
	brain	0.042±0.009	0.002±0.001	0.016±0.003	0.004±0.001

Note. Here and in tables 2, 3: *$p<0.05$ *vs.* intact control.

injection of labeled MSC to rats, decreased much slower than in other tissues.

Exposure to a nonlethal dose of 2 Gy led to more intense loss of radioactivity by the peripheral blood 24 h after intravenous injection of labeled MSC; in other words, MSC were more rapidly eliminated from the circulation, and were homed in tissues and organs of animals irradiated in this dose. The same effect was characteristic of exposure to a dose of 6 Gy. Irradiation in a dose of 2 Gy led to a statistically significant increase of radioactive label accumulation in renal tissues (percentage and absolute count of cells) 1 h and 24 h after cell injection in comparison with the intact control. For the majority of other tissues this increase was observed during one period (more often 24 h after the label injection) or only for the percent content of the label. This latter fact indicates that irradiation leads to a reduction of cell content of the organ, while homing of labeled MSC remained at the level of the intact control, which was most pronounced in the splenic tissue at this dose of irradiation. A similar picture was observed in the intestine, but in this case the increase in the percent of radioactive label was paralleled by an increase of the absolute accumulation of radioactivity in the entire organ. It is known that the spleen and small intestine are radiosensitive tissues and lymphocytes in these organs rapidly die from apoptosis caused by γ-irradiation [2]. Presumably, cell death determines the increase of MSC homing in damaged tissues. No appreciable changes in the accumulation of ^{188}Re-labeled MSC in the heart and brain tissues (usually referred to radioresistant tissues) were observed after γ-irradiation in a dose of 2 Gy.

The number of tissues with high accumulation of radioactive label increased after exposure of Wistar rats to γ-radiation in doses of 6 and 12 Gy 1 day before intravenous transplantation of ^{188}Re-labeled MSC in comparison with the intact control. In addition to the lung, kidney, and liver tissues, this increase was characteristic also of the small intestine and brain. The level of radioactive label after MSC injection was higher than in intact animals even in the hearts of rats irradiated in these doses. After exposure to the highest studied dose (12 Gy) the level of radioactivity 24 h after injection of ^{188}Re-labeled MSC was higher in all organs and tissues except the blood in comparison with the same organs and tissues of intact animals.

In experimental series II, the relationship between the periods between whole-body γ-irradiation in a dose of 6 Gy (1, 7, or 14 days) and intravenous injection of MSC and the distribution of ^{188}Re-labe-

TABLE 2. Distribution of ^{188}Re-Labeled MSC in Organs and Tissues of Wistar Rats Pre-Exposed to γ-Radiation in Doses of 8 and 12 Gy

Dose of irradiation; organ, tissue		Time after injection of ^{188}Re-labeled MSC			
		% per gram tissue		% per organ	
		1 h	24 h	1 h	24 h
6 Gy	blood	0.441±0.036	0.040±0.010	0.894±0.074	0.048±0.009*
	lungs	6.507±0.562*	0.296±0.054	1.189±0.049*	0.077±0.013*
	liver	1.276±0.045*	0.361±0.029	1.850±0.065*	0.786±0.082*
	kidneys	2.782±0.132*	0.586±0.053*	0.892±0.036*	0.225±0.016*
	heart	0.151±0.019	0.056±0.010	0.016±0.002	0.007±0.001
	spleen	2.272±0.241*	0.774±0.105*	0.093±0.006*	0.050±0.007*
	small intestine	0.320±0.013	0.109±0.026*	0.278±0.011	0.158±0.040*
	brain	0.047±0.006*	0.022±0.004*	0.018±0.002*	0.009±0.001*
12 Gy	blood	0.530±0.045	0.063±0.011	0.928±0.078	0.110±0.019
	lungs	6.475±0.693*	0.799±0.093*	1.305±0.150*	0.151±0.023*
	liver	1.447±0.085*	0.681±0.051*	1.809±0.107*	0.852±0.064*
	kidneys	2.746±0.679*	0.994±0.103*	1.068±0.038*	0.300±0.027*
	heart	0.197±0.023	0.151±0.027*	0.022±0.002	0.016±0.003*
	spleen	2.613±0.274*	1.937±0.451*	0.107±0.009*	0.047±0.007*
	small intestine	0.548±0.137	0.226±0.035*	0.411±0.103	0.169±0.026*
	brain	0.058±0.005*	0.033±0.006*	0.020±0.003*	0.012±0.002*

led MSC in organs and tissues were studied. Tissue specimens for radioactivity measurements in experimental series II were collected only 24 h after transplantation of ^{188}Re-labeled MSC (Table 3).

The number of significant differences in comparison with the intact control group decreased significantly with prolongation of the period between γ-irradiation and injection of labeled MSC to 7 or 14 days, instead of 24 h, as in series I. A significant increase was observed only for the label percent content in the spleen, which was presumably due to radiation-induced reduction in the splenic cell count and hence, its weight. In addition, γ-irradiation 7 days before MSC injection led to a more rapid drop of blood radioactivity level (the percent and absolute content of the label). This drop presumably reflected in general a more rapid homing of labeled MSC in other tissues, in which the levels of label were not measured (for example, in muscle or fatty tissues, *etc*.). This necessitates further studies on measurements of the levels of radioactively labeled MSC in other organs and tissues after intravenous transplantation.

Hence, the main conclusion of this study is increased homing of MSC after systemic transplantation in various organs and tissues of irradiated rats in comparison with the values in intact (non-irradiated) animals. Increase in the irradiation dose, associated with an increase of radiation injury severity led to more intense homing of transplanted cells in organs and tissues; for radiosensitive organs it can be xplained by the appearance of damaged and dying cells in these tissues. Prolongation of the interval between radiation exposure and systemic transplantation of MSC resulted in attenuation of the stimulatory effect of irradiation on MSC homing.

Presumably, the level of radioactivity 1 and 24 h after injection of ^{188}Re-labeled MSC reflects mainly the content of viable MSC homed in the organ for all tissues studied in this work, except the kidneys (one of organs for xenobiotic elimination), as well as the liver and spleen (organs functioning as cemeteries for dying cells circulating in the blood). This is seen from comparison of our results with published data on the distribution of MSC after systemic transplantation, evaluated by other methods for labeling these SC. For example, the time course of MSC labeled with ^{111}In was studied on the model of infarction in pigs; initially high accumulation of MSC in the lungs was followed by their migration to the liver, kidneys, and bone marrow [12]. After transplantation of MSC labeled with ^{99m}Tc, the radioactive label was detected in the lungs, heart, liver, and spleen, the level of radioactivity with consideration for the isotope degradation 2 days after cell injection remaining virtually at the

TABLE 3. Distribution (in %) of ^{188}Re-Labeled MSC in Organs and Tissues of Wistar Rats Pre-Exposed to γ-Radiation in Doses of 8 and 12 Gy

Organ, tissue		Intact rats	Radiation exposure, days before transplantation		
			6 Gy, 1 day	6 Gy, 7 days	6 Gy, 14 days
Per 1 g tissue	blood	0.064±0.013	0.040±0.010	0.034±0.003*	0.057±0.007
	lungs	0.188±0.041	0.296±0.054	0.150±0.012	0.200±0.040
	liver	0.235±0.061	0.361±0.029	0.208±0.017	0.256±0.019
	kidneys	0.362±0.084	0.586±0.053*	0.534±0.047	0.503±0.043
	heart	0.037±0.014	0.056±0.010	0.030±0.007	0.034±0.008
	spleen	0.091±0.013	0.774±0.105*	0.332±0.045*	0.173±0.012*
	small intestine	0.029±0.005	0.109±0.026*	0.041±0.006	0.027±0.005
	brain	0.012±0.002	0.022±0.004*	0.007±0.001	0.007±0.002
Per organ	blood	0.135±0.024	0.084±0.020	0.068±0.007*	0.115±0.014
	lungs	0.039±0.009	0.058±0.009	0.033±0.003	0.050±0.007
	liver	0.367±0.046	0.532±0.048*	0.302±0.024	0.372±0.027
	kidneys	0.141±0.024	0.195±0.014*	0.173±0.016	0.164±0.016
	heart	0.004±0.001	0.006±0.001	0.004±0.001	0.004±0.001
	spleen	0.026±0.005	0.031±0.004	0.017±0.002	0.023±0.003
	small intestine	0.037±0.008	0.095±0.022*	0.035±0.005	0.023±0.004
	brain	0.005±0.001	0.009±0.002*	0.002±0.000	0.003±0.001

Note. The label was injected 24 h before collection of sample.

same level even 10-14 days after systemic transplantation of MSC [7]. Homing of autologous MSC in the brain of traumatized rats and further proliferation and differentiation of these cells were shown using chromosome label [10]. Experimental evidence of MSC homing and subsequent multiplication in tissues are presented for the liver and kidneys [9,18], though it is probable that part of the label recorded in these organs was due to dead MSC. However, MSC getting into various tissues of injured organism can be full-value sources of subsequent reparative processes. The results of studies on baboons exposed to lethal irradiation in a dose of 1000 sGy and protected from radiation death by intravenous transplantation of autologous stem hemopoietic cells and genetically labeled MSC [14] are particularly demonstrative in this respect. Nine and twenty-one months after transplantation of MSC their genetically labeled descendants were found in 16 analyzed baboon organs and tissues, including the heart, brain, lung, liver, kidneys, spleen, urinary bladder, thymus, vascular walls in different tissues, large and small intestine, *etc.* No doubt, that in this case pre-exposure of animals to whole-body irradiation led to extensive homing of subsequently transplanted MSC, which, together with injected hemopoietic SC, provided regeneration of damaged normal tissues belonging to the typical systems of cell regeneration in an adult body and to systems in which cell renewal in adult age is slow or virtually absent.

Few available publications [19,20,26] are in good agreement with our results, indicating increased homing of MSC after systemic transplantation in pre-exposed animals. Injection of human MSC to immunodeficient mice with locally irradiated abdomen led to increase of homing of intravenously injected MSC not only in the intestine, but also in other organs in the exposed zone (stomach, kidneys, spleen) [26].

A similar picture was observed after local irradiation of mouse limb and intravenous injection of human MSC [8]. Injection of xenogenic MSC in both cases promoted more rapid regeneration of damaged mouse tissues. We can assert that systemic transplantation of MSC will become a prospective trend in the development of regenerative medicine in injuries and traumas of various organs and tissues, including the radiation injuries to normal tissues. The specific features of MSC homing, detected in our study, suggest transplantation of MSC during the early period after irradiation.

The study was supported by the Russian Foundation for Basic Research (grant No. 07-04-00671).

REFERENCES

1. A. G. Konoplyannikov, *Radiobiology of Stem Cells* [in Russian], Moscow (1984).
2. A. G. Konoplyannikov, *Radiation Medicine* [in Russian], Vol. 1, Moscow (2004), pp. 189-277.
3. A. G. Rumyantsev and A. A. Maschyan, *Transplantation of Hemopoietic Stem Cells in Children* [in Russian], Moscow (2003).
4. V. Yu. Urbakh, *Biometrical Methods* [in Russian], Moscow (1964).
5. A. F. Tsyb, A. G. Konoplyannikov, A. I. Kolesnikova, and V. V. Pavlov, *Vestn. Rossiisk. Akad. Med. Nauk*, **59**, No. 9, 71-76 (2004).
6. S. P. Yarmonenko and A. A. Vainson, *Human and Animal Radiobiology* [in Russian], Moscow (2004).
7. I. M. Barbash, P. Chouraqui, and J. Baron, *Circulation*, **108**, No. 7, 863-868 (2003).
8. M. Bensidhoum, S. Gobin, A. Chapel, *et al.*, *J. Soc. Biol.*, **199**, No. 4, 337-441 (2005).
9. C. Bos, Y. Delmas, A. Desmouliere, *et al.*, *Radiology*, **233**, No. 3, 781-789 (2004).
10. J. Chen, Y. Li, M. Katakowski, *et al.*, *J. Neurosci. Res.*, **73**, No. 6, 778-786 (2003).
11. K. R. Chien, *J. Clin. Invest.*, **116**, No. 7, 1838-1840 (2006).
12. B. B. Chin, Y. Nakamoto, J. W. Bulte, *et al.*, *J. Nucl. Med. Commun.*, **24**, No. 11, 1149-1154 (2003).
13. I. Dimarakis, N. A. Habib, and M. Y. Gordon, *Eur. J. Cardiovasc. Surg.*, **28**, No. 5, 665-676 (2005).
14. S. M. Devine, C. Carrington Cobbs, and M. Matt Jennings, *Blood*, **101**, No. 8, 2999-3001 (2003).
15. M. Dominici, K. Le Blanc, I. Mueller, *et al.*, *Cytotherapy*, **8**, No. 4, 315-317 (2006).
16. S. Francois, M. Bensidhoum, M. Mouiseddine, *et al.*, *Stem Cells*, **24**, No. 4, 1020-1029 (2006).
17. C. A. Gregory, E. Reyes, M. J. Whitney, and J. L. Spees, *Ibid.* **24**, No. 10, 2232-2243 (2006).
18. O. Hauger, E. E. Frost, R. Ruud van Heeswijk, *et al.*, *Radiology*, **238**, No. 1, 100-210 (2006).
19. M. Mimeault and S. K. Batra, *Stem Cells*, **24**, No. 11, 2319-2345 (2006).
20. M. Mouiseddine, S. Francois, A. Semont, *et al.*, *Brit. J. Radiol.*, **80**, Spec. No. 1, 49-55 (2007).
21. N. Nagaya, T. Fujii, T. Iwase, *et al.*, *Am. J. Physiol. Heart Circ. Physiol.*, **287**, No. 6, 2670-2676 (2004).
22. D. G. Phinney and D. J. Prockop, *Stem Cells*, **25**, No. 11, 2896-2902 (2006).
23. M. F. Pittenger, A. M. Mackay, S. C. Beck, *et al.*, *Science*, **284**, No. 5411, 143-147 (1999).
24. S. Pommey and J. Galipeau, *Bull. Cancer*, **93**, No. 9, 901-907 (2006).
25. C. S. Potten, R. B. Clarke, J. Wilson, and A. G. Renehan, *Tissue Stem Cells*, Taylor, Francis (2007).
26. A. Semont, S. Francois, M. Mouiseddine, *et al.*, *Adv. Exp. Med. Biol.*, **585**, 19-30 (2006).
27. T. Thum, J. Bauersachs, F. P. Barry, *et al.*, *J. Am. Coll. Cardiol.*, **46**, No. 10, 1799-1802 (2005).
28. R. Zhou, D. H. Thomas, H. Qiao, *et al.*, *J. Nucl. Med.*, **46**, No. 5, 816-822 (2005).