Cell Therapy Based on Adipose Tissue-Derived Stromal Cells Promotes Physiological and Pathological Wound Healing

T.G. Ebrahimian, F. Pouzoulet, C. Squiban, V. Buard, M. André, B. Cousin, P. Gourmelon, M. Benderitter, L. Casteilla, R. Tamarat

Objective—We hypothesized that adipose tissue may contain progenitors cells with cutaneous and angiogenic potential.

Methods and Results—Adipose tissue-derived stroma cells (ADSCs) were administrated to skin punched wounds of both nonirradiated and irradiated mice (20 Gy, locally). At day14, ADSCs promoted dermal wound healing and enhanced wound closure, viscolesticity, and collagen tissue secretion in both irradiated and nonirradiated mice. Interestingly, GFP-positive ADSCs incorporated in dermal and epidermal tissue in vivo and expressed epidermal markers K5 and K14. Cultured ADSCs in keratinocyte medium have been shown to differentiate into K5- and K14-positive cells and produced high levels of KGF. At Day 7, ADSCs also improved skin blood perfusion assessed by laser Doppler imaging, capillary density, and VEGF plasma levels in both irradiated and nonirradiated animals. GFP-positive ADSCs incorporated into capillary structures in vivo and expressed the endothelial cell marker CD31. Finally, in situ interphase fluorescence hybridization showed that a small number of ADSCs have the potential to fuse with endogenous keratinocytes.

Conclusion—ADSCs participate in dermal wound healing in physiological and pathological conditions by their ability to promote reepithelialization and angiogenesis. Hence, adipose lineage cells represent a new cell source for therapeutic dermal wound healing. **(*Arterioscler Thromb Vasc Biol*. 2009;29:503-510.)**

Key Words: ADSCs ■ cell therapy ■ radiation ■ reepithelialization ■ angiogenesis

Woundhealing requires a well-orchestrated integration of complex biological and molecular events of cell migration, cell proliferation, and extracellular matrix deposition. Within hours after injury, epidermal and dermal cell migration and proliferation trigger reepithelialization.[1] Injury also induces tissue hypoxia leading to upregulation of growth factor, extracellular matrix degradation, and subsequently activation of angiogenesis. This formation of new blood vessels is required to sustain the newly formed granulation tissue.[2]

A number of diseases leads to keratinocytes loss, angiogenesis dysfunction, and consequently deterioration of skin regeneration. Among them, radiation has been shown to affect wound healing.[3] Skin wounds often occur under the conditions of severe nuclear accidents and radiotherapy accidents. However, a major complication of irradiation is deficient wound repair arising from inadequate reepithelization and angiogenesis.[3] Indeed, several studies demonstrate dry desquamation with atypical keratinisation of the skin, loss of epidermis with ulceration, as a consequence of irradiation.[4] Changes in vasculature also occur and include occlusion, edema, thrombosis, and progressive loss of vessels.[5] Therefore, the development of strategies designed to promote wound healing in the setting of irradiation is a major therapeutic challenge.

Advances in the field of vascular biology lead to the identification of vascular progenitor cells from bone marrow and non–bone marrow origins and the development of cell based therapies to improve vascularization and tissue regeneration. In support of this view, different types of stem or progenitors cells have been shown to accelerate wound healing in animal models.[2,6] Adipose-derived stroma cells (ADSCs) represent a population of cell surrounding adipocyte in fat tissue.[7] This fraction is also reported to be a source of pluripotent cells able to acquire mesenchymal and neurogenic phenotype.[8] ADSCs also participate in vascular-like structure formation in a Matrigel model and enhance neovascularization in ischemic tissue.[7] Morever, adipose lineage cells have been shown to release potent angiogenic factors and antiapoptotic factors such as monobutyril,[9] vascular endothelial growth factor (VEGF),[10] and leptin.[11,12]

We therefore hypothesized that ADSC administration may restore wound healing in physiological and pathological

Received October 20, 2008; revision accepted January 26, 2009.

From the Institut de Radioprotection et de Surete Nucleaire IRSN, Service de Radiobiologie d'Epidemiologie (T.G.E, F.P., C.S., V.B., M.B., R.T.), Direction de la Radioprotection de l'Homme, Laboratoire de RadioPathologie, Fontenay-aux-Roses Cedex, France; and UMR 5241 CNRS UPS, IFR31 (M.A., B.C., L.C.), Toulouse, France.

Correspondence to Radia Tamarat, IRSN, B.P. no. 17, F-92262 Fontenay-aux-Roses Cedex, France. E-mail radia.tamarat@irsn.fr

Arterioscler Thromb Vasc Biol **is available at http://atvb.ahajournals.org** **DOI: 10.1161/ATVBAHA.108.178962**

conditions. In the present study, we evaluated the effect of ADSC transplantation on reepithelization and revascularization in a wound healing mouse model in nonirradiated and irradiated conditions. Administration of bone marrow mononuclear cells (BM-MNCs) has been previously described to activate the healing process and were used as positive control in our experimental conditions. Futhermore, we assessed the mechanisms underlying the beneficial effects of ADSCs by analyzing their capacity to differentiate into epithelial cells or endothelial cells, to fuse with keratinocytes and produce proangiogenic factors.

Materials and Methods

C57Bl/6 male mice (8 weeks old, Janvier, France) were anesthetized by intraperitoneal injection of ketamine and xylazine. The back of the mice were shaved and sterilized with alcohol. A full thickness wound, approximately of 8 mm diameter, was then made using a dermal biopsy punch. Six hours after injury, mice received intravenous or intramuscular 100 μL of PBS solution alone or containing 1.10^6 BMMNC or 1.10^6 ADSCs (2 injections of 50 μL in dorsal muscles located around the wounded area).[13] Animals were sacrificed at day 4, 7, 10, or 14 after cell transplantation. In some groups of animals, local irradiation (20 Gy 1.51 Gy/min) of flank skin was also performed before punch application. Effects on reepithelialization and angiogenesis were assessed, as previously described. Please see supplement materials (available online at http://atvb.ahajournals.org).

Results

ADSC Characterization

ADSCs represent a homogenous population of cells, expressing stem cell markers CD34, CD133, and CD90, but negative for CD45. This cell population is also negative for endothelial cell markers such as CD31 and thus does not contain any mature and functional endothelial cells (supplemental Figure I).

ADSC Transplantation Promotes Wound Healing

After dermal incision at day 0, wound areas were measured and expressed as 100%. The size of wound area was then determined at day 4, 7, 10, and 14 after cell transplantation. The wound healing process was significantly altered in irradiated mice compared to non irradiated animals. Hence, at D14, wound closure in irradiated mice was still largely uncompleted compared to control animals (Figure 1). In nonirradiated condition, after intravenous or local injection of ADSCs, the rate of wound healing was increased when compared to PBS-receiving animals. Interestingly, in irradiated condition, ADSCs also increased the wound healing process. Moreover, the effect of ADSCs was higher than that of BM-MNCs, as early as day 4 of treatment. Cutaneous viscoelasticity of regenerated skin was significantly reduced by 1.29-fold in irradiated animals when compared to nonirradiated mice ($P<0.05$, supplemental Figure II). In physiological condition, local ADSC administration increased by 1.12-fold skin viscoelasticity when compared to PBS-receiving animals ($P<0.05$). In irradiated condition, ADSCs also improved by 1.30- and 1.21-fold the viscoelasticity after local and venous injection, respectively, in reference to untreated irradiated mice ($P<0.05$). Similarly, BMMNC injection improved by 1.41- and 1.22-fold skin elasticity after local and venous administration, respectively, compared to untreated irradiated animals (supplemental Figure II).

These changes in skin viscoelasticity were associated with an increase in tissue collagen content (supplemental Figure III). Collagen levels tended to be decreased by irradiation, but this did not reach statistical difference. Interestingly, in nonirradiated conditions, ADSC administration improved by 1.10- and 1.22-fold collagen levels after local or intravenous injection, respectively ($P<0.05$), when compared to PBS-receiving animals. Furthermore, in irradiated condition, we observed an elevation by 1.29- and 1.45-fold of collagen contents after intramuscular and intravenous cell transplantation, respectively, in reference to noninjected irradiated animals. Similarly, BMMNC injection raised by 1.29-and 1.24-fold collagen content after local and venous administration, respectively, compared to untreated irradiated animals.

ADSCs Trigger Reepithelization

ADSC-induced wound healing result from nonexclusive ADSC properties such as the differentiation of ADSCs toward keratinocyte phenotype, the release of regenerative molecules or the fusion. We successively investigated all these points.

ADSC Differentiation Into Keratinocytes

To determine the fate of ADSCs in tissue regeneration, GFP-ADSCs were transplanted into irradiated animals. Three days after cell transplantation, GFP-positive cells were observed in the epidermis and dermis (Figure 2A). At day 7 and day 14, suprabasal GFP-positive cells in the epidermis (Figure 2A) expressed cytokeratin K5 and K14, 2 representative markers of epidermal keratinocytes, suggesting that ADSCs were able to acquire the keratinocyte phenotype in vivo. We observed that 10% of total ADSC-GFP cells were engrafted in the skin. In vivo, after intramuscular injection, $2.8\pm0.4\%$ of ADSC–GFP expressed K5, 7 days after cell injection in the wound area. In addition, after intravenous ADSC-GFP administration, these cells were similarly localized in the epidermis and dermis but to a lesser extent than after intramuscular injection ($1.5\pm0.6\%$, $P<0.05$, n=5).

In vitro when ADSCs were cultured in conditioned media from keratinocyte, cells displayed specific keratinocyte morphology and were positive for keratinocyte basal markers, K5 and K14 (Figure 2B). The expression for these specific markers were further confirmed by Western-blotting experiments (Figure 2B). In contrast, conditioned medium from irradiated keratinocytes impaired the ability of ADSCs to differentiate into keratinocytes as revealed by the decrease in K5 and K14 protein expression (Figure 2B).

ADSC-Induced Release of KGF

The effect of ADSCs on skin regeneration may also rely on their ability to produce specific growth factors. In particular, owing to its potent cytoprotective properties for epithelial cells, KGF has been shown to stimulate wound healing process in vivo and in vitro.[14] Cultured ADSCs released high levels of KGF with or without cocultured keratinocytes. Irradiation did not affect this ADSC-induced production of KGF (Figure 2C).

ADSC Fusion to Keratinocytes

Part of the beneficial effects of progenitors cells may depend on their capacity to fuse with differentiated cells. The specificity of

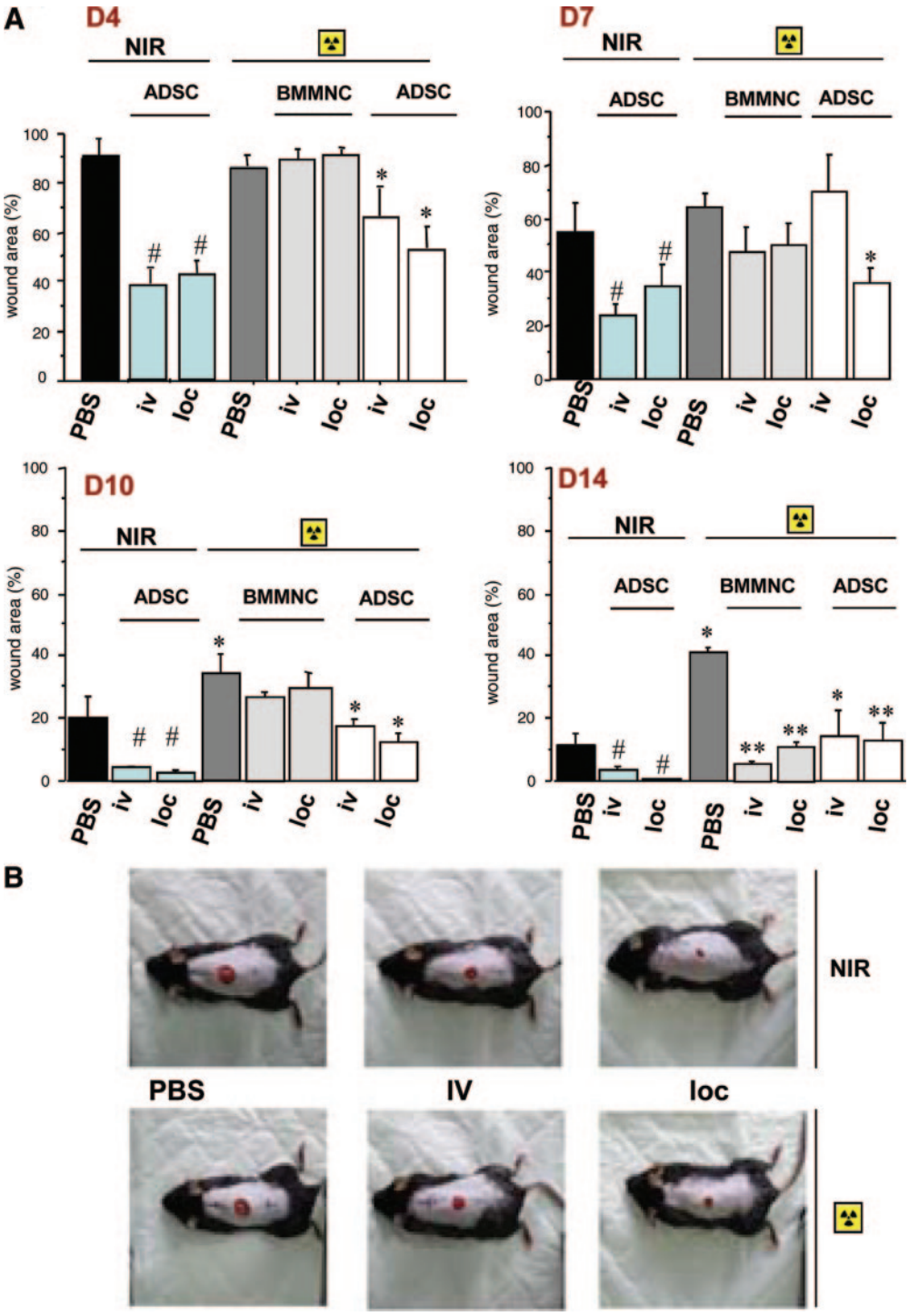

Figure 1. A, Kinetics of skin wound healing in mice treated with or without BMMNC or ADSCs. Data are expressed as percentage of wound healing area (width × length) at day 0. n=6 per groups. #$P<0.05$ vs nonirradiated mice receiving PBS, *$P< 0.05$, **$P<0.01$ vs irradiated mice receiving PBS. B, Representative photomicrographs of dorsal skin wound healing 7 days after PBS and intravenous (iv) or intramuscular (loc) ADSC administration.

DNA probes for X and Y chromosomes was tested in cultured female keratinocytes, and we confirmed that these two probes did not cross-react (data not shown). No cell fusion was observed in normal skin without healing process (data not shown). To assess the ability of ADSCs to fuse with epithelial cells, ADSCs from female mice were transplanted into irradiated male recipients. Tissue sections were stained with X chromosome probe (green arrow) and Y probe (red arrow) Nuclei were stained with DAPI (Blue). Cell fusion was revealed by colocalization between 2 X chromosomes (green arrows) and 1 Y chromosome (red arrow; supplemental Figure IV). However, we observed that less than 0.1% of ADSC-GFP were positive for X chromosome probe. These results further suggest that this process is occasional and cannot explain the therapeutic effect of ADSCs. Finally, no cell fusion was found in vascular network after ADSC cell transplantation.

ADSCs Trigger Angiogenesis

ADSCs Increase Skin Perfusion and Vessel Density

Activation of angiogenesis is required to sustain the newly formed granulation tissue. We therefore assessed the effect of

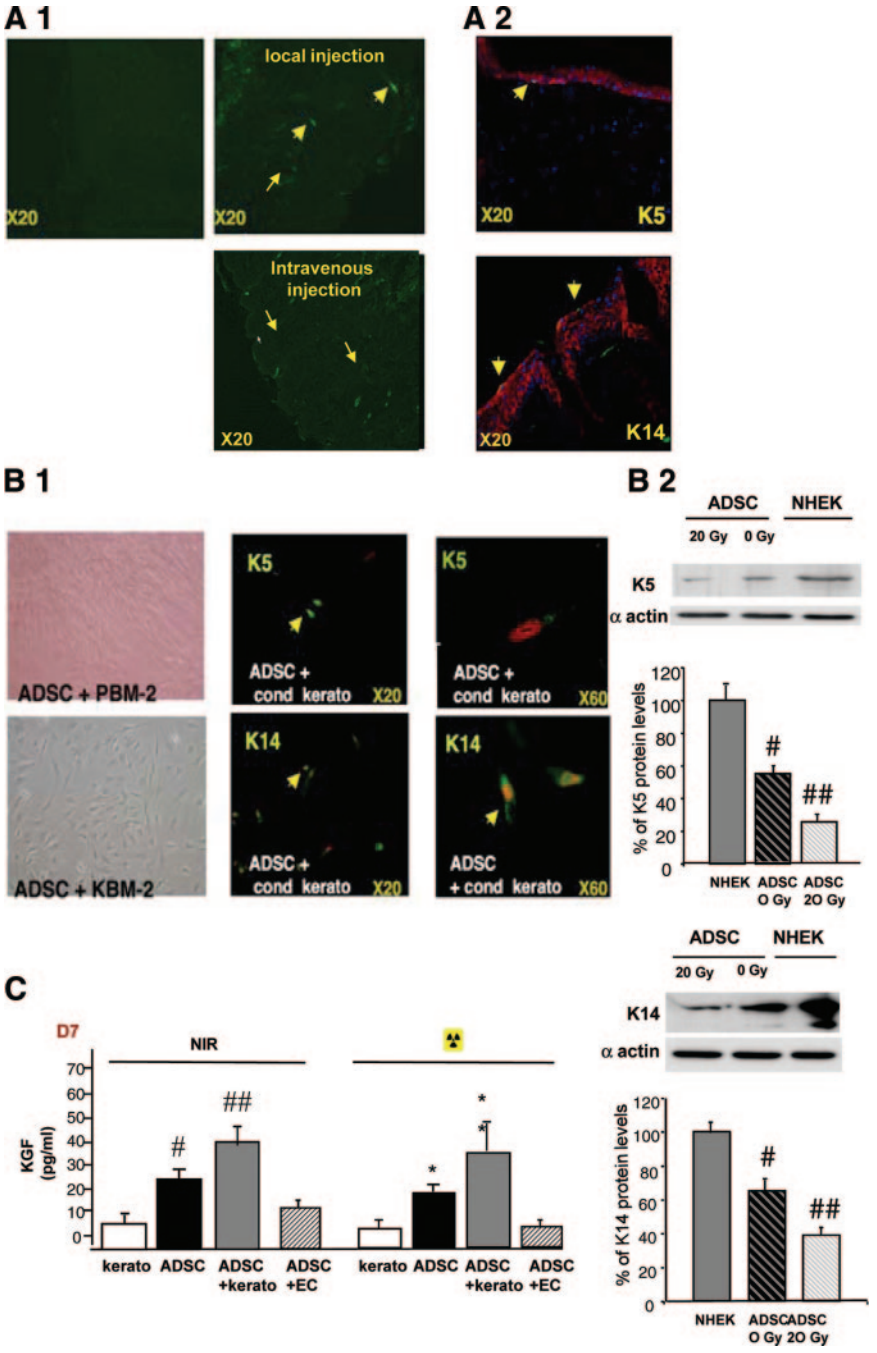

Figure 2. A1, Representative photomicrographs of GFP-ADSCs (arrows) localized in dermal and epidermal tissues after intravenous or intramuscular injection. A2, These GFP-ADSCs expressed K5 and K14 keratinocytes markers, as revealed by positive staining using antibodies directed against K5 or K14 (red). Nuclei were also stained with DAPI (blue). B1, Morphology of ADSCs cultured in Pre-adipocyte Basal Medium-2 (PBM-2) or Keratinocyte Basal Medium-2 (KBM-2) for 21 days (left). Expression of basic epidermal marker K5 and K4 by ADSCs cultured in conditioned medium of non irradiated keratinocytes revealed by immunocytochemistry (middle). B2 Upper, Representative Western Blot of K5 and K14 protein contents. Lower, Quantification of K5 and K14 protein levels expressed as a percentage of control cells NHEK (Human Keratinocytes cells). Values are mean±SEM, n=4 per group #$P<$ 0.05 and ##$P<$ 0.01 vs NHEK cells. C, KGF protein levels in the supernatant of keratinocytes (kerato), ADSCs, and ADSCs cocultured with irradiated or nonirradiated keratinocytes (ADSC+kerato) or endothelial cells (ADSC+ECs), for 7 days. Values are mean±SEM, n=4 per group #$P<0.05$ and ##$P<$ 0.01 vs keratinocytes in nonirradiated condition and *$P<0.05$ and **$P<0.01$ vs keratinocytes in irradiated condition.

ADSCs on new blood vessel formation. Irradiation decreased tissue perfusion compared to nonirradiated condition, this difference reached statistical significance at day 0 (Figure 3A). In nonirradiated condition, intravenous or local transplantation of 1.10^6 ADSCs markedly improved by 1.89- and 1.46-fold, respectively, tissue neovascularization compared to PBS-receiving animals, at D7 (Figure 3; $P<0.05$).

In irradiated conditions, administration of ADSCs increased blood perfusion in reference to untreated animals. Intravenous or intramuscular injection of BMMNCs also raised skin perfusion compared to noninjected animals.

Changes in skin perfusion were associated with modification of vessel number. These effects were mainly observed at day 7. In nonirradiated conditions, ADSC administration was also associated with upregulation of vessel number by 1.80- and 1.66-fold after intravenous and intramuscular injection, respectively. In the irradiated setting, intravenous and local injection of ADSCs increased by 2.22- and 1.85 fold, respectively, vessel density compared to control animals. Similarly, intravenous and local injection of BMMNCs improved vessel density by 1.57 and 1.51, respectively, compared to control animals (Figure 4). No significant differences in the number of vessels were observed at day 14.

ADSC Differentiation Into Endothelial Cells

GFP-ADSCs transplanted into irradiated animals localized around capillary structures and expressed CD31, a specific marker of endothelial cells (supplemental figure VA) suggesting

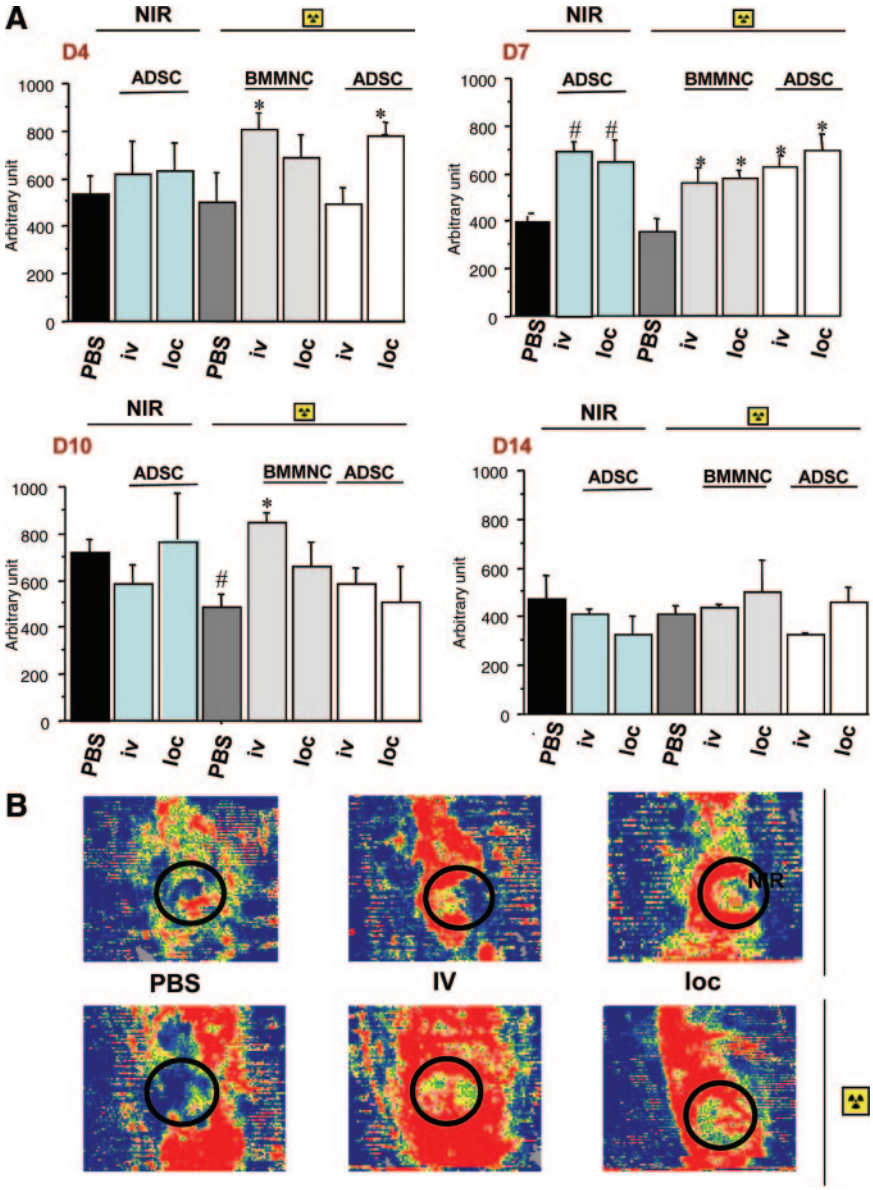

Figure 3. A, Quantification of cutaneaous blood flow perfusion in mice treated with or without ADSCs or BM-MNC. #$P<0.05$ vs nonirradiated mice receiving PBS, *$P<0.05$, vs irradiated mice receiving PBS. NIR indicates nonirradiated mice; iv, intravenous administration; loc, local administration. B, Representative photomicrographs of skin perfusion in irradiated and nonirradiated mice treated with or without ADSCs, 7 days after cell transplantation.

that ADSCs differentiate into endothelial cells. In vivo, 2.6±0.5% of ADSC–GFP expressed the endothelial cell marker CD31 7 days after cell injection in the wound area (n=6).

Furthermore, when ADSCs where cultured in conditioned media from endothelial cells most of the cell displayed spindle shape morphology and expressed CD31 (supplemental Figure V). In vitro, we showed that 58±6% of ADSCs cultured in endothelial medium for 3 weeks expressed CD31 marker in nonirradiated conditions, whereas 40±7% expressed CD31 in irradiated conditions. It is noteworthy that irradiation affected ADSC capacity to differentiate into endothelial cells, as revealed by downregulation of CD31 protein levels assessed by immunohistochemistry and Western blot (supplemental Figure V).

ADSC-Induced Release of VEGF

Changes in capillary number and skin perfusion were associated with modulation of VEGF protein content in plasma. At day 10, VEGF levels was significantly reduced by 0.76-fold in plasma of irradiated animals compared to nonirradiated animals (supplemental Figure VI). In physiological condition, at day 7, VEGF plasma content was higher by 3.00- and 4.75-fold in mice receiving intravenous or intramuscular injection of ADSCs, respectively, compared to noninjected animals ($P<0.05$). Interestingly, in irradiated conditions, at day 7, intravenous and intramuscular administration of ADSCs raised by 2.35- and 6.00-fold VEGF plasma content, respectively, compared to control animals. VEGF levels were unchanged at day 10 and day 14 whatever the experimental groups. In addition, ADSCs cocultured with endothelial cells produced high level of VEGF. Irradiation hampered by 2.60- fold the ability of ADSCs to release VEGF (supplemental Figure VI).

Discussion

The main results of our study are that ADSCs promote wound healing and have the potential to differentiate into keratino-

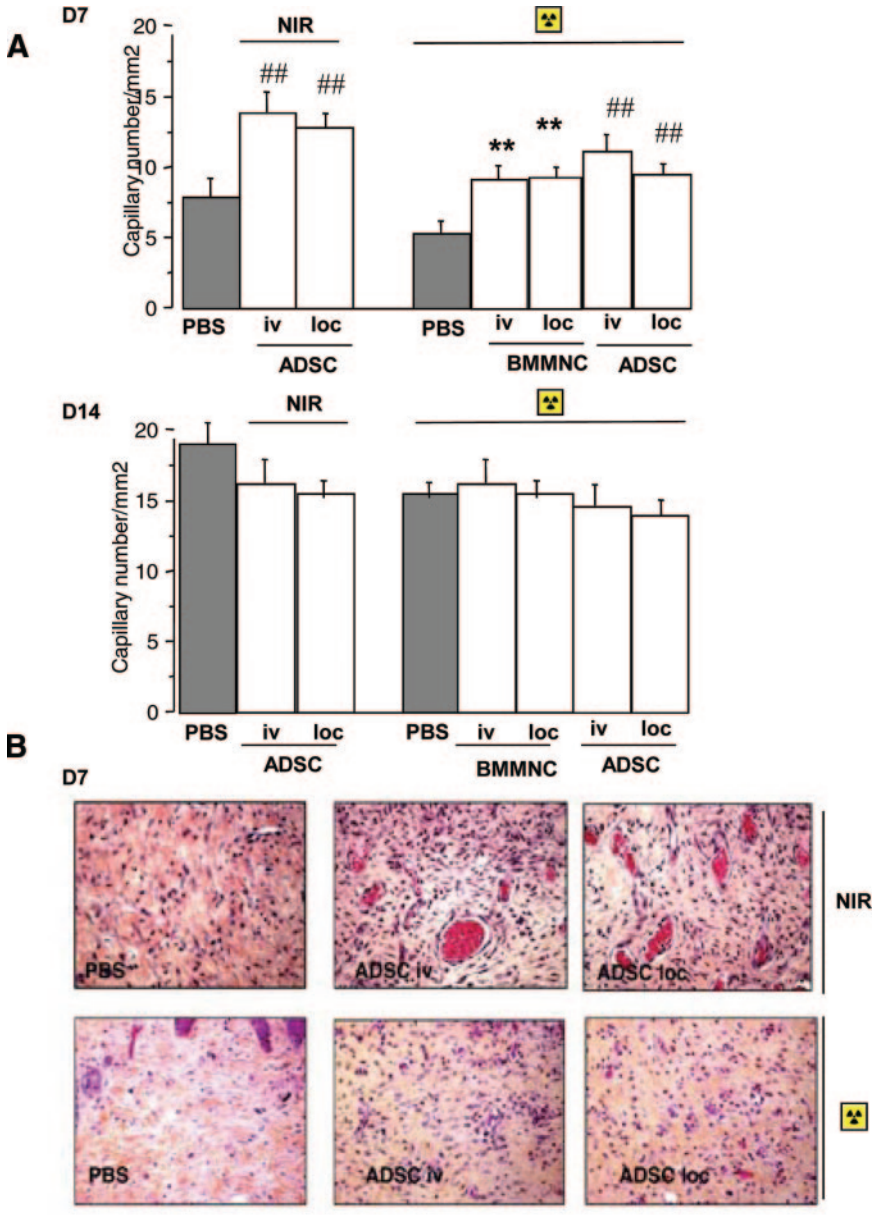

Figure 4. A, Quantification of capillary number in mice treated with or without ADSCs or BM-MNC. #$P<0.05$ vs nonirradiated mice receiving PBS, *$P<0.05$, vs irradiated mice receiving PBS. NIR indicates nonirradiated mice; iv, intravenous administration; loc, local administration. B, Representative photomicrographs of skin capillary density measured by Hematoxylin Eosin and Safran staining in irradiated and nonirradiated mice treated with or without ADSCs, 7 days after cell transplantation.

cyte and produce KGF. ADSCs also trigger neovascularization through their ability to differentiate into endothelial cells and release VEGF. Interestingly, ADSC therapeutic potential is observed during normal and irradiated wound healing process.

Reestablishing an epithelial barrier in injured skin is a crucial wound healing event that protects the body against further water loss and exposure to external pathogens. Epidermal stem cells play a significant role in skin homeostasis and wound repair,[15] but their regenerative capability is often overcome. Particularly, ionizing radiation produces both acute and delayed effects on skin and subcutaneous tissues which have profound implications for surgical wound healing.[16] Radiation also induces acute degenerative changes in basement membranes, increases vascular permeability, and may reduce neovascularization.[17,18] Fibroblasts and myofibroblats from irradiated tissue exhibit abnormalities in collagen production and contractile proprieties[19,20] and may be permanently altered by radiation. They do not produce sufficient collagen to keep up with the demands of wound, or the collagen that is produced does not mature quickly enough to meet these demands during the acute phase of wound healing. In irradiated tissue, a reduction in synthesis of the alpha 1 chain of type I collagen is found 7 days after wounding and is significantly different when compared to nonirradiated animals.[21] Hence, cell therapy may be a promising therapeutic approach to improve wound healing in physiological and pathological conditions. In recent studies, transplantation of BMMNCs has been reported to activate the healing process by their capacity to differentiate into perifollicular cells, perisebaceous gland cells, and blood vessel cells.[15] Furthermore, in a skin defect model in irradiation conditions, human mesenchymal stem cells accelerate cutaneous wound healing and localize in the epithelium.[22] Trans-

plantation of cultured embryonic fibroblasts in both irradiated and nonirradiated settings has also been shown to partly restore wound healing.[23,24] The present study clearly demonstrates that the topical administration of ADSCs into the full thickness wounds normalized the defect of irradiated mice regarding the rate of wound healing. Interestingly, in addition to the degree of healing rate, ADSCs also increase the thickness of the granulation and enhance collagen content. Furthermore, ADSC-related effects do not depend on the mode of administration.

ADSC beneficial effects may rely on different cellular mechanisms: a true ADSC differentiation toward keratinocyte, fusion events, paracrine effects, and proangiogenic potential. The present study identified for the first time the potential of few ADSCs to fuse with epidermal cells suggesting that ADSC lineage can acquire keratinocyte phenotype by cell fusion. However, our results suggest that this process is occasional and cannot explain the therapeutic effect of ADSCs. However, ADSCs have a real potential to differentiate into keratinocyte as revealed by in vivo transplantation experiments. Indeed, female GFP-ADSCs transplanted into wound injury in nonirradiated and irradiated male mice expressed K5 and K14 markers. At the wound site, engraftment of ADSCs as epidermal cells increased within 3 days and continued to be present by 2 weeks after injury. Moreover when ADSCs were cultured in keratinocyte conditioned medium for 3 weeks, numerous cells showed morphology of mature keratinocyte cells and expressed K5 and K14, 2 basic epidermal markers. This strongly suggests a true differentiation potential of ADSCs into keratinocyte. This potential seems to be hampered by irradiation. Such potential is consistent with a previous study showing that all-trans retinoic acid induce cytokeratin 18 expression, a marker of epithelial cells in adipose cells.[25]

We also demonstrated that ADSCs secrete KGF in both irradiated and nonirradiated conditions. Recently, it has been shown that peripheral blood mononuclear cells transdifferentiate into epithelial-like cells with the capacity to release keratinocyte-derived 14-3-3σ protein.[26] KGF, a member of the fibroblast growth family, plays prominent role in epithelial morphogenesis and wound healing.[27,28] In skin, KGF is strongly upregulated after injury, suggesting that KGF may be crucial in early stages of healing process.[29] Exogenous KGF significantly enhanced reepithelization in full and partial thickness wounds in porcine and rabbit ear wound models.[30,31] In addition to epithelization, KGF increases new granulation tissue formation in an ischemic rabbit ear wound model. Therefore, it is likely that ADSC-induced KGF release may regulate the healing of the cutaneous wounds.

A period of robust angiogenesis occurs during adult wound healing and is presumed to be essential for appropriate wound repair. New blood vessels within the wound likely supply oxygen and nutrients to support the cellular proliferation involved in tissue restoration. Radiation therapy affects the time course and end result of wound healing. Arterioles and small arteries exhibit progressive vascular sclerosis, resulting in marked narrowing or obliteration of the lumen.[32] We confirmed and extended these previous results because we evidenced that irradiation hampered skin perfusion, capillary number, and VEGF plasma levels.

Interestingly, we showed that GFP-ADSCs incorporated into vessels and differentiated into CD31 expressing endothelial cells and upregulated blood perfusion and vessel density. When ADSCs were cultured in endothelial conditioned medium, cells showed morphology of mature endothelial cells and expressed CD31. Similarly, cultured human ADSCs differentiated into endothelial cells and enhanced postischemic neovascularization in nude mice and vessel-like structure formation in Matrigel plug.[7] In addition, ADSCs cultured in presence of VEGF and bFGF are able to differentiate into endothelial cells in vitro within 2 days.[33] Although irradiation hampers this potential, ADSCs cocultured with nonirradiated and irradiated endothelial cells secrete VEGF. Many studies have shown that VEGF is a critical regulator of angiogenesis in wound healing.[34,35] The positive effects of VEGF on wound repair were thought to be completely angiogenesis-driven. However, a role of VEGF R1 in the proliferation of keratinocytes as well as in reepithelization after injury has been suggested.[32]

Future therapies for wound healing complications have to be based on correcting several parameters of tissue regeneration and neovascularization. Our work provides evidences that ADSCs have the potential to accelerate reepithelization and vessel growth highlighting new therapeutic alternative for different dermal clinical settings. In addition, these cells can be easily harvested from patients in a simple minimally invasive lipoaspiration procedure and can be expanded ex vivo, suggesting that ADSCs are suitable for autologous cell therapy in patients with skin wounds.

Sources of Funding

This work was supported by grants from DGA ≪ Délégation Générale pour l'Armement (DA 8866).

Disclosures

None.

References

1. Martin P. Wound healing–aiming for perfect skin regeneration. *Science*. 1997;276:75–81.
2. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. *J Clin Invest*. 2007;117:1219–1222.
3. Liu X, Liu JZ, Zhang E, Li P, Zhou P, Cheng TM, Zhou YG. Impaired wound healing after local soft x-ray irradiation in rat skin: time course study of pathology, proliferation, cell cycle, and apoptosis. *J Trauma*. 2005;59:682–690.
4. Wang J, Boerma M, Fu Q, Hauer-Jensen M. Radiation responses in skin and connective tissues: effect on wound healing and surgical outcome. *Hernia*. 2006;10:502–506.
5. Tibbs MK. Wound healing following radiation therapy: a review. *Radiother Oncol*. 1997;42:99–106.
6. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature*. 2003;425:968–973.
7. Planat-Benard V, Silvestre JS, Cousin B, Andre M, Nibbelink M, Tamarat R, Clergue M, Manneville C, Saillan-Barreau C, Duriez M, Tedgui A, Levy B, Penicaud L, Casteilla L. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. *Circulation*. 2004;109:656–663.
8. Safford KM, Hicok KC, Safford SD, Halvorsen YD, Wilkison WO, Gimble JM, Rice HE. Neurogenic differentiation of murine and human

adipose-derived stromal cells. *Biochem Biophys Res Commun*. 2002;294: 371–379.
9. Dobson DE, Kambe A, Block E, Dion T, Lu H, Castellot JJ Jr, Spiegelman BM. 1-Butyryl-glycerol: a novel angiogenesis factor secreted by differentiating adipocytes. *Cell*. 1990;61:223–230.
10. Claffey KP, Wilkison WO, Spiegelman BM. Vascular endothelial growth factor. Regulation by cell differentiation and activated second messenger pathways. *J Biol Chem*. 1992;267:16317–16322.
11. Bouloumie A, Drexler HC, Lafontan M, Busse R. Leptin, the product of Ob gene, promotes angiogenesis. *Circ Res*. 1998;83:1059–1066.
12. Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR. Biological action of leptin as an angiogenic factor. *Science*. 1998;281:1683–1686.
13. Bjorntorp P, Karlsson M, Pertoft H, Pettersson P, Sjostrom L, Smith U. Isolation and characterization of cells from rat adipose tissue developing into adipocytes. *J Lipid Res*. 1978;19:316–324.
14. Geer DJ, Swartz DD, Andreadis ST. Biomimetic delivery of keratinocyte growth factor upon cellular demand for accelerated wound healing in vitro and in vivo. *Am J Pathol*. 2005;167:1575–1586.
15. Borue X, Lee S, Grove J, Herzog EL, Harris R, Diflo T, Glusac E, Hyman K, Theise ND, Krause DS. Bone marrow-derived cells contribute to epithelial engraftment during wound healing. *Am J Pathol*. 2004;165: 1767–1772.
16. Bernstein EF, Sullivan FJ, Mitchell JB, Salomon GD, Glatstein E. Biology of chronic radiation effect on tissues and wound healing. *Clin Plast Surg*. 1993;20:435–453.
17. Doyle JW, Li YQ, Salloum A, FitzGerald TJ, Walton RL. The effects of radiation on neovascularization in a rat model. *Plast Reconstr Surg*. 98:129–135, 1996; discussion 136–129.
18. van den Brenk HA, Sharpington C, Orton C, Stone M. Effects of x-radiation on growth and function of the repair blastema (granulation tissue). II. Measurements of angiogenesis in the Selye pouch in the rat. *Int J Radiat Biol Relat Stud Phys Chem Med*. 1974;25:277–289.
19. Metson R, Freehling DJ, Wang CC. Surgical complications following twice-a-day versus once-a-day radiation therapy. *Laryngoscope*. 1988;98: 30–34.
20. Ormsby MV, Hilaris BS, Nori D, Brennan MF. Wound complications of adjuvant radiation therapy in patients with soft-tissue sarcomas. *Ann Surg*. 1989;210:93–99.
21. Bernstein EF, Salomon GD, Harisiadis L, Talbot T, Harrington F, Russo A, Uitto J. Collagen gene expression and wound strength in normal and radiation-impaired wounds. A model of radiation-impaired wound healing. *J Dermatol Surg Oncol*. 1993;19:564–570.
22. Francois S, Mouiseddine M, Mathieu N, Semont A, Monti P, Dudoignon N, Sache A, Boutarfa A, Thierry D, Gourmelon P, Chapel A. Human mesenchymal stem cells favour healing of the cutaneous radiation syndrome in a xenogenic transplant model. *Ann Hematol*. 2007;86:1–8.
23. Gorodetsky R, McBride WH, Withers HR, Miller GG. Effect of fibroblast implants on wound healing of irradiated skin: assay of wound strength and quantitative immunohistology of collagen. *Radiat Res*. 1991;125: 181–186.
24. Ferguson PC, Boynton EL, Wunder JS, Hill RP, O'Sullivan B, Sandhu JS, Bell RS. Intradermal injection of autologous dermal fibroblasts improves wound healing in irradiated skin. *J Surg Res*. 1999;85:331–338.
25. Brzoska M, Geiger H, Gauer S, Baer P. Epithelial differentiation of human adipose tissue-derived adult stem cells. *Biochem Biophys Res Commun*. 2005;330:142–150.
26. Medina A, Kilani RT, Carr N, Brown E, Ghahary A. Transdifferentiation of peripheral blood mononuclear cells into epithelial-like cells. *Am J Pathol*. 2007;171:1140–1152.
27. Aaronson SA, Bottaro DP, Miki T, Ron D, Finch PW, Fleming TP, Ahn J, Taylor WG, Rubin JS. Keratinocyte growth factor. A fibroblast growth factor family member with unusual target cell specificity. *Ann N Y Acad Sci*. 1991;638:62–77.
28. Miki T, Bottaro DP, Fleming TP, Smith CL, Burgess WH, Chan AM, Aaronson SA. Determination of ligand-binding specificity by alternative splicing: two distinct growth factor receptors encoded by a single gene. *Proc Natl Acad Sci U S A*. 1992;89:246–250.
29. Werner S, Peters KG, Longaker MT, Fuller-Pace F, Banda MJ, Williams LT. Large induction of keratinocyte growth factor expression in the dermis during wound healing. *Proc Natl Acad Sci U S A*. 1992;89: 6896–6900.
30. Staiano-Coico L, Krueger JG, Rubin JS, D'Limi S, Vallat VP, Valentino L, Fahey T, III, Hawes A, Kingston G, Madden MR, et al. Human keratinocyte growth factor effects in a porcine model of epidermal wound healing. *J Exp Med*. 1993;178:865–878.
31. Pierce GF, Yanagihara D, Klopchin K, Danilenko DM, Hsu E, Kenney WC, Morris CF. Stimulation of all epithelial elements during skin regeneration by keratinocyte growth factor. *J Exp Med*. 1994;179:831–840.
32. Wilgus TA, Matthies AM, Radek KA, Dovi JV, Burns AL, Shankar R, DiPietro LA. Novel function for vascular endothelial growth factor receptor-1 on epidermal keratinocytes. *Am J Pathol*. 2005;167: 1257–1266.
33. Cao Y, Meng Y, Sun Z, Liao LM, Han Q, Li J, Liu YN, Zhao CH. [Potential of human adipose tissue derived adult stem cells differentiate into endothelial cells]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*. 2005; 27:678–682.
34. Rossiter H, Barresi C, Pammer J, Rendl M, Haigh J, Wagner EF, Tschachler E. Loss of vascular endothelial growth factor a activity in murine epidermal keratinocytes delays wound healing and inhibits tumor formation. *Cancer Res*. 2004;64:3508–3516.
35. Deodato B, Arsic N, Zentilin L, Galeano M, Santoro D, Torre V, Altavilla D, Valdembri D, Bussolino F, Squadrito F, Giacca M. Recombinant AAV vector encoding human VEGF165 enhances wound healing. *Gene Ther*. 2002;9:777–785.