Stem Cells, Tissue Engineering and Hematopoietic Elements

Bone Marrow-Derived Cells Are Not the Origin of the Cancer Stem Cells in Ultraviolet-Induced Skin Cancer

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Several lines of evidence have demonstrated that various cancers are derived from cancer stem cells (CSCs), which are thought to originate from either tissue stem or progenitor cells. However, recent studies have suggested that the origin of CSCs could be bone marrow-derived cells (BMDCs); for example, gastric cancer, which follows persistent gastric inflammation, appears to originate from BMDCs. Although our previous research showed the capability of BMDCs to differentiate into epidermal keratinocytes, it has yet to be determined whether skin CSCs originate from BMDCs. To assess the possibility that BMDCs could be the origin of CSCs in skin squamous cell carcinoma (SCC), we used a mouse model of UVB-induced skin SCC. We detected a low percentage of BMDCs in the lesions of epidermal dysplasia (0.59%), SCC *in situ* (0.15%), and SCC (0.03%). Furthermore, we could not find any evidence of clonal BMDC expansion. In SCC lesions, we also found that most of the BMDCs were tumor-infiltrating hematopoietic cells. In addition, BMDCs in the SCC lesions lacked characteristics of epidermal stem cells, including expression of stem cell markers (CD34, high $\alpha 6$ integrin) and the potential retention of BrdU label. These results indicate that BMDCs are not a major source of malignant keratinocytes in UVB-induced SCC. Therefore, we conclude that BMDCs are not the origin of CSCs in UVB-induced SCC. (Am J Pathol 2009, 174:595–601; DOI: 10.2353/ajpath.2009.080362)

Stem cells, which have the capacity to self-renew and to differentiate into the various mature cells that constitute the tissue of organ, are found in many adult tissues including the skin.¹ Stem cells are critical for replenishing

and maintaining the balance of cells (homeostasis) within the tissue and reconstituting tissue damaged during injury. Numerous studies have shown that the specific stem cell properties and the characteristics of stem-cell systems (populations of cells that derive from stem cells are organized in a hierarchical manner) are relevant to some forms of human cancer.^{2,3} In cancers, cancer stem cells (CSCs) are thought to exist. CSCs, like tissue stem cells, would have a capacity for self-renewal and a proliferative ability with successive expansion potential promoting tumor structure organization. Tumor-initiating cells, which are considered to be a population rich in CSCs, have been identified in cancers of the hematopoietic system^{4,5} and various organs.^{6–10}

Although several lines of evidence indicate that CSCs can arise from tissue stem cells^{6,8,11,12} or mutated progenitor cells^{13,14} current reports showed that gastric cancer, which follows persistent gastric inflammation because of the infection with *Helicobacter felis* (*H. felis*), appears to originate from bone marrow-derived cells (BMDCs).¹⁵ Indeed, some populations of BMDCs have the potential to differentiate into mature cells of various nonhematopoietic organs including liver,¹⁶ skeletal-muscle,¹⁷ brain,¹⁸ and skin.¹⁹ We also showed that BMDCs and mesenchymal stem cells are able to transdifferentiate into keratinocytes.^{20,21} BMDCs with this plasticity are frequently recruited to sites of injured or inflamed tissue, where they differentiate into mature tissue cells to contribute to tissue repair.²² Results from *H. felis*-induced gastric cancer suggest that BMDCs with plasticity would differentiate into tissue stem or mature cells to reconstitute the damaged tissue, they then convert into CSCs, and contribute to carcinoma formation. Although recent investigations have demonstrated that BMDCs could contribute to cancers of small intestine, colon, lung,²³ larynx, and brain,²⁴ it is yet to be determined whether cancers originating from BMDCs certainly exist.

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Skin cancer is currently the most common malignancy in humans.²⁵ The skin has the role to protect our bodies from a wide range of environmental assaults including UVB irradiation, chemical carcinogens, and the entry of viruses and other pathogens. Therefore, epidermal keratinocytes have more opportunity to manifest maturation arrest. Particular epidemiological and scientific evidence has shown that UVB is one of the most important factors affecting skin carcinogenesis in the physical environment.^{25,26}

As in the case of BMDC-originated gastric cancer after persistent inflammation with *H. felis* infection, it is presumed that BMDCs, which are recruited to the UVB-damaged epidermis and differentiate into epidermal keratinocytes to reconstitute the damaged skin, could then give rise to the maturation arrest during continuous UVB irradiation, convert into CSCs, and finally propagate to form bone marrow (BM)-derived skin cancer. Such a novel hypothesis, if true, would have profound implications for our present understanding of the pathogenesis of squamous cell carcinoma (SCC).

To investigate the possible role of BMDCs in skin cancer, we used a mouse model of UVB-induced skin SCC and evaluated the number and marker expressions of labeled BMDCs that differentiated into keratinocytes in skin SCC.

Materials and Methods

BM Transplantation

All animal procedures were conducted according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee under an approved protocol. BM was isolated from the femurs and tibias of male C57BL/6JGtosa26 (ROSA26) or C57BL/6-TgN(ACTB-EGFP)1Osb/J (GFP) mice (The Jackson Laboratory, Bar Harbor, ME). After lethal irradiation (9 Gy), 1×10^6 BM cells from donor mice in a volume of 200 μ l of sterile phosphate-buffered saline were transplanted to recipient C57BL/6 female mice via a single tail vein injection. Hematopoietic reconstitution was subsequently evaluated in peripheral blood 4 weeks after transplantation and more than 94% of BM cells were donor-derived cells.

Induction of UVB Radiation-Induced SCC

UVB-induced carcinogenesis was performed as previously reported (Figure 1A).²⁷ The UVB light source was a FL20SE30 fluorescent lamp (Clinical Supply, Tokyo, Japan). The UVB irradiation (180 mJ/cm²) was continued daily for 10 days for tumor initiation to mice ($n = 20$). One week after the initiation, UVB exposure (180 mJ/cm²) was performed twice a week until the end of the experiment at 10 months from the last UVB exposure. At 5 months, all irradiated mice ($n = 8$) had small papules (at least two papules) and erosion. At 10 months, all irradiated mice ($n = 6$) had tumors (at least three tumors), papules (at least five papules), and ulcer.

Figure 1. UVB-induced SCC model mice in which BMDCs are labeled with β -Gal enzyme or GFP. **A:** Lethally irradiated mice were transplanted with BM from ROSA26 mice expressing β -Gal enzyme or GFP mice expressing GFP. After confirmation of BM reconstitution, mice were UVB-irradiated. Intermittent UVB irradiation leads mice skin to form SCC. **B:** Tumors were histologically classified as unaffected, dysplasia, SCC *in situ*, or SCC based on tumor architecture, keratinocyte differentiation, and cytological atypia.

Histological Analysis

Mice were sacrificed and tissue was removed, embedded in OCT compound (Sakura, Torrance, CA), snap-frozen or fixed in 4% formalin, and embedded in paraffin. Tumor sections were visualized by routine staining with hematoxylin and eosin (H&E). All of the slides were reviewed twice in blinded manner by three dermatologists, and assessed for tumor architecture, keratinocyte differentiation, cytological atypia, and inflammation. Tumors were classified as dysplasia (typical papilloma), SCC *in situ*, or SCC based on tumor architecture and cytological atypia as described previously.²⁸ Some lesions exhibiting nonpapillomatous architecture and comprising one to three layers with well-differentiated keratinocytes were classified as normal. Ten samples were analyzed in each normal growth, dysplasia, SCC *in situ*, and SCC. Counts were averaged from eight or nine separate fields in each histological category.

Determination of Enzyme (X-Gal) Activity

Frozen sections (5 μ m) were fixed for 30 minutes in 0.2% glutaraldehyde, washed in sodium phosphate buffer containing 0.01% sodium deoxycholate and 0.02% Nonidet P-40 and 1 mmol/L MgCl and incubated for 10 hours at 37°C in a 1-mg/ml X-Gal solution [5-bromo-4-chloro-3-indolyl- β -galactopyranoside: X-Gal, dissolved in dimethyl sulfoxide, 5 mmol/L K₃Fe(CN)₆, 5 mmol/L K₄Fe(CN)₆ 3H₂O in 0.1mol/L sodium phosphate buffer] and counter-stained with H&E.

Figure 2. BMDCs in UVB-irradiated mouse skin. **A:** X-Gal-positive cells located within the basal layer in the unaffected epidermis lesions. In the epidermal dysplasia lesions, most X-Gal-positive cells (arrows) were found in the suprabasal layers. In the SCC *in situ* lesions, X-Gal-positive cells were found at the inner part of the tumor. In the SCC lesions, X-Gal-positive cells were also found at the inner part of the tumor. **B:** After 5 months of UVB irradiation, the percentage of X-Gal-positive cells was found at $0.15 \pm 0.21\%$ in the unaffected epidermis lesions, increased to $0.58 \pm 0.25\%$ in the epidermal dysplasia lesions, and decreased to $0.25 \pm 0.20\%$ in the SCC *in situ* lesions ($*P < 0.05$, $**P < 0.01$). **C:** After 10 months of UVB irradiation, the percentage of X-Gal-positive cells was $0.59 \pm 0.57\%$ in the epidermal dysplasia lesions and $0.15 \pm 0.22\%$ in the SCC *in situ* lesions. In the SCC lesions, the percentage of X-Gal-positive cells in the tumor was decreased to $0.03 \pm 0.06\%$ ($*P < 0.05$, $**P < 0.01$).

Immunofluorescence

Frozen blocks were prepared and sectioned as described above. Sections were fixed in 4% paraformaldehyde and analyzed for β -galactosidase-expressing cells by using polyclonal antibodies (Cappel, Aurora, OH) and fluorescent secondary antibodies (fluorescein isothiocyanate-labeled goat anti-rabbit antibody; Jackson ImmunoResearch, West Grove, PA). Sections fixed in 4% paraformaldehyde were also analyzed for GFP-expressing cells by using polyclonal antibodies (Molecular Probes, Carlsbad, CA). β -Galactosidase-expressing cells were also stained with antibodies to CD45 (BD Biosciences, San Diego, CA), pan cytokeratins (Progen, Heidelberg, Germany), CD34 (BD Biosciences), or $\alpha 6$ integrin (BD Biosciences). Sections were viewed with a confocal laser-scanning fluorescence microscope (FV1000; Olympus, Tokyo, Japan).

BrdU Assay

The procedure for BrdU pulse labeling and the subsequent detection were performed as previously reported.²⁹ In brief, at the time of 9-month UVB irradiation, the tumor-bearing model mice were fed with water containing BrdU (1 mg/ml) for 10 days. Forty-five days after BrdU labeling, the tissues were removed. Frozen sections were fixed with 4% paraformaldehyde or 70% ethanol, stained with antibodies to BrdU (Roche, Penzberg, Germany) and fluorescent second antibodies (tetramethyl-rhodamine isothiocyanate-labeled goat anti-mouse antibody; Southern Biotechnology, Birmingham, AL).

Fluorescence in Situ Hybridization

X and Y chromosomes were detected on sections from the UVB-irradiated mice skin using a dual-color detection kit (Cambio, Cambridge, UK) according to the manufacturer's protocol (Cy5 for Y chromosomes and Cy3 for X chromosomes) and immediately viewed with a confocal microscope.

Results

Low Frequency of BMDCs in UVB-Irradiated Skin

To investigate the possible role of BMDCs in UVB-induced skin dysplasia/carcinoma progression, we used a model mouse whose BMDCs are labeled with β -galactosidase (β -Gal) or green fluorescent protein (GFP). Lethally irradiated mice were transplanted with BM from ROSA26 mice or GFP transgenic mice (Figure 1A). After the confirmation of BM reconstitution, mice were irradiated with UVB and developing tumors in mice skin were evaluated histologically. Each section was divided into four categories of unaffected, dysplasia, SCC *in situ*, and SCC (Figure 1B).²⁸ After 5 months of UVB irradiation, we found the dysplasia lesions and the SCC *in situ* lesions, whereas we found no SCC lesions in irradiated skin. After 10 months of UVB irradiation, the dysplasia lesions and the SCC *in situ* lesions were found to be continuous with the SCC lesions, whereas the unaffected epidermis lesions were completely absent.

To detect the presence of BMDCs in UVB-irradiated mouse skin, X-galactosidase (X-Gal) staining was performed. The numbers of BMDCs were quantified by counting the number of X-Gal-positive cells in the UVB-irradiated mouse skin (Figure 2A). After 5 months of UVB irradiation, even in the unaffected epidermis lesions, some X-Gal-positive cells, indicating BMDCs, were located within the basal layer. In the epidermal dysplasia lesions, some X-Gal-positive cells were also found within the basal layer, but most X-Gal-positive cells were found within the suprabasal layers. In the SCC *in situ* lesions, X-Gal-positive cells were found within the inner parts of the tumor. The percentage of occurrence of X-Gal-positive cells was 0.15% in the unaffected epidermis lesions. Since we previously showed that wounded skin contained BMDCs (0.03%),²⁰ repeated UVB irradiation might induce BMDC accumulation. The percentage of X-Gal-positive cells in the epidermal dysplasia lesions increased to 0.58%, whereas the percentage of X-Gal-

Figure 3. BM-derived infiltrating cells in the UVB-irradiated skin. Triple staining of β -Gal (green) (A), CD45 (red) (B), and pancytokeratin (cyan) was performed. C: Merged image showed β -Gal⁺/CD45⁺ (arrow) or β -Gal⁺/CD45⁻ (arrowheads) cells in the tumor. D: Percentage of CD45⁺ of all β -Gal⁺ cells in the UVB-irradiated mice skin. In all β -Gal⁺ cells, 10.1 ± 15.3% was positive for CD45 in the epidermal dysplasia lesions, 27.3 ± 44.1% in the SCC *in situ* lesions, and at 78.7 ± 27.4% in the SCC lesions (**P* < 0.05, ***P* < 0.01). Original magnifications, ×600.

positive cells in the SCC *in situ* lesions was decreased to 0.25% (Figure 2B). The number of β -Gal-positive cells and total epidermal cells of the UVB-irradiated skin were as follow; unaffected (6 of 2276), dysplasia (28 of 4804), SCC *in situ* (15 of 5445). We further confirmed that no X-Gal-positive cells were detected in untreated (unirradiated) mice. We failed to find any clusters of X-Gal-positive cells in either the unaffected epidermis or the tumor. These results indicate that BMDCs in the UVB-irradiated skin do not commonly give rise to a monoclonal expansion.

After 10 months of UVB irradiation, in the epidermal dysplasia lesions and SCC *in situ* lesions, we found X-Gal-positive cells in a similar location as mice skin that received 5 months of UVB irradiation. In the SCC lesions, X-Gal-positive cells were found within the inner part of the tumor (Figure 2A). X-Gal-positive cells were found at a percentage of 0.59% in the epidermal dysplasia lesions and 0.15% in the SCC *in situ* lesions. These percentages of X-Gal-positive cells in 10-month UVB-irradiated mouse skin were similar to the percentage in 5-month UVB-irradiated mouse skin. In the SCC lesions, the percentage of X-Gal-positive cells was at 0.03%, which decreased in comparison with the percentage in the SCC *in situ* lesions (Figure 2C). The number of β -Gal-positive cells and total epidermal cells of the UVB-irradiated skin were as follow; dysplasia (28 of 5141), SCC *in situ* (9 of 6559), SCC (4 of 13,701).

As an additional test for BM origin, we used a mouse model in which BMDCs were GFP⁺ using BMT from GFP transgenic mice. Although we evaluated the percentages of BMDCs in UVB-irradiated skin, the GFP⁺/pancytokeratin⁺ cells were found at an extremely low percentage, ~0.12% in the epidermal dysplasia lesions and 0% in the SCC *in situ* lesions (data not shown). Previous reports about the *H. felis* gastric cancer also showed a similar tendency that the percentages of malignant cells with the marker of BMDCs was much lower in GFP-labeled model mice than in β -Gal-labeled model mice.¹⁵ Therefore we used an UVB-irradiated mouse model with labeled BMDCs with β -Gal in the following experiments.

Most BMDCs in the SCC Are Inflammatory Hematopoietic Cells

We considered that some X-Gal-positive cells in the UVB-irradiated skin were likely to be the tumor-infiltrating he-

matopoietic cells. To investigate the presence of these cells, triple staining for β -Gal, CD45 (hematopoietic marker), and a pancytokeratin (cytokeratin marker) was performed (Figure 3, A–C). The number of β -Gal⁺/CD45⁺ of all β -Gal⁺ cells per field was counted in UVB-irradiated mouse skin. In all β -Gal⁺ cells, 10.1% were positive for CD45 in the epidermal dysplasia lesions. Percentages of CD45⁺ cells of all β -Gal⁺ cells were 27.3% and 78.7% in the SCC *in situ* lesions and in the SCC lesions, respectively (Figure 3D). Some of the CD45⁺ cells were fused with carcinoma cells. Indeed, CD45 has been found to be expressed by cancer cells.^{30–32} However, we were unable to find X-Gal-positive cells that co-expressed CD45 and pancytokeratin. The result of our experiments clearly shows that some β -Gal⁺ cells are tumor-infiltrating hematopoietic cells, whereas other β -Gal⁺/CD45⁻ cells might be BMDCs that differentiated into tumor keratinocytes. However, the percentage of β -Gal⁺/CD45⁺ cells (indicating tumor-infiltrating hematopoietic cells) is increased in the SCC lesions. This observation would indicate that the actual occurrence rate of BM-derived keratinocytes is lower than our counting of BMDCs that were detected with X-Gal staining.

Small Number of BMDCs in the SCC Exhibited Donor XY Chromosomes

To further confirm BM origin, we analyzed UVB-induced skin SCC cells from female hosts (XX chromosomes) transplanted with male donor BM (XY chromosomes) using fluorescence *in situ* hybridization technique. We counted more than 10,000 cells and detected some donor-derived keratinocytes with XY chromosome expression, indicating BM origin (less than 0.05%) (Figure 4A).

In various organs, BMDCs contribute to the tissue reconstitution by either fusion²² or transdifferentiation.¹⁹ To determine whether BMDC engraftment into the specific tissue cells was because of differentiation or somatic cell fusion, fluorescence *in situ* hybridization was used because the fused cells would be expected to possess XXXY chromosomes. Although we observed keratinocytes with Y chromosomes in the tumor, none of them expressed an XXXY chromosome. However, fusion hybrids notoriously lose chromosomes and the absence of tetraploid cells does not rule out fusion.^{33–35} Therefore, we could not exclude the possibility of cell fusion with the present data.

Figure 4. XY chromosome expressions and epidermal stem cell markers of the BMDCs in the UVB-irradiated skin. **A:** Fluorescence *in situ* hybridization showed cells with single X chromosome (red, **arrow**) and single Y chromosome (cyan, **arrowhead**) in the UVB-induced skin SCC. XY chromosome cells, indicating BMDCs were indicated. **B:** Triple staining of β -Gal (green), $\alpha 6$ integrin (red), and pancytokeratin (cyan) was performed. **Arrow** shows β -Gal⁺ tumor keratinocytes. Although $\alpha 6$ integrin was positive within the edge of the tumor, we could not find any significant overexpression of $\alpha 6$ integrin of β -Gal⁺/cytokeratin⁺ cells. **C:** Triple staining of β -Gal (green), BrdU (red), pancytokeratin (cyan). **Arrows** show β -Gal⁺ tumor keratinocytes. **Arrowhead** shows a BrdU⁺ tumor keratinocyte. We found no β -Gal⁺/BrdU⁺ tumor keratinocytes.

BMDCs in the SCC Failed to Express Epidermal Stem Cell Marker

Although the CSC markers of skin SCC have yet to be defined, published studies suggest that tumor-initiating cells might be positive for the stem cell marker of the original organs.^{6,8} To investigate the possibility that BMDCs in the UVB-irradiated skin could share some characteristics of CSCs of skin SCC, we assayed the location of these presumptive CSCs that are positive for epidermal stem cell markers in the UVB-induced skin SCC.

Although CD34 is an established marker of skin epithelial stem cells,³⁶ none of the keratinocytes (including BM-derived keratinocytes) in the UVB-induced skin SCC expressed CD34 (data not shown). Furthermore, skin epithelial stem cells express elevated levels of $\alpha 6$ integrin compared with differentiated keratinocytes.³⁷ Although some keratinocytes in the edge of SCC showed $\alpha 6$ integrin expression, β -Gal⁺/pancytokeratin⁺ cells (indicating BM-derived keratinocytes) did not show significantly up-regulated $\alpha 6$ integrin expression compared with non-BM-derived keratinocytes (Figure 4B). In addition, tissue stem cells can be distinguished from transit-amplifying cells by their ability to incorporate and retain 5-bromo-2'-deoxyuridine (BrdU) throughout a long period of time. Therefore, tissue stem cells can be identified as label-retaining cells (LRCs).²⁹ To determine whether BMDCs in the UVB-irradiated mouse skin exhibit any LRC characteristics, the tumor-bearing mice were fed water containing BrdU. In the UVB-irradiated mice skin, no LRCs expressed β -Gal (Figure 4C). These results indicate that BMDCs in the UVB-induced skin SCC did not share any of these characters of the presumptive CSCs of the skin SCC.

Discussion

Based on recent investigations that suggest the possibility for BMDCs to be the origin of cancers,^{15,38} we used a labeled BMDC mouse model and investigated the role of BMDCs during UVB-induced carcinogenesis. With intermittent UVB irradiation, the epidermal morphology in mouse skin changed from the normal state through dysplasia, SCC *in situ*, and finally to SCC. These histological changes are analogous to the natural phenomenon observed in UVB-induced human skin carcinogenesis. We

certainly found BMDCs in UVB-irradiated mouse skin. Our data further suggests that BMDCs are recruited to the UVB-damaged skin and transdifferentiate into epidermal keratinocytes to reconstitute the skin, as we previously reported in wound repair.²⁰ We show the accelerated recruitment of BMDCs in the epidermal dysplasia lesions and the decreased rate of BMDCs in the SCC lesions. We propose this is attributable to the propagation of non-BM-derived malignant keratinocytes. Although BMDCs are recruited to the UVB-damaged skin and transdifferentiate into unaffected epidermal keratinocytes, BMDCs do not convert into malignant keratinocytes so that the rate of BMDCs relatively decreases as non-BM-derived tumor keratinocytes propagate to form skin SCC.

As a result, we found very few instances of BM-derived keratinocytes in the UVB-irradiated mouse skin. This observation strongly suggests that BMDCs are unlikely to be the origin of UVB-induced skin SCC. The objection will no doubt be raised that BMDCs might lose the expression of BM markers during the continuous UVB irradiation. Therefore we were careful to examine BM-derived keratinocytes in skin SCC with three different BMDC markers (β -Gal, GFP, Y chromosome analysis). Our conclusion is exactly the opposite of the *H. felis*-induced murine gastric carcinoma study.¹⁵ It is reasonable to suppose that the difference in the results between *H. felis*-induced gastric carcinoma study and our UVB-induced skin carcinoma study is partially attributable to the process of carcinogenesis including the type of genetic damage and degree of inflammation. In *H. felis*-induced gastric carcinoma, the pathogenic factor, namely CagA, increases the proliferation of host cells or inhibits cell apoptosis, stimulating the malignant transformation of host cells.^{39,40} These processes would be important for cancer progression from BMDCs. In humans, previous reports showed that solid cancers contain BM-derived cancer cells at a low level of 0 to 6% except for lung carcinoma that contains ~20% of BM-derived cancer cells.^{23,24} These data further showed that BMDCs do not contribute to skin cancers.²³ Our results are consistent with these observations.

The epidermis is continuously supplied with keratinocytes from the hair follicle bulge stem cells throughout adult life.⁴¹ Most epidermal keratinocytes that acquire

oncogenic mutations are lost during differentiation. Therefore, only long-term resident cells, such as stem cells, have the capacity to accumulate the required number of genetic hits necessary for tumor development. For this reason, it is not unreasonable to assume that these epidermal stem cells in the bulge could acquire oncogenic mutations, transdifferentiate into CSCs, and proliferate as malignant cells in the skin cancer. Although a previous report showed that BMDCs were more frequently found in the bulge area,⁴² we could not find such a tendency in our experiments in UVB-induced carcinogenesis. Our previous research in the damaged skin also showed no tendency of BMDC accumulation at specific skin sites.²⁰ Furthermore, we failed to find any evidence of BMDC clonal expansion in the UVB-irradiated mice skin. We also showed that BMDCs express no epidermal stem cell markers and fail to behave as LRCs, one of the main characteristics of tissue stem cells. Although the existence of the CSCs in the skin cancer has yet to be properly defined, we suggest that the CSCs in the UVB-induced skin SCC, if present, do not commonly originate from BMDCs.

It is important to determine the origin of the CSCs for the elucidation of carcinogenic mechanisms or for the treatment of cancer. Because of the recent reports that showed sarcoma derived from mesenchymal stem cells,^{43,44} an objection against transferring cells with the potential to have properties of stem or progenitor cells has arisen in regenerative medicine. However we can conclude from the results of our experiments that cancer cells in the UVB-induced skin SCC do not originate from BMDCs. Therefore we consider that in adopting or using BMDCs for regenerative medicine, the possibility of unexpected carcinogenesis can primarily be excluded and that BMDCs should be further tested and adapted for use in regenerative medicine, especially for skin.

We demonstrated the existence of BM-derived keratinocytes in the UVB-irradiated skin. These BM-derived keratinocytes were considered to be the result of transdifferentiation, not fusion. However, the number of BM-derived keratinocytes was extremely few, with no clonal expansion. Furthermore, BM-derived keratinocytes failed to express the epidermal stem cell markers (CD34, high $\alpha 6$ integrin and LRCs). Through our laboratory experiments, the possibility that BMDCs are the origin of UVB-induced skin SCC is extremely low.

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