Biochemical and Biophysical Research Communications 461 (2015) 695–701

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Epidermis–dermis junction as a novel location for bone marrow-derived cells to reside in response to ionizing radiation

Junko Okano [a, *], Hideto Kojima [b], Miwako Katagi [b], Yuki Nakae [c], Tomoya Terashima [b], Takahiko Nakagawa [d], Takeshi Kurakane [a], Naoki Okamoto [a], Keita Morohashi [a], Hiroshi Maegawa [c], Jun Udagawa [a]

[a] Division of Anatomy and Cell Biology, Shiga University of Medical Science, Shiga, Japan
[b] Department of Stem Cell Biology and Regenerative Medicine, Shiga University of Medical Science, Shiga, Japan
[c] Department of Internal Medicine, Shiga University of Medical Science, Shiga, Japan
[d] TMK Project, Medical Innovation Center, Kyoto University Graduate School of Medicine, Kyoto, Japan

ARTICLE INFO

Article history:
Received 13 April 2015
Available online 25 April 2015

Keywords:
Bone marrow transplantation
Bone marrow-derived cells
Ionizing radiation
Skin
Epidermis–dermis junction
Inflammation

ABSTRACT

Bone marrow-derived cells (BMDCs) can migrate into the various organs in the mice irradiated by ionizing radiation (IR). However, it may not be the case in the skin. While IR is used for bone marrow (BM) transplantation, studying with the epidermal sheets demonstrated that the BMDC recruitment is extraordinarily rare in epidermis in the mouse. Herein, using the chimera mice with BM from green fluorescent protein (GFP) transgenic mice, we simply examined if BMDCs migrate into any layers in the total skin, as opposed to the epidermal sheets, in response to IR. Interestingly, we identified the presence of GFP-positive (GFP^+) cells in the epidermis-dermis junction in the total skin sections although the epidermal cell sheets failed to have any GFP cells. To examine a possibility that the cells in the junction could be mechanically dissociated during separating epidermal sheets, we then salvaged such dissociated cells and examined its characteristics. Surprisingly, some GFP^+ cells were found in the salvaged cells, indicating that these cells could be derived from BM. In addition, such BMDCs were also associated with inflammation in the junction. In conclusion, BMDCs can migrate to and reside in the epidermis-dermis junction after IR.

1. Introduction

Ionizing radiation (IR) at ~10 Gy, a dose necessary for bone marrow (BM) transplantation, is known to deplete the resident dendritic cells (DCs), followed by the migration of BM-derived DCs in many tissues where those cells may contribute to the repair process after IR injury [1]. In contrast, the epidermis of the skin undergoes a unique repair process. The resident epidermal DCs called Langerhans cells (LCs) are not depleted due to their radioresistant nature so that the recruitment of BM-derived LCs is extraordinarily rare in the epidermis [1]. Likewise, BM-derived keratinocytes may not be commonly involved in skin repair except for such situations as grafting onto bone marrow transplantation (BMT) mice [2]. For these reasons, the contribution of BM-derived cells (BMDCs) to epidermal tissue repair has been widely believed to be extraordinarily rare in response to IR.

BMT with green fluorescent protein (GFP)-tagged cells to irradiated mice has been recognized as a highly efficient tool to analyze the role of BMDCs [3]. By using BMT mice, our research group has demonstrated that a small subset of BMDCs was capable of migrating into several organs to contribute maintenance of homeostasis in target organs [3–8]. In line with these studies, we simply examined both whole layers of ear skin sections and the epidermal sheets in our BMT model. As a result, a small number of BMDCs was interestingly detected in the epidermis in the close vicinity of dermis in total skin sections in response to IR. However, this finding was not confirmed by studying the epidermal sheets, which is a different approach to assess epidermis as consistent with previous notion [1,12],

In general, the epidermal sheet is a commonly-used tool to analyze various cell populations in the epidermis. However, a

* Corresponding author. Division of Anatomy and Cell Biology, Shiga University of Medical Science, Setatsukinowa-cho, Otsu-City, Shiga, 520-2192, Japan. Fax: +81 77 548 2642.
E-mail address: jokano@belle.shiga-med.ac.jp (J. Okano).

http://dx.doi.org/10.1016/j.bbrc.2015.04.094
0006-291X/

possibility has been pointed out that some basal cells including LCs from epidermal sheets can be lost in the preparation process [9]. In particular, cells localizing in the epidermis-dermis junction can be mechanically stretched out during preparation of epidermal sheets. Given these facts, we have assumed that BMDCs could be dissociated in the process if such cells reside in the junction. Here, we found that GFP$^+$ cells were present in the dissociated cells from the peeling process. Our data suggest that BMDCs can migrate into the special location of skin, the epidermal-dermis junction, after IR in the mouse.

2. Materials and methods

2.1. Mouse models

Wild-type C57BL/6 and C57BL/6-Tg (UBC-GFP) 30Scha/J (UBC-GFP hereafter) mice were purchased from CLEA (Osaka, Japan) and Jackson Laboratory (Bar Harbor, ME), respectively. MBR-1520R (Hitachi Medical Corporation, Tokyo, Japan) was operated at 150 kVp, 20 mA, with 0.5 mm Al and 0.1 mm Cu filters for irradiation. Adult mice aged 8 weeks or older were irradiated with a single dose of 10 Gy of X-ray. For BMT, 4×10^6 bone marrow cells from UBC-GFP mice were injected into the irradiated wild-type mice. Another set of mice was exposed to 1.5 J/cm^2 of ultraviolet C (UVC) after BMT as this model is known to show BMDCs migration into the skin and can be used as a positive control [1]. The other set of mice were protected by lead shields except the left ears and then irradiated with 20 Gy of X-ray three months after BMT. A lead shield resulted in a reduction of IR by 50-fold. The Animal Care Committee of Shiga University approved all experimental protocols (#2013-10-1H).

2.2. Immunohistochemistry

After exsanguination, a half of the right ear was excised and fixed in 4% paraformaldehyde for frozen sections (10 μm thick) to examine BMDCs in total skin sections. The other half was further split into dorsal and ventral halves, and incubated in 0.5% dispase (Invitrogen, Carlsbad, CA) for 30 min at 37 °C [10]. Then, the skin was carefully floated on RPMI (Wako, Kyoto, Japan) containing 10% fetal bovine serum not to sink epidermal surface (Fig. 1A) [11]. Epidermis was mechanically peeled with one stroke using curved tweezers [11]. The peeled epidermis was fixed and made into frozen sections (referred to as epidermal sections, hereafter) similarly as total skin sections. Both total skin and epidermal sections were stained with anti-rabbit Keratin 14 antibody (1:1000; Covance, Princeton, NJ) and mounted with Vector Shield with 4, 6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA). The secondary antibody was anti-rabbit immunoglobulins conjugated to Alexa Fluor 555 (1:1000; Life Technologies, Grand Island, NY). The sections were photographed using a laser-scanning confocal microscope (Nikon EZ-C1, Tokyo, Japan).

2.3. Flow cytometry

The epidermal cell suspension was prepared as previously described with slight modification [10]. After 0.5% dispase digestion of split ear skin, dermal side was rinsed with PBS to remove isolated contaminated dermal cells and skin was carefully floated on RPMI containing 10% FBS as described in the previous section to peel epidermis. A cluster of cells dissociated from the epidermis-dermis junction was salvaged into RPMI medium in this peeling process. After addition of 0.1% DNase (Sigma, St. Louis, MO) to RPMI, it was centrifuged at 1400 rpm for 10 min at 4 °C to collect the dissociated cells. Epidermal sheets were put in 0.3% trypsin containing 0.1% DNase solution and incubated at 37 °C for 10 min with shaking to release epidermal cells. Then, the same volume of RPMI containing 10% FBS was added to inactivate trypsin. Tubes were inverted gently twenty times and filtrated to obtain epidermal cell suspension. An amine-reactive dye (LIVE/DEAD Fixable Violet Dead Cell Stain Kit (Life Technologies)) was used to detect and remove dead cells in the dissociated cells and epidermal cell suspension. Epidermal cell suspension from UBC-GFP and wild-type mice was prepared for positive and negative control to determine GFP$^+$ population, respectively. Data was collected with FACSAria Fusion™ (BD Biosciences, San Jose, CA).

2.4. Statistics

Unpaired Student's *t*-test (two-tailed) was used to assess significance of the data, and $P<0.05$ was taken as statistically significant.

3. Results

First of all, the efficacy of BMT in our model was confirmed by the finding that 85.6 ± 7.52% (mean ± SD) of white blood cells were replaced with those of donor origin (n = 3) (Supplemental Fig. S1). Exsanguination was conducted to avoid BMDC contamination derived from blood in the immunohistological and flow cytometry analyses (Fig. 1, right images).

With the purpose of confirming our technical skill to identify BMDCs in the skin, we examined a BMT mouse model with UVC exposure as this model is known to exhibit BMDCs migration in the epidermis of the ear skin [1]. In this model, both total skin and epidermal sections were prepared from the ears 2 weeks after UVC exposure. Detection of Keratin 14 (K14) in the basal layer of epidermal sections indicated that the epidermis was successfully separated from the dermis by dispase digestion (right figure in Fig. 2A). We found that GFP$^+$ cells were present in the epidermis of the epidermal sheets as well as the total skin sections in this model (Fig. 2A). In particular, GFP$^+$ cells were distributed from the suprabasal layers to the basal layer of the epidermis. These data suggest that BMDCs can migrate into the epidermis in response to UVC. As this is consisted with previous studies [1,12], we confirmed that our skill was capable of detecting BMDCs in the skin.

We next examined the mouse ears with IR alone in the same way. We found that IR did not recruit any GFP cells in the epidermis of the separated epidermal sections as consistent with previous studies (Fig. 2B) [1,12]. In contrast, a substantial amount of GFP$^+$ cells was detected in the epidermis of total skin sections [(7.01 ± 0.744) x 10^4 cells/ear (mean ± SD)] (left figure in Fig. 2B and C; Supplemental Table S1). Interestingly, such GFP$^+$ cells were located only within the K14 positive layer, most of which were in the close vicinity of dermis in the total skin sections (Fig. 2B).

Such discrepancy led us to hypothesize that BMDCs can be actually migrate onto the epidermis-dermis junction, but could be lost in the preparation process of epidermal sheets. In other words, most of GFP positive cells were located on the epidermis–dermis junction and therefore there might be a chance for such cells to be dissociated in the process of peeling the epidermis. To address this issue, the dissociated cells were salvaged during peeling of the epidermis and compared with the single cell suspension prepared from epidermal sheets by flow cytometry (Fig. 1). After excluding the amine-reactive dye$^+$ cells as dead cells (Fig. 3A), we found substantial percentage of the salvaged cells was positive for GFP (25.3 ± 2.60% in the dissociated cells) (Fig. 3B, C and D). In contrast, epidermal cell suspension had very small

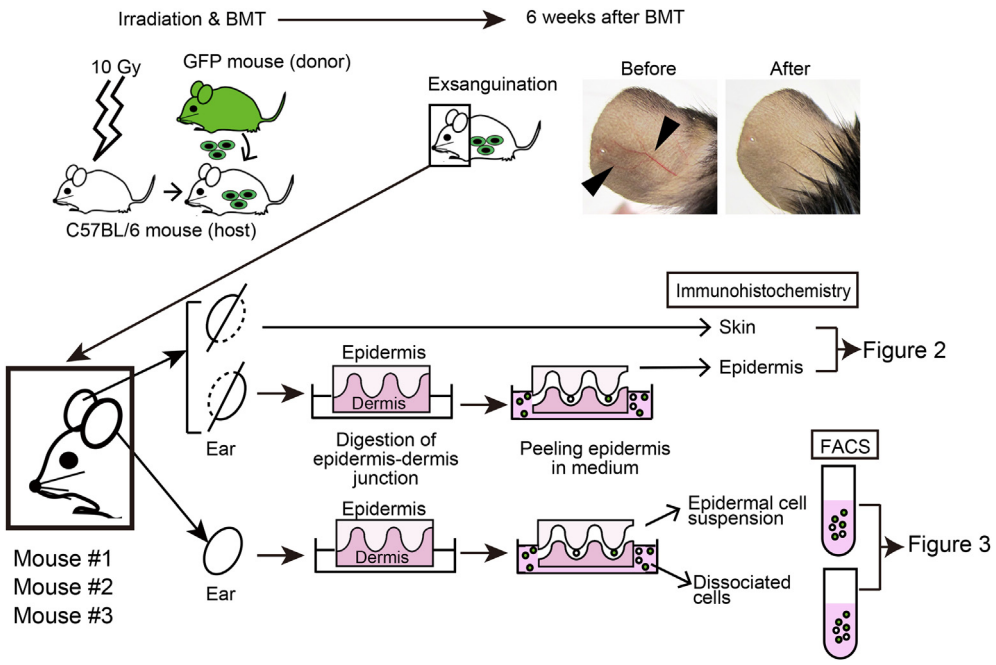

Fig. 1. Experimental design of this study is schematically shown. Photos at right upper corner show the ears before and after exsanguination. Arrowheads indicate blood vessel with blood cells.

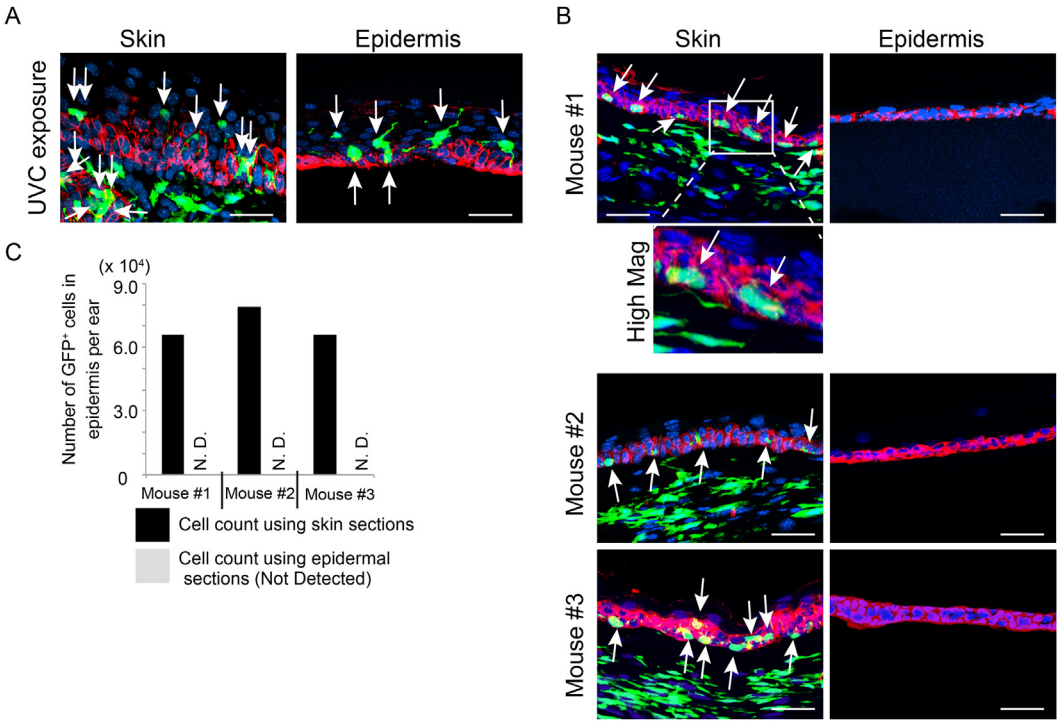

Fig. 2. Histological analyses using immunofluorescence Ear skin exposed to Ultraviolet C (UVC) contains substantial number of GFP-positive (green, arrows) cells in basal and suprabasal layers of epidermis in both total skin (Skin) and epidermal sections prepared from epidermal sheets (Epidermis). Basal layer of epidermis is stained by Keratin 14 (red color), while nuclei are labeled with DAPI (4, 6-diamidino-2-phenylindole, blue) (A). Immunohistological sections of skin and epidermal sheet from three mice (Mouse #1, #2, and #3) are shown. The white box in Mouse #1 is magnified below ("High Mag") to clearly show GFP^+ cells in the epidermis-demis junction. Arrows indicate GFP^+ cells in the epidermis. Scale bars = 25 μm (B). The graph shows the number of GFP^+ cells in the epidermis per ear as calculated in total skin (black bars) and epidermal (light grey bars; not detected; N. D.) sections. While total skin contains substantial number of GFP^+ cells in the epidermis, no cells are detected in the epidermal sections. Values are shown as mean ± SD (C). Data are from one experiment representative of two or three independent experiments with three mice per group.

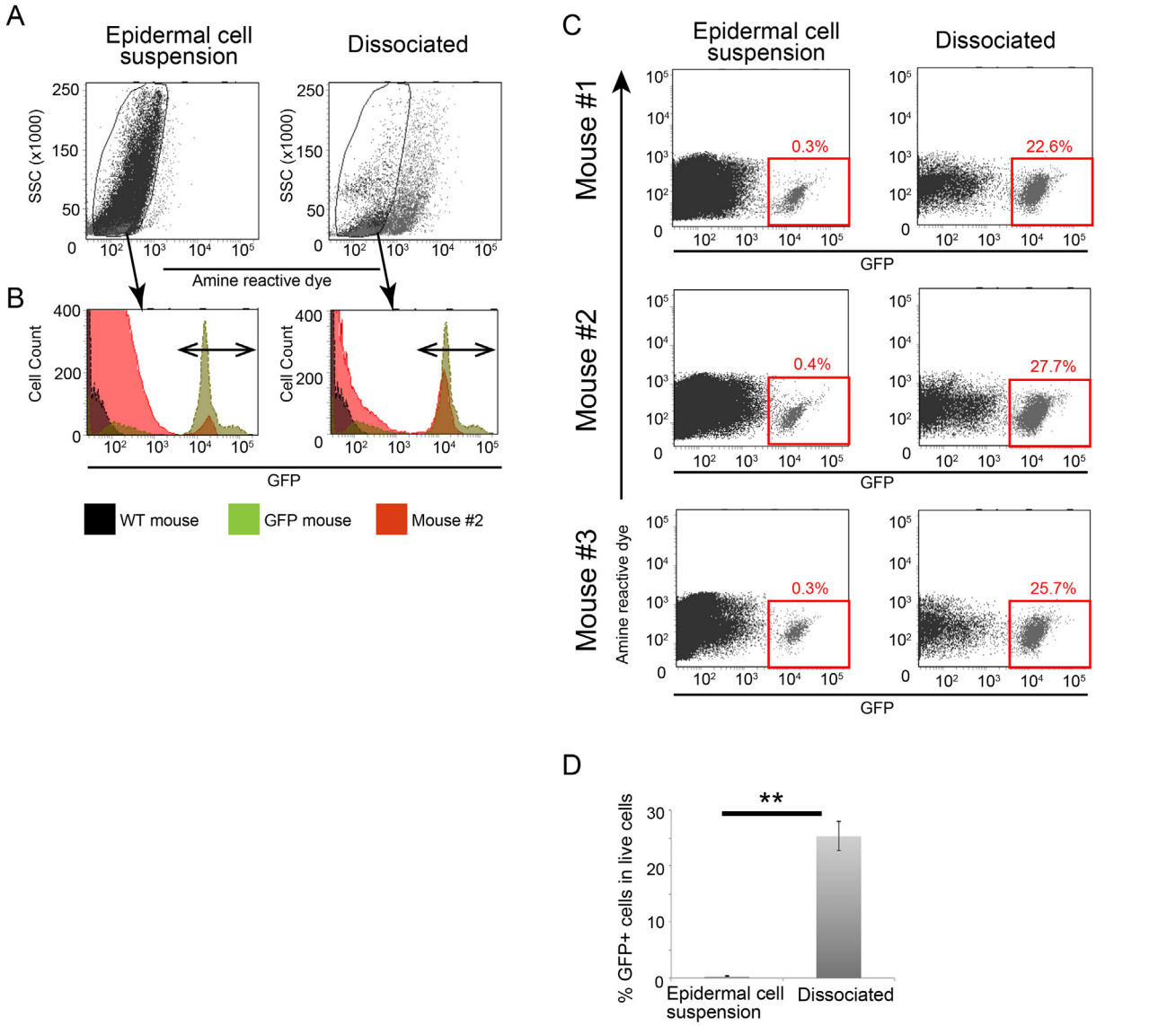

Fig. 3. Analyses using flow cytometry After side and forward scatter gating, amine reactive dye$^-$ cells were selected as live cells (A). Histograms to determine the GFP$^+$ population (two-headed arrows) in Mouse #2 (red), wild-type mouse as negative control (black), and GFP mouse as positive control (green) (B). The proportion of GFP$^+$ cells in each sample from three different mice is shown in red box and font (C). The percentage of dissociated GFP$^+$ cells was significantly higher than that in epidermal cell suspension. Values are shown as mean ± SD. $^{**}P < 0.01$ (D). Data are from one experiment representative of two independent experiments with three mice per group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

percentage of GFP positive cells (0.33 ± 0.06% (mean ± SD)) (Fig. 3B, C and D). This is consistent with previous findings [13]. Altogether, GFP$^+$ cells can be located in the epidermal-dermal junction and may be a reaction from IR.

The recruitment of BMDCs was found to correlate with the degree of inflammation in case of UVC exposure [1], suggesting that inflammation could be a driving force to bring BMDCs into the lesions. Then, we examined whether a 10 Gy of IR could cause inflammation in the skin using Keratin 6 (K6) antibody, which is a marker of activated keratinocytes and positive in inflamed epidermis [14]. In wild-type mice, there were no K6 positive cells in the interfollicular epidermis although some positive cells were observed around hair follicles (Fig. 4A, upper panels). In contrast, UVC exposure appeared to increase K6 expression along with hyperplastic response in the epidermis as previously suggested (Fig. 4A, middle panels) [1]. In turn, IR could also induce K6 expression, which was predominantly observed in the cells located basally in the epidermis (Fig. 4A, bottom panels). Importantly, some BMDCs were co-localized with K6-positive keratinocytes under IR stimulation (Fig. 4A), suggesting that BMDCs recruitment was associated with inflammation caused by IR.

Finally, we examined if higher dose of IR could induce proportionally the BMDCs migration in the epidermis. To address this issue, the left ears of BMT mice were irradiated with 20 Gy, followed by the preparation of epidermal cell suspension after 2 weeks (Fig. 4B). Flow cytometry revealed that BMDCs migration in the epidermis after additional 20 Gy exposure significantly increased compared with those exposed to 10 Gy alone in performing BMT (Fig. 4C, left). Consistently, IR tended to increase the percentages of BMDCs in dissociated cells in dose dependent manner. (Fig. 4C, right). These data indicate that higher dose IR is more potent to induce BMDCs migration into the epidermis.

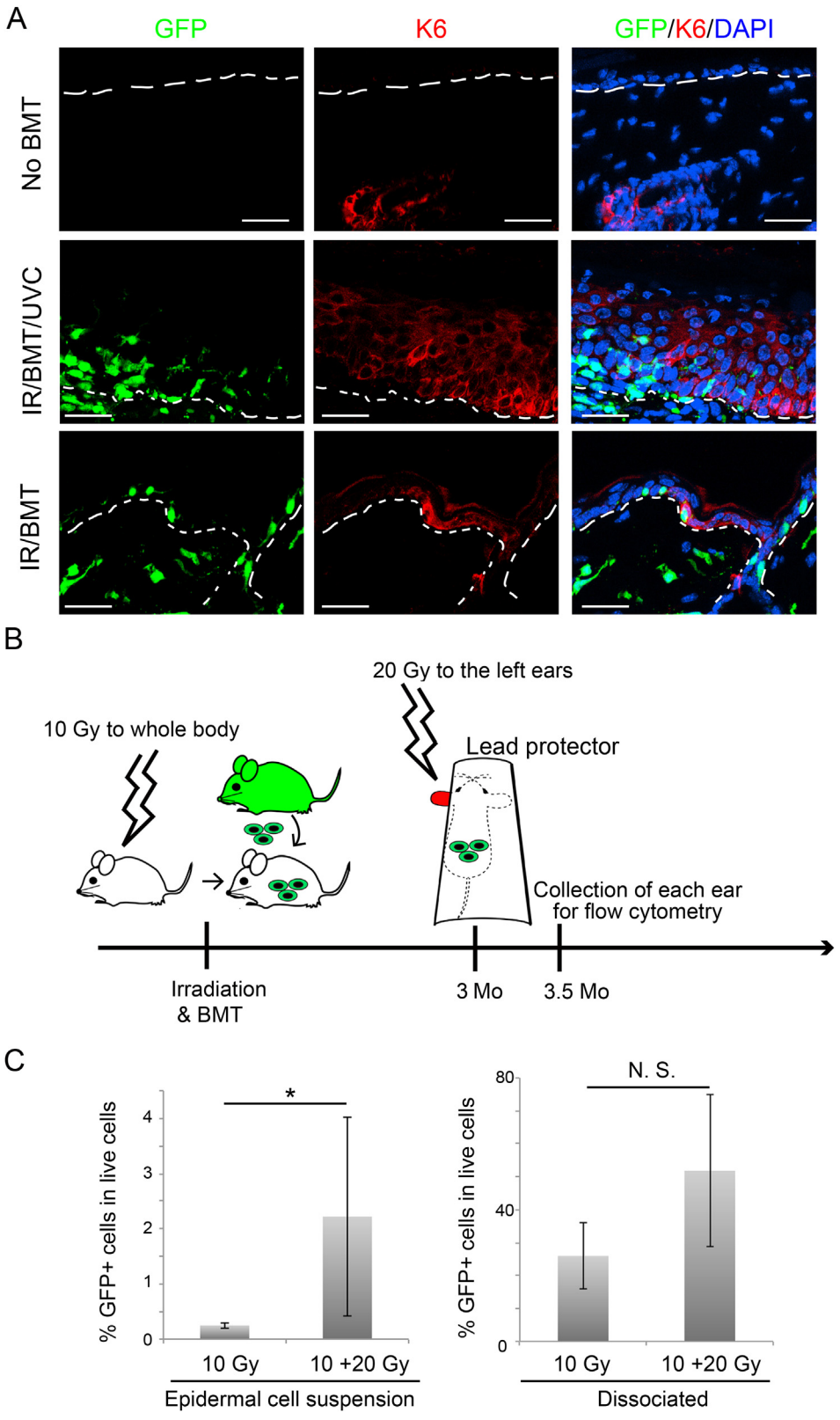

Fig. 4. Association of BMDCs with inflammation and IR dose Compared to wild-type (No BMT), both UVC exposure (IR/BMT/UVC) and IR (IR/BMT) increases the number of Keratin 6 (K6) positive interfollicular epidermal cells (red), some of which are overlapped with GFP signals in the epidermis. DAPI (blue) signal indicates nucleus. Dot lines indicate the boundary between epidermis and dermis. Scale bars = 25 μm (A). Experimental protocol to compare the effects between 10 Gy exposure in the left ear and 10 + 20 Gy in the right ear in terms of the percentage of BMDCs. Mo; months after IR/BMT (B). The percentages of GFP^+ cells in epidermal cell suspension and in dissociated cells after 10 Gy of IR alone and 10 + 20 Gy are shown, respectively. Values are shown as mean ± SD. $^*P < 0.03$, N.S.: not significant (C). Data are from one experiment representative of two independent experiments with three mice per group.

4. Discussion

In the present study, we demonstrated evidence that BMDCs could migrate into the skin in response to IR. Importantly, BMDCs were restricted to be located in the epidermis-dermis junction where inflammation was also mildly induced in response to IR. We found that cells residing in this location could fall off during the preparation of the epidermal sheets as the junction was a special location which could be stretch out in the peeling off the epidermis. This could be a reason why there were no BMDCs in the epidermal sheets.

An advantage of using the epidermal sheets is that epidermal cell suspension prepared from epidermal sheets allows us to perform flow cytometry which could quantify and obtain the cells of interest in total cells by using various antibodies against proteins on the cell surfaces. In contrast, it is much more difficult to obtain single cell suspension from whole skin because dermis consists of connective tissue which is consists of collagen, elastic fibers and extracellular matrices [15]. For those reasons, the epidermal sheets are generally preferred to whole skin in order to prepare single cell suspension. In particular, ear skin is the most intensively examined as dermis and subcutaneous fat is so thin that the chances of their contamination are lower than other regions.

The epidermis-dermis junction is anatomically a unique region where basement membrane lies between epidermis and dermis, and the basement membrane should be enzymatically digested prior to peeling out the epidermis from dermis. We used dispase in this experiment as it is commonly used. However, this enzyme lacks an ability to digest laminin, a component of basement membrane although fibronectin and type IV collagen, both of which are components of the basement membrane,can be digested [16,17]. As a result, dispase digestion is usually heterogeneous, and therefore physical force must be required to peel out the epidermis from dermis for the preparation for epidermal sheets. In other words, BMDCs residing in the epidermis in the vicinity of dermis are mechanically stretched out during the peeling of epidermis. Trypsin, a serine protease, is also commonly used to separate epidermis from dermis, followed by triturating epidermal sheet into single cells to make the epidermal cell suspension [19]. This enzyme makes it possible to separate epidermis from dermis by digesting specifically heparin sulfate proteoglycan, another component of basement membrane but not laminin and type IV collagen [18].

Inflammation is often concomitant with a recruitment of BMDCs while potent stimuli have been thought to be required to cause the epidermal inflammation [1,2,20]. In this regard, IR at ~10 Gy has been thought not to be potent enough to induce inflammation as no obvious dermatological symptoms (such as erythema, desquamation and thickening of the skin) was induced in rodents [21]. However, recent technological development in science found that 4–8 Gy of IR was able to induce apoptosis in mouse hair bulb cells *in vivo*. Likewise, IR doses less than 2 Gy was potent enough to perturb the expression patterns of secreted proteins in the human skin [22,23]. Interestingly, the alterations in inflammatory protein profiles in response to less than 0.1 Gy was found to be identical to that by higher-dose, 2 Gy, of IR [24]. Consistently, this study demonstrated that IR at ~10 Gy induced inflammation, as evidenced by the presence of K6-positive keratinocytes, in the epidermal-dermis junction where BMDCs were recruited. Taken together, low-doses of IR are likely capable of inducing subtle inflammation in the mouse skin. Such inflammation could account for the BMDC migration in this study.

A potential mechanism for inflammation-induced BMDCs entry in the epidermis is that chemokine (C–C motif) ligand 2 (CCL2) and CCL7 secreted by inflamed keratinocytes force BMDCs expressing CCR2, a receptor for CCL2 [1]. Recently, inflammatory keratinocytes in the epidermis of hair follicles has been shown to regulate the entry of BMDCs by secreting CCL1, 8, and 20 as well as CCL2 [20]. Therefore, some of these chemokines appear to play important roles on the recruitment of BMDCs by IR.

In summary, we demonstrated that low grade IR could recruit BMDCs into the epidermal-dermis junction in concomitant with mild inflammation.

Conflict of interest

The authors state no conflict of interest.

Acknowledgments

We thank T Yamamoto and Y Mori for technical assistance. This work was supported by a grant-in–aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology (no. 26462730 to JO and 23590378 to HK), the President's Discretionary Funds from Shiga University of Medical Science (no. 515503ZZG and 1515503ZZA to JO, and 151550322J to HK) and a grant in-aid from Banyu Life Science Foundation International to JO.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.04.094.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.094.

References

[1] M. Merad, M.G. Manz, H. Karsunky, A. Wagers, W. Peters, I. Charo, I.L. Weissman, J.G. Cyster, E.G. Engleman, Langerhans cells renew in the skin throughout life under steady-state conditions, Nat. Immunol. 3 (2002) 1135–1141.
[2] K. Tamai, T. Yamazaki, T. Chino, M. Ishii, S. Otsuru, Y. Kikuchi, S. Iinuma, K. Saga, K. Nimura, T. Shimbo, N. Umegaki, I. Katayama, J. Miyazaki, J. Takeda, J.A. McGrath, J. Uitto, Y. Kaneda, PDGFRalpha-positive cells in bone marrow are mobilized by high mobility group box 1 (HMGB1) to regenerate injured epithelia, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 6609–6614.
[3] M. Katagi, T. Terashima, J. Okano, H. Urabe, Y. Nakae, N. Ogawa, J. Udagawa, H. Maegawa, K. Matsumura, L. Chan, H. Kojima, Hyperglycemia induces abnormal gene expression in hematopoietic stem cells and their progeny in diabetic neuropathy, FEBS Lett. 588 (2014) 1080–1086.
[4] H. Kojima, M. Fujimiya, K. Matsumura, T. Nakahara, M. Hara, L. Chan, Extrapancreatic insulin-producing cells in multiple organs in diabetes, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 2458–2463.
[5] Y. Li, H. Kojima, K. Fujino, K. Matsumura, M. Katagi, H. Urabe, L. Chan, Y. Eguchi, L. Zhao, H. Kimura, Homing of the bone marrow-derived interstitial cells of cajal is decreased in diabetic mouse intestine, J. Gastroenterol. Hepatol. 26 (2011) 1072–1078.
[6] T. Terashima, H. Kojima, M. Fujimiya, K. Matsumura, J. Oi, M. Hara, A. Kashiwagi, H. Kimura, H. Yasuda, L. Chan, The fusion of bone-marrow-derived proinsulin-expressing cells with nerve cells underlies diabetic neuropathy, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 12525–12530.
[7] M. Fujimiya, H. Kojima, M. Ichinose, R. Arai, H. Kimura, A. Kashiwagi, L. Chan, Fusion of proinsulin-producing bone marrow-derived cells with hepatocytes in diabetes, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 4030–4035.
[8] H. Urabe, H. Kojima, L. Chan, T. Terashima, N. Ogawa, M. Katagi, K. Fujino, A. Kumagai, H. Kawai, A. Asakawa, A. Inui, H. Yasuda, Y. Eguchi, K. Oka, H. Maegawa, A. Kashiwagi, H. Kimura, Haematopoietic cells produce BDNF and regulate appetite upon migration to the hypothalamus, Nat. Commun. 4 (2013) 1526.
[9] G. Schuler, Epidermal Langerhans Cells, CRC Press, 1990.
[10] Y. Nagaoka, K. Nakamura, N. Yasaka, T. Watanabe, A. Asahina, K. Tamaki, Identification and characterization of the low-affinity receptor for immunoglobulin E (Fc|[egr]|RII|[sol]|CD23) on murine langerhans cells, J. Invest Dermatol 119 (2002) 130–136.

[11] A. Kubo, K. Nagao, M. Amagai, 3D visualization of epidermal langerhans cells, Methods Mol. Biol. 961 (2013) 119–127.
[12] N. Romani, B.E. Clausen, P. Stoitzner, Langerhans cells and more: langerin-expressing dendritic cell subsets in the skin, Immunol. Rev. 234 (2010) 120–141.
[13] C. Ghigo, I. Mondor, A. Jorquera, J. Nowak, S. Wienert, S.P. Zahner, B.E. Clausen, H. Luche, B. Malissen, F. Klauschen, M. Bajenoff, Multicolor fate mapping of langerhans cell homeostasis, J. Exp. Med. 210 (2013) 1657–1664.
[14] R.P. Hobbs, J.C. Lessard, P.A. Coulombe, Keratin intermediate filament proteins – novel regulators of inflammation and immunity in skin, J. Cell. Sci. 125 (2012) 5257–5258.
[15] R.R. Driskell, F.M. Watt, Understanding fibroblast heterogeneity in the skin, Trends Cell. Biol. 25 (2015) 92–99.
[16] K.S. Stenn, R. Link, G. Moellmann, J. Madri, E. Kuklinska, Dispase, a neutral protease from Bacillus polymyxa, is a powerful fibronectinase and type IV collagenase, J. Invest Dermatol 93 (1989) 287–290.
[17] G.W. Laurie, C.P. Leblond, G.R. Martin, Localization of type IV collagen, laminin, heparan sulfate proteoglycan, and fibronectin to the basal lamina of basement membranes, J. Cell. Biol. 95 (1982) 340–344.
[18] D. Woodley, D. Sauder, M.J. Talley, M. Silver, G. Grotendorst, E. Qwarnstrom, Localization of basement membrane components after dermal-epidermal junction separation, J. Invest Dermatol 81 (1983) 149–153.
[19] H. Takahashi, K. Sano, K. Yoshizato, N. Shioya, K. Sasaki, Comparative studies on methods of isolating rat epidermal cells, Ann. Plast. Surg. 14 (1985) 258–266.
[20] K. Nagao, T. Kobayashi, K. Moro, M. Ohyama, T. Adachi, D.Y. Kitashima, S. Ueha, K. Horiuchi, H. Tanizaki, K. Kabashima, A. Kubo, Y.H. Cho, B.E. Clausen, K. Matsushima, M. Suematsu, G.C. Furtado, S.A. Lira, J.M. Farber, M.C. Udey, M. Amagai, Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin, Nat. Immunol. 13 (2012) 744–752.
[21] R.J. Cummings, S. Mitra, T.H. Foster, E.M. Lord, Migration of skin dendritic cells in response to ionizing radiation exposure, Radiat. Res. 171 (2009) 687–697.
[22] F. Nakayama, A. Hagiwara, M. Kimura, M. Akashi, T. Imamura, Evaluation of radiation-induced hair follicle apoptosis in mice and the preventive effects of fibroblast growth factor-1, Exp. Dermatol Den. (2009) 889–892.
[23] Q. Zhang, M. Matzke, A.A. Schepmoes, R.J. Moore, B.J. Webb-Robertson, Z. Hu, M.E. Monroe, W.J. Qian, R.D. Smith, W.F. Morgan, High and low doses of ionizing radiation induce different secretome profiles in a human skin model, PLoS One 9 (2014) e92332.
[24] S.M. Varnum, D.L. Springer, M.E. Chaffee, K.A. Lien, B.J. Webb-Robertson, K.M. Waters, C.A. Sacksteder, The effects of low-dose irradiation on inflammatory response proteins in a 3D reconstituted human skin tissue model, Radiat. Res. 178 (2012) 591–599.