

# 1 $\alpha$ ,25-Dihydroxyvitamin D<sub>3</sub> Modulates the Response of Human Keratinocytes to Ionizing Radiation Exposure

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**Abstract.** *Background:* Exposure of human skin to ionizing radiation may result in various effects such as inflammation, keratosis, fibrosis and cancer. 1 $\alpha$ ,25-Dihydroxyvitamin D<sub>3</sub> (1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>), the biologically active metabolite of vitamin D, has been shown to exert pleiotropic effects on the skin. The aim of the study was to evaluate the effect of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> on the radiation response of human keratinocytes. *Materials and Methods:* Keratinocytes (HaCaT), either untreated or pretreated with 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>, were irradiated with 0-7.5 Gy. Growth curves were generated to determine cell proliferation. Cell survival was examined using a clonogenic assay. The cell surface expression of adhesion molecules was investigated by flow cytometry. *Results:* The cell growth and clonogenic survival of irradiated keratinocytes were both significantly increased by 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>. Ionizing radiation caused an up-regulation of the cell surface expression of intercellular adhesion molecule-1 (ICAM-1) and integrins beta1 (CD29), alpha2 (CD49b), alpha5 (CD49e) and alpha6 (CD49f) in keratinocytes, which was inhibited by 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>. *Conclusion:* The data suggest that 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> may be a promising agent to modify the radiation reaction, thus offering new options in radiotherapy and oncology.

1 $\alpha$ ,25-Dihydroxyvitamin D<sub>3</sub> (1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>), the biologically active form of vitamin D, exerts its actions through a nuclear receptor protein named the vitamin D receptor (VDR) (1). Beyond its prominent role in calcium homeostasis and bone metabolism, 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> is widely recognized to possess growth-regulatory and immunomodulatory properties in a wide variety of tissues and cells (2). The skin is a target organ for 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>, and the vitamin D receptor is expressed in cells of both the epidermis and the dermis (3-6).

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Radiation effects on skin are a common consequence of routine clinical radiotherapy (7). Clarification of the pathophysiology of the cutaneous radiation reaction and the evaluation of therapeutical interventions are, therefore, mandatory to benefit patients suffering from dermatological complications after radiotherapy.

Exposure of the skin to ionizing radiation is known to induce acute inflammatory responses including alterations in adhesive interactions (8). These are mediated by cellular adhesion molecules, which interact with extracellular matrix proteins and also play important roles in mediating cell-cell adhesions (9). Among the many types of adhesion receptors on the cell surface, two major families stand out, the integrins and the immunoglobulin superfamily. Structurally, each integrin is a heterodimer consisting of an alpha subunit non-covalently associated with a beta subunit. The integrin family comprises 18 alpha and eight beta transmembrane subunits, to date known to assemble 24 different alphabeta heterodimers (9). Human keratinocytes have been demonstrated to express several members of the beta1 subfamily of integrins, including alpha2beta1 (CD49b/CD29), a receptor for collagen and laminin, alpha5beta1 (CD49e/CD29), the keratinocyte fibronectin receptor, and alpha6beta1 (CD49f/CD29), a receptor for laminin (10). The intercellular adhesion molecule-1 (ICAM-1), an important member of the immunoglobulin superfamily of cellular adhesion molecules, has also been identified on human keratinocytes (11-14). ICAM-1 has been proposed to function as a mediator of adhesive interactions by binding to two integrins belonging to the  $\beta$ 2 subfamily *i.e.*, CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1) on the surface of leukocytes, which is important for their transendothelial migration to sites of inflammation (11). Previous studies have shown that the cell surface expression of both beta1 integrin (CD29) and ICAM-1 in human skin cells is up-regulated in response to ionizing radiation (12-15). Although a few studies have addressed the effects of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> on the expression of adhesion molecules (16-21), to our knowledge, no data on the impact of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> on adhesion molecule expression in skin cells after ionizing radiation are available.

Several *in vitro* and *in vivo* studies had been devoted to the influence of  $1\alpha,25(\text{OH})_2\text{D}_3$  on the cell proliferation of epidermal cells (22). It has been proposed that  $1\alpha,25(\text{OH})_2\text{D}_3$  acts as a physiological regulator of keratinocyte growth and differentiation. At low concentrations,  $1\alpha,25(\text{OH})_2\text{D}_3$  promoted the proliferation of keratinocytes *in vitro*; at higher pharmacological doses, keratinocyte proliferation was inhibited (23).

The aim of the present study was to investigate whether  $1\alpha,25(\text{OH})_2\text{D}_3$  affects the cell growth and clonogenic cell survival of human HaCaT keratinocytes after ionizing radiation exposure. In addition, the cell surface expression of adhesion molecules on irradiated HaCaT cells was assayed by flow cytometry, and the impact of pretreatment with  $1\alpha,25(\text{OH})_2\text{D}_3$  prior to ionizing radiation was evaluated. The results suggest that  $1\alpha,25(\text{OH})_2\text{D}_3$  may have therapeutic potential in cutaneous immune disorders *in vivo* caused by ionizing radiation.

## Materials and Methods

**Reagents.**  $1\alpha,25(\text{OH})_2\text{D}_3$  was obtained from Sigma (Munich, Germany). The following antibodies were purchased from Chemicon (Hampshire, UK): fluorescein 5-isothiocyanate (FITC)-conjugated mouse monoclonal antibodies (mAbs) to human ICAM-1 (clone 15.2), alpha2 integrin (clone AK7) and alpha6 integrin (clone 4F10); the phycoerythrin (PE)-conjugated mouse mAbs to alpha5 integrin (clone SAM-1) and beta1 integrin (clone TDM29); mouse IgG1 (clone DD7) and IgG2b (clone DD311) negative isotype control antibodies conjugated to either FITC or PE.

**Cell culture.** The spontaneously immortalized, non-tumorigenic keratinocyte cell line HaCaT (24) was a kind gift from N. Fusenig (DKFZ, Heidelberg, Germany). The HaCaT cells were cultured in Dulbecco's modified Eagle medium (DMEM, PAA Laboratories, Linz, Austria) supplemented with 10% fetal bovine serum (FBS, PAA Laboratories) at 37°C in a humidified atmosphere in the presence of 10% CO<sub>2</sub>; the pH was maintained at 7.4.

**Irradiation of cells.** Exponentially growing HaCaT cells were irradiated with 240 kV X-rays (Isovolt 320/10, Seifert, Ahrensburg, Germany) filtered through 3 mm Be with a focus-to-culture distance of 40 cm. The absorbed dose was measured using a Duplex dosimeter (PTW, Freiburg, Germany). The dose rate was 1 Gy min<sup>-1</sup> at 13 mA. The applied doses ranged from 0-7.5 Gy.

**Growth curve determination.** Exponentially growing HaCaT cells were incubated with 10<sup>-8</sup> M  $1\alpha,25(\text{OH})_2\text{D}_3$  for 48 h before irradiation with 0-5 Gy. At different time-points after irradiation, the cells were harvested by gentle trypsinization and mixed with an equal volume of Trypan blue. The number of living cells was counted with a hemocytometer.

**Clonogenic assay.** The clonogenic assay was applied for measurement of clonogenic cell survival. Single HaCaT cells were seeded in small petri dishes and allowed to attach overnight before treatment with 10<sup>-8</sup> M  $1\alpha,25(\text{OH})_2\text{D}_3$  for 48 h. The cells were

irradiated with 0-7.5 Gy. After 10-14 days, colonies with >50 cells were stained with Coomassie blue and counted. The fit of the dose-effect curves was calculated using the linear-quadratic model.

**Flow cytometry.** The cell surface expression of adhesion molecules on HaCaT cells was analyzed by flow cytometry. In brief, exponentially growing cells, either untreated or pretreated with 10<sup>-8</sup> M  $1\alpha,25(\text{OH})_2\text{D}_3$  for 48 h, were irradiated with 5 Gy. At different time-points after irradiation, non-irradiated and irradiated cells were trypsinized and washed with phosphate-buffered saline (PBS) prior to incubation with mAbs to ICAM-1, integrins beta1, alpha2, alpha5 and alpha6 or isotype-matched negative controls on ice in the dark. After washing twice with ice-cold PBS, the cells were fixed in PBS/1% paraformaldehyde. Flow cytometric analysis was performed using a Becton-Dickinson FACS Calibur flow cytometer and Cell Quest Software (Becton-Dickinson, Heidelberg, Germany). The fluorescence intensity of the FITC or PE emission of at least 10,000 cells was quantified and the mean channel fluorescence (MCF) served as an indicator of the amount of the respective adhesion molecule. The results were expressed as the difference between the MCF of cells stained with an antibody to an adhesion molecule and the MCF of cells stained with an isotype control antibody normalized to the basal expression of non-irradiated, untreated cells. The cell viability, as determined by flow cytometry using propidium iodide dye exclusion, was consistently more than 90%.

**Statistical analysis.** The results were expressed as mean±SD. The Student's *t*-test was used for comparison between the control and experimental groups. *P*<0.05 was considered to be statistically significant.

## Results

**Cell growth of keratinocytes after ionizing radiation is enhanced by  $1\alpha,25(\text{OH})_2\text{D}_3$ .** To evaluate the effect of ionizing radiation on the cell proliferation of human keratinocytes, HaCaT cells were irradiated with doses between 0 and 5 Gy and subjected to growth curve determination at different time-points after irradiation. The analysis revealed that the proliferation of keratinocytes was dose-dependently inhibited by ionizing radiation (Figure 1A). In order to investigate whether the radiation effect on cell proliferation can be modulated by  $1\alpha,25(\text{OH})_2\text{D}_3$ , HaCaT cells were pretreated with  $1\alpha,25(\text{OH})_2\text{D}_3$  at 48 h prior to irradiation. As shown in Figure 1B, the cell growth of the irradiated keratinocytes was significantly increased when the cells had been treated with  $1\alpha,25(\text{OH})_2\text{D}_3$  prior to irradiation, as shown exemplarily for day 4 and day 6 after ionizing radiation.

**$1\alpha,25(\text{OH})_2\text{D}_3$  causes an increase in cell survival of irradiated keratinocytes.** After establishing the influence of  $1\alpha,25(\text{OH})_2\text{D}_3$  on the cell growth of irradiated keratinocytes, we investigated whether  $1\alpha,25(\text{OH})_2\text{D}_3$  also affects the cell survival of keratinocytes after ionizing radiation. Therefore, cell survival curves of keratinocytes after irradiation with doses between 0 and 7.5 Gy, either with or without

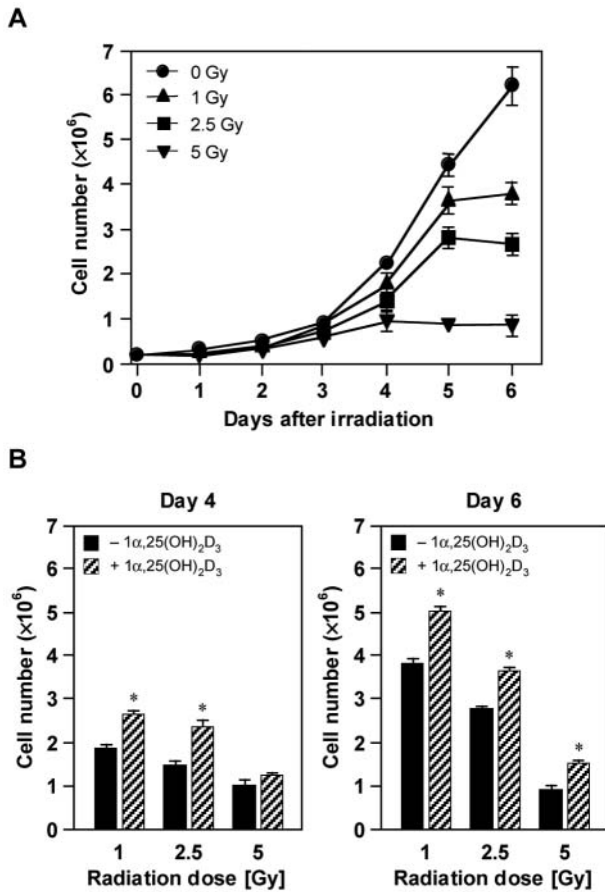


Figure 1. Pretreatment of human keratinocytes with  $1\alpha,25(OH)_2D_3$  prior to ionizing radiation elevated cell growth. (A) HaCaT cells were irradiated with 0, 1, 2.5 or 5 Gy. At the indicated time-points after irradiation, the number of living cells was counted using a hemocytometer. (B) HaCaT cells, either untreated or treated with  $10^{-8}$  M  $1\alpha,25(OH)_2D_3$  for 48 h, were irradiated with 0, 1, 2.5 or 5 Gy. Cell growth was determined every day up to one week after irradiation. The cell numbers of irradiated keratinocytes are exemplarily shown for day 4 and day 6 after irradiation. The results are shown as mean  $\pm$  SD from three independent experiments. A statistically significant difference between control cells and cells pretreated with  $1\alpha,25(OH)_2D_3$  was determined using the Student's *t*-test, and is indicated as \*,  $p < 0.05$ .

pretreatment with  $1\alpha,25(OH)_2D_3$  for 48 h, were generated. Clonogenic studies showed a significant suppression in colony number after ionizing radiation (Figure 2). Treatment of keratinocytes with  $1\alpha,25(OH)_2D_3$  prior to ionizing radiation resulted in a significantly higher number of colonies after 14 days in culture, as compared to the untreated controls (Figure 2).

*Ionizing radiation induces an up-regulation of adhesion molecule expression on keratinocytes.* Radiation exposure of human skin causes various acute effects, one of which is inflammation. As inflammatory processes involve cell-cell

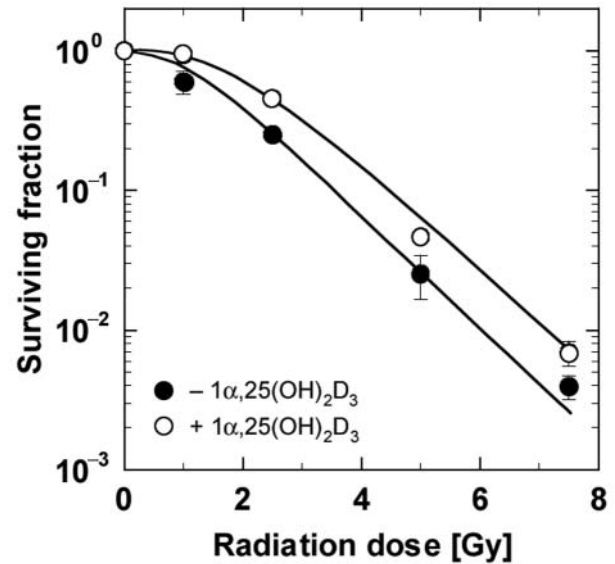


Figure 2. Cell survival of human keratinocytes after ionizing radiation was significantly increased by  $1\alpha,25(OH)_2D_3$ . HaCaT cells, either untreated or treated with  $10^{-8}$  M  $1\alpha,25(OH)_2D_3$  for 48 h, were irradiated with different doses. Cell survival was investigated by the clonogenic assay. The fit of the dose-effect curves was calculated using the linear-quadratic model. The results are expressed as mean  $\pm$  SD from three independent experiments.

and cell-matrix interactions which are mediated by cellular adhesion molecules, the effect of ionizing radiation on adhesion molecule expression on keratinocytes was evaluated next. Therefore, HaCaT cells were irradiated with 5 Gy and investigated for cell surface expression of adhesion molecules at 48 h after irradiation. The flow cytometric analysis revealed that HaCaT cells constitutively expressed ICAM-1 and integrins beta1, alpha2, alpha5 and alpha6 on their cell surface (Figure 3A). Exposure of HaCaT cells to ionizing radiation with 5 Gy caused an up-regulation of the cell surface expression of all five adhesion molecules, as compared to the non-irradiated controls (Figure 3A).

*$1\alpha,25(OH)_2D_3$  suppresses the radiation-induced up-regulation of adhesion molecule expression.* To determine the impact of  $1\alpha,25(OH)_2D_3$  on the increase of the cell surface expression of adhesion molecules on HaCaT cells caused by ionizing radiation, cells were pretreated with  $1\alpha,25(OH)_2D_3$  prior to ionizing radiation with 5 Gy and analyzed using flow cytometry at 48 h after irradiation. The results showed that the radiation-induced up-regulation of expression of the adhesion molecules ICAM-1 and integrins beta1, alpha2, alpha5 and alpha6 on HaCaT cells was inhibited by  $1\alpha,25(OH)_2D_3$ , in comparison to untreated controls (Figure 3B).

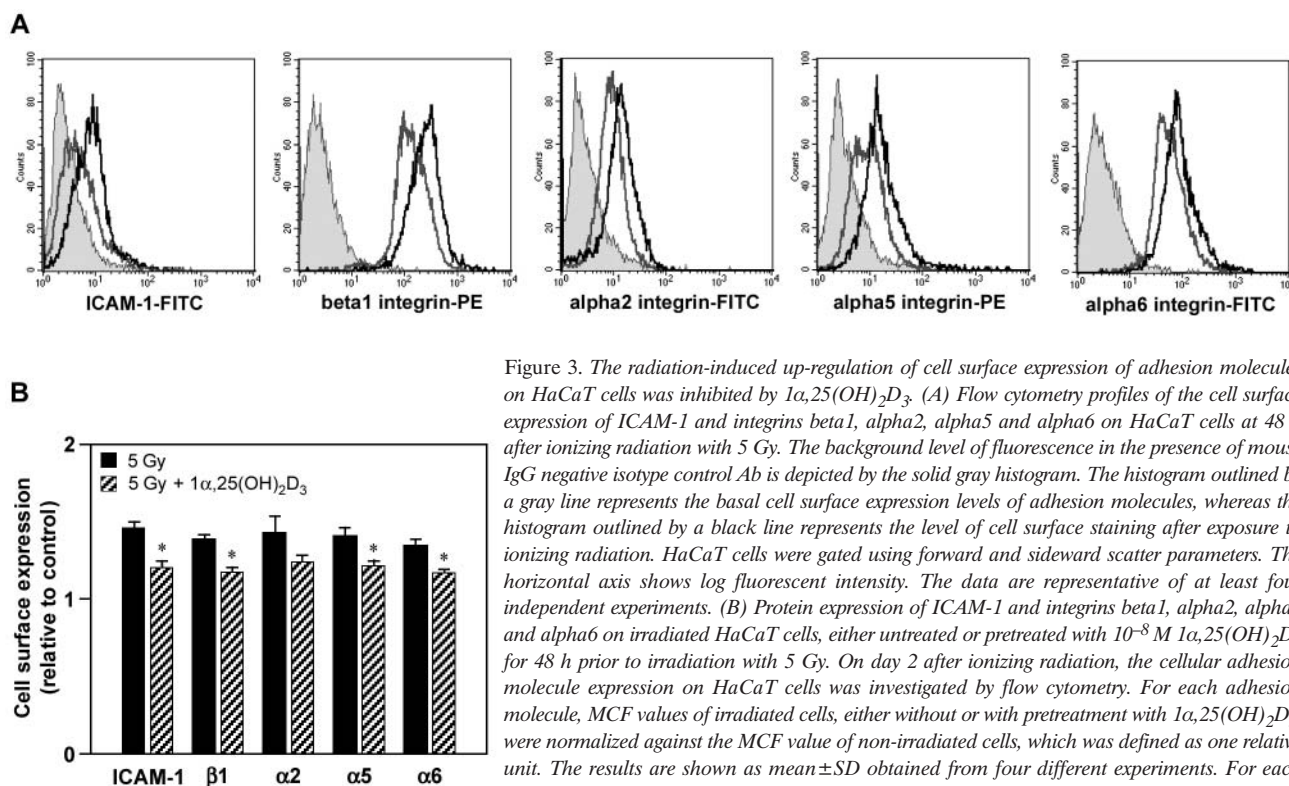


Figure 3. The radiation-induced up-regulation of cell surface expression of adhesion molecules on HaCaT cells was inhibited by  $1\alpha,25(\text{OH})_2\text{D}_3$ . (A) Flow cytometry profiles of the cell surface expression of ICAM-1 and integrins beta1, alpha2, alpha5 and alpha6 on HaCaT cells at 48 h after ionizing radiation with 5 Gy. The background level of fluorescence in the presence of mouse IgG negative isotype control Ab is depicted by the solid gray histogram. The histogram outlined by a gray line represents the basal cell surface expression levels of adhesion molecules, whereas the histogram outlined by a black line represents the level of cell surface staining after exposure to ionizing radiation. HaCaT cells were gated using forward and sideward scatter parameters. The horizontal axis shows log fluorescent intensity. The data are representative of at least four independent experiments. (B) Protein expression of ICAM-1 and integrins beta1, alpha2, alpha5 and alpha6 on irradiated HaCaT cells, either untreated or pretreated with  $10^{-8}$  M  $1\alpha,25(\text{OH})_2\text{D}_3$  for 48 h prior to irradiation with 5 Gy. On day 2 after ionizing radiation, the cellular adhesion molecule expression on HaCaT cells was investigated by flow cytometry. For each adhesion molecule, MCF values of irradiated cells, either without or with pretreatment with  $1\alpha,25(\text{OH})_2\text{D}_3$ , were normalized against the MCF value of non-irradiated cells, which was defined as one relative unit. The results are shown as mean  $\pm$  SD obtained from four different experiments. For each adhesion molecule, a significant difference between the expression value from irradiated cells and the expression value from cells pretreated with  $1\alpha,25(\text{OH})_2\text{D}_3$  prior to irradiation was determined using the Student's t-test and is indicated as \*,  $p < 0.05$ .

**Discussion**

It was demonstrated that the cell growth and clonogenic cell survival of human irradiated keratinocytes (HaCaT) were increased when the cells had been treated with  $1\alpha,25(\text{OH})_2\text{D}_3$  prior to ionizing radiation, as compared to untreated cells. Moreover, it was shown that the radiation-induced up-regulation of the cell surface expression of adhesion molecules on HaCaT cells was inhibited by pretreatment with  $1\alpha,25(\text{OH})_2\text{D}_3$ .

Numerous *in vitro* and *in vivo* studies have addressed the effects of  $1\alpha,25(\text{OH})_2\text{D}_3$  on the cell proliferation of epidermal cells (22). In cell culture systems,  $1\alpha,25(\text{OH})_2\text{D}_3$  was shown to inhibit the proliferation of keratinocytes (25, 26). Growth inhibition of keratinocytes has also been observed *in vivo*, where  $1\alpha,25(\text{OH})_2\text{D}_3$  had been topically or systemically administered to treat psoriasis (27-31). However, several studies demonstrated a stimulatory effect of  $1\alpha,25(\text{OH})_2\text{D}_3$  on keratinocyte proliferation both *in vitro* and *in vivo* (32-38). Available data suggest that the effect of  $1\alpha,25(\text{OH})_2\text{D}_3$  on keratinocyte proliferation *in vitro* depends on cell culture conditions, namely on specific growth factors, cell density and the concentration of  $1\alpha,25(\text{OH})_2\text{D}_3$  (23, 35). At low concentrations,  $1\alpha,25(\text{OH})_2\text{D}_3$  promoted the

proliferation of keratinocytes; at higher pharmacological doses, keratinocyte proliferation was inhibited (23). Recently, cell proliferation studies were performed on human epidermal keratinocytes that had been stimulated with sulfur mustard and afterwards treated with  $1\alpha,25(\text{OH})_2\text{D}_3$  (32). The results revealed that stimulation of keratinocytes with sulfur mustard decreased the cell number. However, treatment of stimulated keratinocytes with  $1\alpha,25(\text{OH})_2\text{D}_3$  dose-dependently increased the cell proliferation with a maximum effect at  $10^{-9}$  M  $1\alpha,25(\text{OH})_2\text{D}_3$  (32). Here, it was demonstrated, for the first time, that human keratinocytes exposed to ionizing radiation showed improved cell growth in the presence of  $1\alpha,25(\text{OH})_2\text{D}_3$ , as compared to untreated cells. In this work HaCaT cells, a spontaneously immortalized and non-tumorigenic human keratinocyte cell line (24) that is able to proliferate in culture in the absence of exogenous growth factors, were used. It is well established that growth factors acting in an autocrine manner play a major role in controlling keratinocyte proliferation (39). Of utmost importance among the autocrine growth factors produced by keratinocytes are members of the epidermal growth factor (EGF) family (36). Their binding to membrane proteoglycans, followed by the formation of a ternary

complex with the EGF receptor (EGFR), is a prerequisite for signal transduction. This interaction results in the activation of mitogen-activated protein (MAP) kinases and, finally, in the activation of transcription factors and cell mitogenesis (40). Recently, it has been demonstrated that the  $1\alpha,25(\text{OH})_2\text{D}_3$ -stimulated proliferation of HaCaT keratinocytes and mitogenic signaling were mediated by the autocrine network of proteoglycan-dependent EGFR ligands. It was assumed that the stimulatory effect of  $1\alpha,25(\text{OH})_2\text{D}_3$  was due to the up-regulation of proteins belonging to the ErbB family of receptor tyrosine kinases (36). An increase in the levels of activated MAP kinase after treatment with  $1\alpha,25(\text{OH})_2\text{D}_3$  was shown in HaCaT cells (36) and in primary keratinocyte cultures (41). Based on these findings, it is tempting to speculate that the intracellular mechanisms postulated above for the effect of  $1\alpha,25(\text{OH})_2\text{D}_3$  also account for its stimulatory effect on the cell proliferation of keratinocytes after being exposed to ionizing radiation.

To investigate the capacity of  $1\alpha,25(\text{OH})_2\text{D}_3$  to improve the clonogenic cell survival of keratinocytes after ionizing radiation, cell survival curves were generated. The results revealed that irradiated keratinocytes exhibited an increase in clonogenic cell survival when the cells had been pretreated with  $1\alpha,25(\text{OH})_2\text{D}_3$  before irradiation, as compared to untreated controls. To our knowledge, this is the first report about the stimulatory effect of  $1\alpha,25(\text{OH})_2\text{D}_3$  on the clonogenic cell survival of epidermal cells exposed to ionizing radiation.

Human keratinocytes were shown to express ICAM-1 (11-14) and several members of the beta1 subfamily of integrins, including alpha2beta1 (CD49b/CD29), alpha5beta1 (CD49e/CD29) and alpha6beta1 (CD49f/CD29) (10). In previous studies, the cell surface expressions of beta1 integrin (CD29) and ICAM-1 were found to be up-regulated in response to ionizing radiation in human skin cells (12-15). Here, it was demonstrated, for the first time, that ionizing radiation also caused an increase in the expressions of integrins alpha2 (CD49b), alpha5 (CD49e) and alpha6 (CD49f) on HaCaT cells in addition to beta1 integrin and ICAM-1. Beta1 integrin is known to form a non-covalent dimer with CD49 chains, of which a number have been shown to be involved in inflammation. Integrins have been identified as adhesive receptors for extracellular matrix proteins, as counter-receptors for neighboring cells, and as signaling elements in migration, tissue and cytoskeletal organization (42). Alpha5 integrin binds to fibronectin and alpha6 integrin binds to laminin, whereas alpha2 integrin has been shown to mediate binding to both laminin and collagen (10). This study explored the effect of  $1\alpha,25(\text{OH})_2\text{D}_3$  on the expression of cellular adhesion molecules in HaCaT cells exposed to ionizing radiation. Our findings suggest that  $1\alpha,25(\text{OH})_2\text{D}_3$  decreases the radiation-

induced up-regulation of ICAM-1 and integrins beta1, alpha2, alpha5 and alpha6 cell surface expressions on HaCaT cells as compared to untreated cells. To date, only limited data have been available regarding the effects of  $1\alpha,25(\text{OH})_2\text{D}_3$  on cellular adhesion molecule expression. In agreement with our results, studies in human renal proximal tubular cells, mouse pulmonary endothelial cells and keratinocytes found a decrease in ICAM-1 expression by treatment with  $1\alpha,25(\text{OH})_2\text{D}_3$  (16-17, 43). A  $1\alpha,25(\text{OH})_2\text{D}_3$ -induced down-regulation of the alpha6 integrin expression was demonstrated in a human melanoma cell line, in prostate cancer cell lines and in basal and suprabasal keratinocytes in skin biopsies from patients with psoriasis (17-18, 21). In a study by Kaneko *et al.* (20),  $1\alpha,25(\text{OH})_2\text{D}_3$  was shown to suppress the expression of alpha4 integrin in human leukemic HL-60 cells. The molecular mechanism by which  $1\alpha,25(\text{OH})_2\text{D}_3$  reduces the expression of adhesion molecules has not been well established. Previous studies have postulated that  $1\alpha,25(\text{OH})_2\text{D}_3$  regulates the expression of various molecules, *i.e.*, cytokines, by acting on both the transcriptional and translational level (44). Recently,  $1\alpha,25(\text{OH})_2\text{D}_3$  was reported to down-regulate nuclear factor- $\kappa\text{B}$  (NF- $\kappa\text{B}$ ) protein levels in activated human lymphocytes (45). Based on these data, one might assume that, in addition to direct binding of a VDR/ $1\alpha,25(\text{OH})_2\text{D}_3$  complex to vitamin D response elements in the promoter region of a target gene,  $1\alpha,25(\text{OH})_2\text{D}_3$  might act by modulation of nuclear transcription factors. The ICAM-1 promoter contains a large number of binding sites for inducible transcription factors, the most important of which is NF- $\kappa\text{B}$  (11). Furthermore, it was shown for granulocyte macrophage-colony stimulating factor that VDR can inhibit transcription by interfering with the transactivation function of c-Jun (46). In contrast to the above considerations, previous studies on human renal tubular epithelial cells revealed stimulation of ICAM-1 expression in response to  $1\alpha,25(\text{OH})_2\text{D}_3$  (19). These different observations might originate from the diverse cell types tested.

In summary, the present study demonstrated, for the first time, that the radiation-induced up-regulation of adhesion molecule expression on human keratinocytes can be inhibited by pretreatment of the cells with  $1\alpha,25(\text{OH})_2\text{D}_3$  prior to ionizing radiation. It was also shown that cell growth and clonogenic cell survival of human irradiated keratinocytes was increased when the cells had been pretreated with  $1\alpha,25(\text{OH})_2\text{D}_3$  prior to ionizing radiation. These findings support our hypothesis that  $1\alpha,25(\text{OH})_2\text{D}_3$ , besides its prominent role in calcium homeostasis and bone metabolism, may be a promising agent to modify the radiation reaction, thus offering new options in radiotherapy and oncology.

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