A Study on DNA Repair Mechanisms Effects of UVB Irradiation in a Human Skin Basal Cell Carcinoma Cell Line

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Abstract

Exposition of solar ultraviolet (UV) radiation has been identified as one of the major risk factors for skin cancer development in Americans. UV radiation induces two of the most abundant mutagenic and cytotoxic DNA lesions such as cyclobutane–pyrimidine dimmers (CPDs) and 6–4 photoproducts (6–4PPs) and their Dewar valence isomers. However, cells have developed a number of repair mechanisms to counteract the DNA damage caused by UV.

In the present study, we observed differential susceptibility to UV radiation exposure between a spontaneously immortalized human keratinocyte HaCaT cell line and a human skin basal cell carcinoma (BCC) cell line. We investigated numerous candidate genes in different DNA repair mechanisms affected by UV radiation. Our data indicated that treatment with 100 mJ/cm² UV-B-radiation in HaCaT cells induced higher RAD51 protein expression (approximately 2 fold of control), measured by Western blot, than in BCC cells (approximately 1.4 fold of control). In addition, the reduction level of TDG protein expression was seriously in BCC cells (approximately 22% of control) than in HaCaT cells (approximately 80% of control). Similarly results were also observed in the level of RAD51 and TDG mRNA, measured by Quantitative real-time polymerase chain reaction (qRT-PCR) method. Our results suggest that DNA repair mechanisms may be involved in the development of skin carcinogenesis. Furthermore, UV-B phototherapy may be is a helpful strategy to skin cancer patients as a pretreatment for radiation therapy.

Introduction

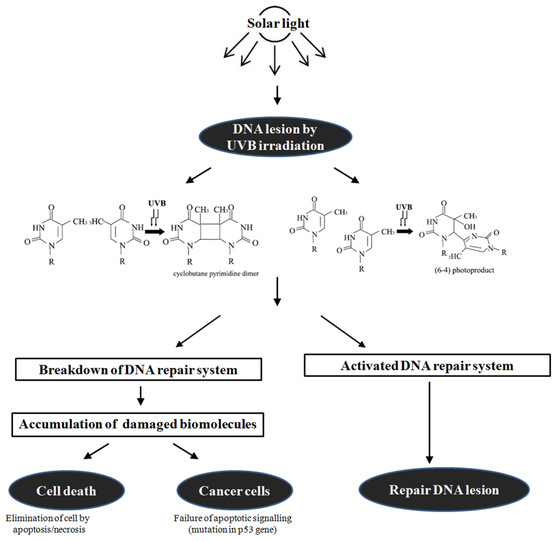

Figure 1. Schematic overview of the mechanism of UV mutagenesis. After UV irradiation the CPDs are the most abundant and probably most cytotoxic lesions but the 6–4PPs may have more serious, potentially lethal, mutagenic effects. Therefore, these DNA lesions, if unrepaired, may interfere with DNA transcription and replication and can lead to misreading of the genetic code and cause mutations and death.

Results

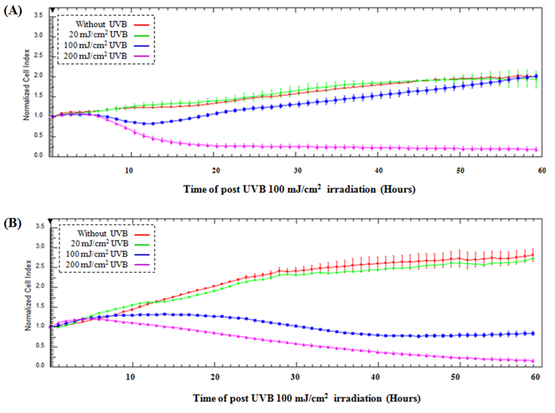

Figure 2. Cell proliferation assay with UVB irradiation using the xCELLigence System. Cells were unirradiated, UVB irradiated with 20 mJ/cm² , 100 mJ/cm² , or 200 mJ/cm²and then incubated in a CO_2-incubator at 37◦ C.(A) immortalized human keratinocyte HaCaT cell line. (B) human skin basal cell carcinoma (BCC) cell line.

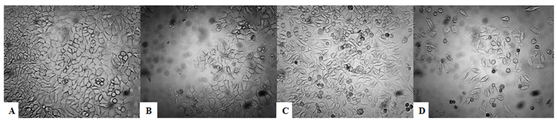

Figure 3. Morphological changes in UVB-irradiated HaCaT cells and BCC cells. HaCaT cells and BCC cells were unirradiated, irradiated with UVB 100 mJ/cm², and then incubated for 12 hours in a CO_2-incubator at 37ºC. (A)HaCaT cells were unirradiated. (B) HaCaT cells were irradiated with UVB 100 mJ/cm². (C) BCC cells were unirradiated. (D) BCC cells were irradiated with UVB 100 mJ/cm².

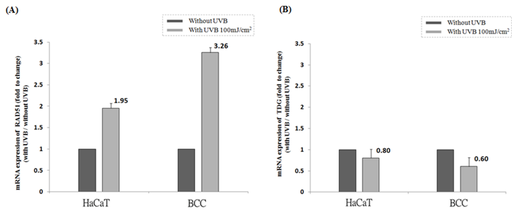

Figure 4. The expression of mRNA of (A) RAD51 and (B) TDG in HaCaT cells and BCC cells with UVB 100 mJ/cm² after 12 hours.11

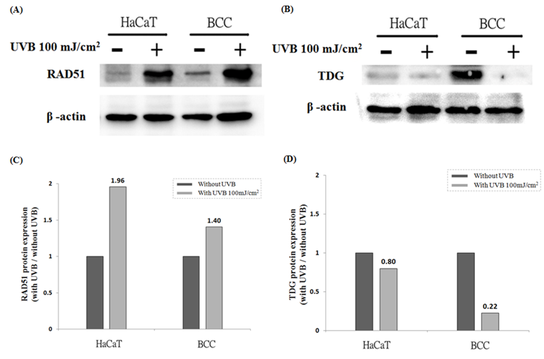

Figure 5. The expression of protein of RAD51 and TDG in HaCaT cells and BCC cells with UVB 100 mJ/cm² after 12 hours. (C) and (D) represented quantification of band intensity(irradiated with UVB / without UVB)

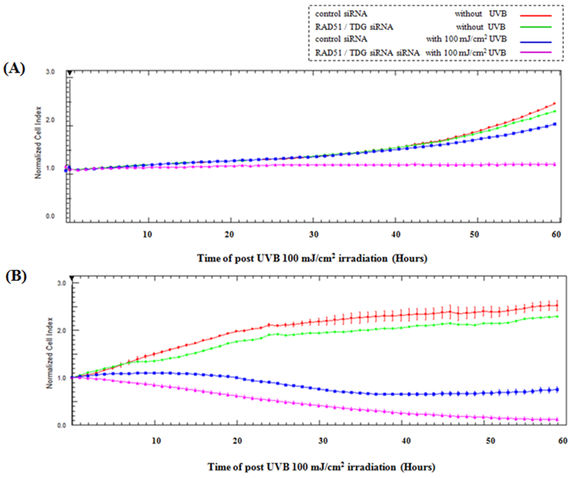

Figure 6. Cell proliferation assay with UVB irradiation using the xCELLigence System. Cells were transfected RAD51 siRNA and TDG siRNA. After 72 hours UVB irradiated with 100mJ/cm² and then incubated in a CO_2-incubator at 37◦ C.(A) immortalized human keratinocyte HaCaT cell line. (B) human skin basal cell carcinoma (BCC) cell line.

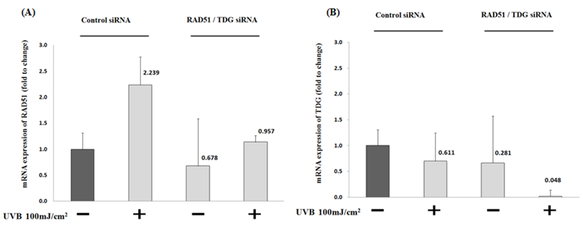

Figure 7. The expression of mRNA of (A) RAD51 and (B) TDG in BCC cells were transfected RAD51 siRNA and TDG siRNA . After 72 hours with UVB 100 mJ/cm² .

Conclusion

Differential Susceptibility to UVB irradiation in Human Skin Keratinocytes, BCC and HaCaT cells. Our results suggest that DNA repair mechanisms may be involved in the development of skin carcinogenesis. BCC cells were more sensitive to UV-B, therefore phototherapy may be is a helpful strategy to skin cancer patients as a pretreatment for radiation therapy.

Acknowledgement

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