

SHP-2 tyrosine phosphate-dependent focal adhesion kinase mediated cell migration of primary endothelial cells upon low LET radiation exposure

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The influence and mechanism of low LET radiation exposure on wound healing process is not yet fully understood. Based on the vital role of endothelial cells in migration and wound healing, this study investigated the response of endothelial cells to low-dose irradiation and the underlying mechanism. A wound was inflicted in the confluent monolayer of primary vascular endothelial cells exposing a cell-free zone on the plate. The cells were then exposed to acute dose of 10 cGy, 2 Gy or 10 Gy at a dose rate of 1.29 Gy/min using the ¹³⁷Cs source. The number of cells crossing the wound border and the migration distance in the wounded region were measured. Integrin signaling pathway was examined by $\alpha_v\beta_3$ integrins, focal adhesion kinase (FAK), phosphorylated FAK (Tyr³⁹⁷) and actin fiber expression. Also, the reverse transcriptase polymerase chain reaction was performed to analyze the transcription level of SHP-2 tyrosine phosphatase, the regulator of FAK. The results revealed a decrease in cell motility in terms of migration distance and the number of migrating cells after 2 and 10 Gy exposed cells. The migrating distance in 2 and 10 Gy exposed cells were only 0.5 and 0.1 mm, respectively compared to 3 mm observed in either mock-irradiated control cells or 10 cGy exposed cells. Confocal immunofluorescence showed impaired distribution and morphological alteration of FAK Tyr³⁹⁷ and actins after radiation exposure. With 10 Gy exposure, the expression of β_3 domain of integrins was inhibited compared to the control group. The level of β_3 integrins after 2 Gy was similar to that of controls. There was no detectable difference in α_v domain and α -actin among all groups. Although the level of total FAK was invariable, phosphor-FAK Tyr³⁹⁷ was higher in the irradiated groups. At 10 cGy the migration was enhanced and $\alpha_v\beta_3$ integrins were upregulated. The RT-PCR analysis of SHP-2 demonstrated a significantly dose-dependent decrease in transcription of SHP-2 mRNA. These results suggested that radiation at higher doses inhibited endothelial cell migration. Unlike chemical factors that induce chemotaxis through the reaction with integrins receptors and the ensuing signaling cascade, radiation-induced inhibition of cell migration could be attribute to the direct irradiative damage to SHP-2 transcription. Subsequent accumulation of phosphor-FAK mediated through SHP-2 transcription abrogated the contraction of cytoskeletons. Unlike high doses, the low dose range (10 cGy) that enhances the cell migration suggests a positive role in wound healing.

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