Tumor Cell-mediated bystander response on the angiogenic potentials of endothelium.

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Evidence strongly supports a possible involvement of a process called 'bystander effect' in which the irradiated cells convey the manifestation of the damage to non-irradiated bystander cells. Surviving tumor cells after exposure to low LET radiation may elicit signaling to the nearby endothelial cells. Those signaling events may be responsible for the initiation and establishment of microvessels for continued tumor cell survival and clonal expansion. In this study, cells derived from human squamous cell carcinoma of tongue (SCC-4 cells) were co-cultured with primary endothelial cells, where the cells were separated by porous membrane but shared the common medium, and examined for the enhanced angiogenic potentials of endothelial cells. The SCC-4 cells irradiated with low LET radiation at a total dose of 10 cGy, 2 Gy or 10 Gy at a dose rate of 1.29 Gy/min were co-cultured with endothelial cells. The proliferative response, endothelial cell migration and tube network formation were examined. Second, the induced levels of angiogenic factors from the SCC-4 cells were examined. Third, the levels of expression of receptors on endothelial cells that are involved in angiogenesis were determined. The results showed an increased expression of VEGF, FGF and PDGF in irradiated SCC-4 cells compared to mock irradiated cells. Similarly, in the endothelial cells the RNase protection assay revealed an induced expression of receptors, flt-1, flt-4, endoglin, and VEGF-RII as early as 3h after co-culture with irradiated SCC-4 cells. These increases in the expression of angiogenic receptors were maintained even after 24 h. No significant difference was observed in the viability of endothelial cells co-cultured either with mock-irradiated SCC-4 cells or irradiated SCC-4 cells. However, there is an augmented response in endothelial cell migration determined by *in vitro* scratch wound model. Correspondingly, there was enhanced angiogenic potential of cells grown on matrigel determined by tube network formation. These results clearly indicated that radiation enhances tumor-cell dependent responses on endothelial cells for the morphogenesis of microvessels.

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