Is the In-Vitro Production of Fibrosis-Associated Signal Protein TGFβ Radiation-Induced?

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We have investigated whether irradiation of human fibroblasts leads to an enhanced synthesis and secretion of the cytokine TGFβ. Fibrotic in vitro-parameters known to be TGFβ mediated are the accelerated terminal differentiation of fibroblasts and the concomitant enhanced production of proteins [2] both shown to change dose- and LET-dependently [3]. Earlier studies on tissue and animal models reported in literature, revealed a key role of TGFβ in the signal transduction after irradiation resulting in the fibrotic phenotype [reviewed in 1]. Accordingly, the TGFβ-mediated in vitro effects can be suppressed by immuno-neutralisation at non-lethal doses [4,5], but the influence of dose and LET on the expression of TGFβ protein is less pronounced than expected [5,6].

In order to determine the influence of radiation quality on TGFβ synthesis, we irradiated semi-confluent human fibroblasts originating from different tissues (foreskin, skin and lung) with 250 kV x-rays and 11 MeV/u carbon ions (LET 153,5 keV/μm). The total TGFβ content (latent plus activated form) in the cell culture supernatant was measured up to 48 hours after irradiation using an immunoabsorbent assay (ELISA) under serum free conditions or in the presence of 10% fetal calf serum (fcs) containing appr. 8 ng TGFβ per ml. In unirradiated cells the TGFβ amount released into the cell culture medium increases proportionally to cell density, but the calculated production per cell decreases with increasing cell density. This effect is more drastic in the presence of 10% fcs than under serum free conditions (not shown). Under serum-free conditions the cells are arrested in G_1-phase and the cell density remains constant in each flask. After irradiation, the amount of TGFβ per flask increases in controls as well as in irradiated probes independently from dose (not shown). In the presence of 10% fcs the control cells continue to proliferate (1-2 population doublings within 48 hours), whereas the irradiated cells undergo a cell cycle delay, whose duration is dependent on the applied dose, resulting in significantly different cell densities. As shown in figure 1 (top) after 11 MeV/u carbon irradiation, the amount of TGFβ per flask in the presence of 10% fcs increases with time, but independently from dose, leading to an increment of TGFβ per cell (already shown in [5,6]). The observed correlations appear more clearly by directly comparing TGFβ production and cell density (figure 1, bottom): The control cells proliferate and the released TGFβ amount per flask increases proportionally. In contrast, the irradiated cells do not increase in cell density, but attain a comparable level of TGFβ within the same period of time. We could demonstrate this for the three fibroblast cell lines after X-irradiation and for foreskin fibroblasts (AG) after carbon irradiation.

These data confirm our preliminary results after X-irradiation [5] and are in line with data from literature [7]. We could show that the effects are not linked to radiation quality. The data presented sustain our hypothesis, that a fast regulatory step could be the crucial event leading to dose- and LET-dependent changes in fibrosis-related parameters after irradiation [2,5]. The measured changes in TGFβ production after irradiation could be regulated in a subsequent step in a way to assure a constant TGFβ level in the microenvironment of the cells. Such a regulation could normally depend on cell density and become independent from it after irradiation.

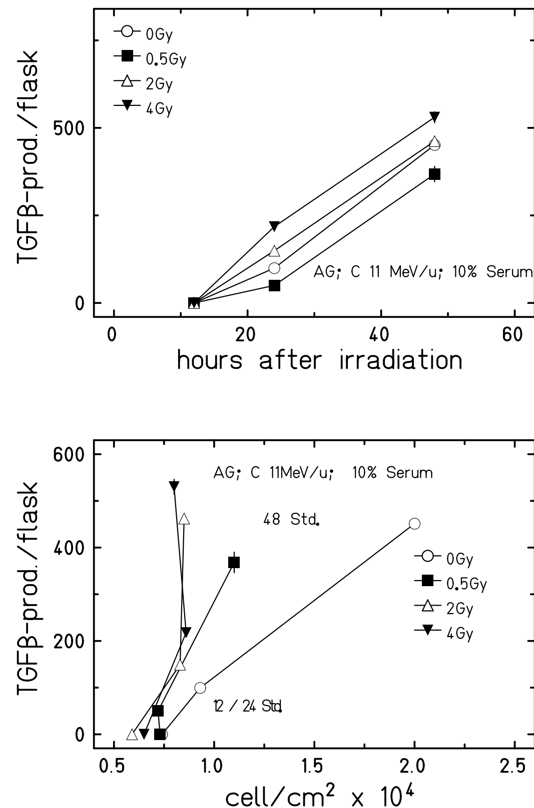

Figure 1: Time-course (top) and influence of cell density (bottom) on TGFβ production in human foreskin fibroblasts after exposure to carbon ions (153,5 keV/μm). Comparable and time-dependent levels of TGFβ are obtained independently of irradiation and concomittant growth inhibition. The increase is measured on top of a basal TGFβ level.

References

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