A non-linear regulation of the antioxidative system in endothelial cells contributes to the anti- inflammatory effect of low dose X-irradiation*

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Introduction

Since ionizing radiation exerts its cytotoxic activity in large parts by the generation of reactive oxygen species (ROS), antioxidant systems to maintain cellular redox balances are relevant for radiation response. Moreover, ROS are involved in immunological defense mechanisms and have a physiological role in cellular signaling [1]. A major mechanism in the regulation of oxidative stress/damage homeostasis is activation of the transcription factor nuclear factor E2-related factor 2 (Nrf2), which tightly controls the expression of genes encoding antioxidant proteins [2]. We have recently reported on a diminished activity of the enzyme superoxide dismutase (SOD) and a discontinuous induction of ROS in EA.hy926 endothelial cells (ECs) most pronounced at 24 h after a 0.5 Gy exposure [3]. Based on these findings, analyses were expanded to Nrf2 as a common regulatory element in the antioxidative defense and on functional properties of ROS in the anti-adhesive effects of low dose X-irradiation [4].

Material and Methods

EA.hy926 ECs were either stimulated in a proinflammatory manner by tumor necrosis factor-α (TNF-α; 20 ng/ml) 4 h before or at 20 h after irradiation with X-ray doses ranging from 0.3 to 1 Gy. Expression and DNA binding activity of Nrf2 were analyzed by flow cytometry and colorimetric assays. The impact of ROS on peripheral blood mononuclear cell (PBMC) adhesion was assayed in the presence of the scavenger N-acetyl cysteine (NAC).

Results and Conclusion

As depicted in Figure 1, a non-linear dose response relationship was evident with a relative minimum of protein detection at 0.5 Gy (Fig. 1A) in line with a reduction in Nrf2 DNA-binding activity as quantitated by colorimetric assays (Fig. 1B). Next, to investigate whether low dose X-irradiation modulated induction of ROS is associated with altered functional properties of EA.hy926 ECs, leukocyte adhesion was quantitated in the presence of the ROS scavenger NAC. As shown in figure 1C, PBMC/EA.hy926 EC adhesion was suppressed by more than 50 % after a single dose irradiation with 0.5 Gy in control treated ECs. On the contrary, ROS scavenging by NAC resulted a pronounced increase of adhesion events and thus a partial, but significant ($p< 0.05$) diminution of the anti-adhesive effect of low dose exposure.

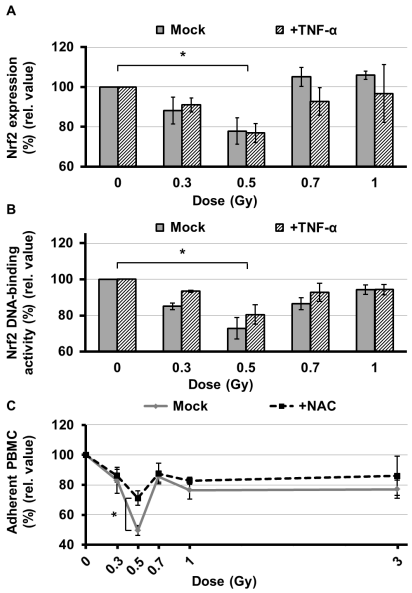

Figure 1: Relative Nrf2 protein detection (A) and colorimetric Nrf2 DNA-binding activity (B) as measured at 24 h after irradiation with single doses as indicated. Data represent mean $\pm$ s.e.m from at least three independent experiments. $*$ $p < 0.05$ versus non irradiated ECs. (C) Adhesion of PBMC to NAC (10 mM) treated and stimulated (TNF-α: 20 ng/ml) EA.hy.926 EC subsequent to low-dose irradiation. Mock treated cells served as a control. Data are given as mean relative values ($\pm$ s.e.m) from three independent experiments. $*$ $p < 0.05$ versus mock treated controls.

In conclusion, these findings implicate a non-linear expression and activity of a major compound of the antioxidative system that may contribute to a differential regulation of ROS production and via modulation of the adhesion process to the anti-inflammatory effects of low dose radiation therapy.

References

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