

A discontinuous detection of phospho-histone H2AX in endothelial cells following low-dose irradiation is mediated by reactive oxygen species (ROS)*

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Introduction

Since decades an anti-inflammatory efficacy of radiation therapy with single doses < 1 Gy is clinically well established in the treatment of benign and chronic degenerative diseases [1]. In line with that, recent experimental data indicate an involvement of a variety of cellular and molecular immune-modulatory mechanisms in these effects. Among them, an impact of low-dose irradiation on endothelial cells (EC) and mononuclear/polymorphonuclear leukocyte activity has been proven to comprise key elements in the modulation of inflammatory cascades [2]. Moreover, a non-linear dose response relationship, shared with non (DNA)-targeted properties of ionizing radiation [3], is a major characteristic of the mechanisms explored so far [4]. Recently, a putative interrelationship between DNA damage repair, induction of reactive oxygen species (ROS) and factors implicated in the regulation of antioxidant response pathways have been proposed to contribute to these discontinuous responses [5].

Material and Methods

HUVEC derived immortalized EA.hy926 cells were stimulated in a pro-inflammatory manner by tumor necrosis factor- α (20 ng/ml) 4 hours before irradiation with doses ranging from 0.3 to 1 Gy. To analyse DNA repair capacity, γ -H2AX foci were assayed at 1 hour, 4 hours and 24 hours after irradiation by immunofluorescence. ROS production and superoxide dismutase (SOD) activity were analysed by fluorometric 2',7'-dichlorodihydrofluorescein-diacetate (H2DCFDA) and colorimetric assays, respectively.

Results and Conclusion

Irrespective of stimulation by TNF- α , we observed in EA.hy926 EC a linear dose-response characteristic of γ -H2AX foci detection at early times (1 hours and 4 hours) after irradiation. On the contrary, at 24 hours the number of residual γ -H2AX foci was significantly ($p \leq 0.05$) elevated after a 0.5 Gy exposure (Figure 1a). To further analyse molecular mechanisms implicated in the biphasic induction of γ -H2AX foci the impact of ROS expression and enzymatic activity of SOD, reported to be involved in anti-oxidant defence [6] were investigated. As a result, a biphasic induction of ROS became evident at 24 hours after irradiation concomitant with a significant decrease in

SOD activity, most pronounced at a dose of 0.5 Gy (Figure 1b).

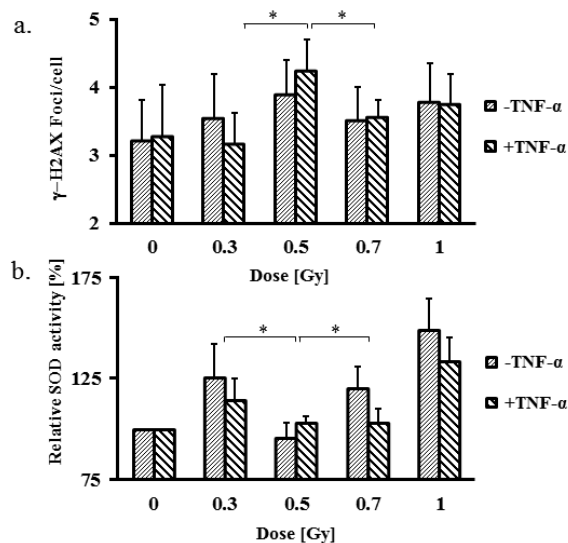


Figure 1: Dose kinetics of γ -H2AX foci detection (a) and relative SOD enzymatic activity (b) as measured by a colorimetric assay in stimulated (TNF- α , 20 ng/ml) EA.hy926 EC at 24 h following irradiation with the doses indicated. Data represent means \pm SD from at least three independent experiments. Asterisks indicate significant differences ($p < 0.05$) vs. 0.3 Gy and 0.7 Gy irradiated ECs.

In conclusion, these data implicate a non-linear regulation of SOD activity and ROS production in EA.hy926 EC following irradiation with doses < 1 Gy that contributes to a discontinuous dose response relationship of γ -H2AX detection.

References

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