Inflammatory cytokine release by vascular cells after X-irradiation*

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Introduction

Exposure to high radiation doses is considered to be responsible for the development of cardiovascular diseases (CVD), but also low doses are suspected to be a risk factor [1,2]. The development of CVD is mediated by changes in the cytokine release of endothelial and immune cells, whereas the role of smooth muscle cells is underinvestigated up to now [3]. We used a co-culture model of both cell types in order determine the cytokine release up to two weeks after irradiation, comparing low and high doses of X-rays. Here we present the results of Interleukin-6 (IL-6), a cytokine which can have both pro- and anti-inflammatory effects.

Materials and Methods

Human coronary artery endothelial cells (HCAEC) and human smooth muscle cells (SMC) were immortalized by hTERT overexpression. Cell types were either cultured separately or in co-culture for 3 d prior irradiation. After irradiation with 0.05, 0.1, 0.5 and 2 Gy X-rays, medium supernatants were collected after 4 h, 24 h, 1 week and 2 weeks and frozen at $-80\ ^{\circ}\mathrm{C}$. Medium was replaced 24 h before each time point. Concentrations of 21 inflammation-related cytokines in the supernatants of mono- and co-cultures were determined by bead-based protein arrays. Based on these results, IL-6 concentrations were quantified using ELISA (eBioscience) and data were analyzed using a custom pipeline written in the R language.

Results and Discussion

In Figure 1, the IL-6 release of unirradiated cells is shown over the time. While the IL-6 release rate of HCAEC remained almost unchanged, SMC and the coculture released initially high amounts of IL-6 that decreased rapidly during the following week. The IL-6 production of the co-culture after assembly exceedes the total amount of the two monocultures, pointing towards signaling processes occuring in the co-cultured cells.

There was no significant radiation-induced change of IL-6 release 4 h, 24 h or 2 weeks after radiation exposure (not shown). In contrast, 1 week after irradiation the IL-6 concentrations were $1.5\times$ (0.5 Gy) and $2\times$ (2 Gy) increased relative to control (Fig. 2). So far, this indicates no influence of very low doses (≤ 0.1 Gy) on the release of this inflammatory mediator. Higher doses (≥ 0.5 Gy), however, are able to increase the cytokine release in a vascular co-culture model, but mono-cultures remain unaffected by irradiation. Preliminary results from proteomics studies showed an induction of proteins that could be indicative of ongoing inflammation, thus supporting our results (not shown). Preliminary transcriptomic data of HCAEC show that genes involved in regulation of mitosis were strongly downregulated at 24 h post-IR, pointing towards a G2/M arrest (not shown). After 7 days, these genes are slightly but significantly upregulated after 2 Gy irradiation.

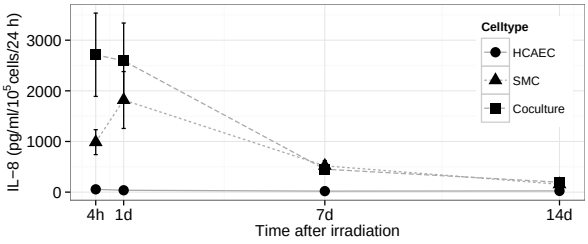

Figure 1: IL-6 cytokine release rate of unirradiated cells as a function of the time. Each sample was measured at least twice. Error bars show SEM. $N = 2, n = 6$

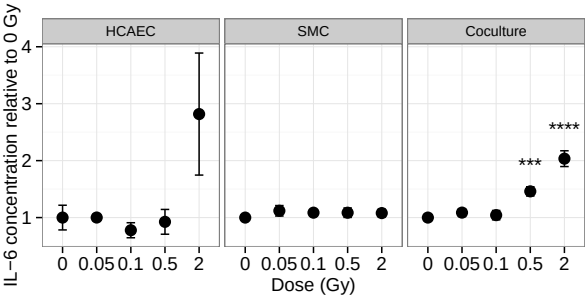

Figure 2: IL-6 cytokine release one week post irradiation. Concentrations have been normalized to 0 Gy sample. Error bars show SEM. *** $p < 0.001$; **** $p < 0.0001$. $N = 3, n \geq 9$

References

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