Finding NEMO – radiation induced bystander effects elicit NF-κB dependent survival

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Abstract

Radiation-induced bystander effects (RIBE) are an acknowledged issue of radiation therapy. Radiation of tumor tissue has been shown to affect non-irradiated neighboring cells in a paracrine and endocrine manner. Transduction of bystander signaling though remains to be investigated in detail. A part of the transduction is the receptor-initiated activation of signaling pathways by secreted factors of the irradiated cell during irradiation damage response. This work focusses on the activation of the transcription factor Nuclear Factor κB (NF-κB) in bystander cells after irradiation. NF-κB is a well-known contributor to inflammatory processes like cyto- / chemokine production as well as to stress reactions such as the DNA damage response and cell cycle regulation. Using a mouse embryonic fibroblasts (MEF) *in vitro* model with a genetic knock-out of an NF-κB regulator (NEMO, NF-κB essential modulator), clonogenic survival and cell cycle distribution was determined in directly irradiated cells and in cells incubated with conditioned medium from X-irradiated cells (bystander treatment). Directly irradiated NEMO ko cells, plated for clonogenic survival immediately after X-irradiation, display the same dose-effect curve as the wildtype (wt) ($a/b_{NEMO\ ko}$ = 13.92 ± 2.4 vs. a/b_{wt} = 12.37 ± 2.6). But when allowed to recover for 24 h, the wt cells show a broader shoulder in the curve (a/b =3.5 ± 2.9), indicating a role of NF-kB in the repair of radiation induced DNA damages. Looking into the survival of bystander cells, the survival curves show a statistically different slope, with NEMO ko cells surviving better than wt cells ($S_{16\ Gy}$: NEMO ko = 1.66 vs wt = 0.83). The different behavior may correlate with NF-kB dependent DNA repair in bystander cells for NEMO ko and wt cells. Cell cycle analysis revealed a 6 hour delayed arrest in G2/M phase in directly irradiated NEMO ko cells compared to wt cells. This indicates that NF-kB regulated DNA repair pathways are important for recovery of radiation induced damages. Bystander NEMO ko show an even further delayed arrest at 48 h, while wt bystander cells show no G2/M arrest at all. This supports the assumption that damages have to overcome a certain threshold to be recognized as repair-worthy. As NFkB has been reported to be involved in homologous recombination; cells with impairment in NFkB pathways, such as NEMO ko, register damages caused by bystander treatment differently from wt cells. This leads to G2/M arrest extending time for repair in NEMO ko bystander cells.

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