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# Bystander Effects in the Cellular Radiation Response

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The overall response to tumor radiation therapy results from direct radiation damage and indirect bystander effects (RIBE) mediated by secreted molecules and paracrine transfer of short-lived mediators. RIBE can have both detrimental and protective actions on cancerous as well as healthy tissues. Nuclear Factor κB (NF-κB), which governs immune responses, is a prime candidate for mediating RIBE. NF-κB also modulates DNA repair while promoting cellular survival as part of the DNA damage response. After exposure to ionizing radiation NF-κB is activated in directly targeted and non-targeted bystander cells. We tested the hypothesis that the NF-κB status is relevant for induction of RIBE, using a mouse embryonal fibroblasts (MEF) knock-out variant that is unable to activate NF-κB (NF-κB essential modulator knock-out (NEMO ko)).

To analyze influences of secreted soluble factors, cells were irradiated with X-rays (200 kV) and incubated with fresh culture medium for 24 h. The conditioned medium was then transferred onto non-irradiated cells and incubated for 24 h (bystander treatment). Bystander responses concerning cellular survival were determined with the colony forming ability test. NF-κB activation and DNA double strand break (DSB) response were visualized by immunofluorescence staining (p65, γH2AX).

MEF wildtype (wt) bystander cells (conditioned medium from 4 Gy irradiated cells) showed a significantly stronger relative activation of NF-κB (1.19 ± 0.02, $p<0.001$), indicating a role of the transcription factor in RIBE. In MEF wt and MEF NEMO ko, the number of DNA DSB was increased 6 h after bystander treatment. The number of γH2AX foci per cell after 4 Gy conditioned medium treatment was vastly increased in MEF NEMO ko cells ($Ctrl_{NEMO\ ko}$ 0.01 ± 0.01 vs. 4 $Gy_{NEMO\ ko}$ 1.48 ± 0.18, $p < 0.01$) compared to MEF wt cells with the same treatment ($Ctrl_{wt}$ 0.62 ± 0.05 vs. 4 $Gy_{wt}$ 1.06 ± 0.06, $p < 0.01$). Cellular survival was most prominently affected by bystander treatment. MEF wt bystander cells showed significantly reduced survival above a threshold of 4 Gy conditioning dose (70 %). On the other hand, relative survival of MEF NEMO ko cells increased significantly (140 %) after bystander treatment of 1 to 8 Gy conditioning dose.

We conclude that, while MEF wt cells appear to be negatively affected by RIBE, NF-κB (-) MEF cells seem to thrive on a treatment with conditioned medium despite an increased occurrence of DNA DSB. Mechanistically, the lack of NF-κB activation in MEF NEMO ko cells may abolish an RIBE-induced senescence phenotype and promote intracellular signaling cascades that regulate cellular growth. Therefore, NF-κB is a promising target for immune-assisted radiation therapy.

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Poster

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