

Title: Protective signaling pathways in the cellular radiation response: Nuclear Factor  $\kappa$ B (NF- $\kappa$ B) and Nrf2

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Abstract:

As a prerequisite for developing appropriate countermeasures to mitigate acute effects and late risks of ionizing radiation exposure, the role of protective pathways in the cellular radiation response needs to be better understood. The Nuclear Factor  $\kappa$ B (NF- $\kappa$ B) pathway is generally regarded as protective by upregulating anti-apoptotic genes and is known to be activated by DNA double strand breaks. The transcription factor Nuclear Factor Erythroid 2 Like 2 (Nrf2) is activated in response to oxidative stress and increases the expression of anti-oxidative enzymes. In this work, the production of reactive oxygen species (ROS), the activation of NF- $\kappa$ B and Nrf2 and the expression of selected target genes after ionizing radiation exposure (X-rays, heavy ions) were analyzed in human cell lines.

ROS were determined with CellROX® Green. NF- $\kappa$ B activation was quantified by a NF- $\kappa$ B reporter cell line (HEK-pNF- $\kappa$ B-d2EGFP/Neo L2). Nrf2 activation was measured using the Dual Luciferase Assay. Nrf2 and NF- $\kappa$ B target gene expression was analyzed by real time reverse transcriptase quantitative PCR (RT-qPCR).

X-irradiation increased ROS dose-dependently and they persisted several days after irradiation. NF- $\kappa$ B activation and NF- $\kappa$ B dependent gene expression occurred as an early step in the cellular radiation response. The expression of several chemokines and cytokines (CXCL1, CXCL2, CXCL10, IL-8 and TNF) was up-regulated. Nrf2 was not activated up to 48 hours after exposure, and only NAD(P)H quinone dehydrogenase 1 (NQO1) was transiently upregulated.

Nrf2 seems to play a minor role in the radiation induced signaling compared to NF- $\kappa$ B. The upregulated chemokines and cytokines might be important for cell-cell communication. Their role in the cellular and tissue response to ionizing radiation needs to be further examined as they might induce proinflammatory effects.

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