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RADIATION-INDUCED BLADDER TOXICITY IS ASSOCIATED WITH PURINERGIC-ACTIVATED, PRO-APOPTOTIC SIGNALLING IN UROTHELIAL CELLS

Radiation-induced bladder toxicity (RIBT) is a significant clinical condition negatively impacting the health and well-being of patients who have undergone radiation therapy for prostate, bladder or gynaecological malignancies. RIBT is an unavoidable consequence of pelvic irradiation representing collateral damage to normal cells located close to the tumour (1). The majority of radiation therapy prostate cancer patients experience acute genitourinary toxicity or lower urinary tract symptoms (LUTS) during treatment; a smaller proportion suffer late toxicity months and even years after final treatment. The urothelium is known to be damaged during radiation therapy; morphological impairment of the murine urothelial barrier after irradiation is associated with loss of umbrella cells in both acute and late RIBT (2). A recent study of rat bladder histology after pelvic irradiation showed reduction in urothelium thickness, increased layers of mucosal myofibroblasts and a damaged microvasculature (3). Urothelial loss exposes the underlying smooth muscle, nerves, interstitial cells and microvasculature to the urine which has an irritative effect causing overactivity of the bladder smooth muscle, experienced by the patient as urgency/LUTS. The adverse effects of radiation therapy can occur both through direct radiation damage and in some cells, via bystander signaling mechanisms where non-irradiated cells respond to signalling molecules released from irradiated cells.

Hypothesis / Aims of Study

Our hypothesis that radiation exerts bladder toxicity by induction of pro-apoptotic signalling mechanisms was tested by addressing the study aims: (1) to investigate whether radiation impacts survival of human urothelial cells; (2) to determine if radiation affects urothelial cells via an indirect/bystander mechanism; (3) to elucidate the molecular pathways underpinning radiation-induced urothelium damage.

Study design, materials and methods

Immortalised (SV-HUC) and primary human urothelial cells (HUC) were irradiated over the dose range (0.5, 1, 2, 4, 8 Gy). Cells were either studied directly; or the media, conditioned after irradiation was transferred to naïve recipient cells Clonogenic cell survival assays, DNA damage foci quantification with confocal imaging and Western blot analysis were performed with pharmacological modulation of purinergic signalling. Experiments were performed in triplicate with N=3-5 independent experiments performed in each series. Data sets were compared with Student's t-test or ANOVA followed by post-hoc tests with $P<0.05$ considered as significant.

Results

HUC were sensitive to direct radiation exposure as confirmed by reduction in cell colony formation in clonogenic survival assays ($p<0.05$). Exposure to conditioned medium from irradiated cells also reduced cell survival indicating the presence of a bystander signalling mechanism. Exposure of HUC in flasks where 50% was shielded with lead to radiation resulted in the presence of nuclear DNA damage foci in the shielded region (greater than that predicted by calculating the scattered radiation dose). These findings suggested a bystander signalling mechanism in the flask which was abolished when the experiment were repeated with a physical barrier between the shielded and unshielded regions of the flask. The presence of DNA damage foci (labelled with γH2AX or 53BP1) in non-irradiated urothelial cells in the shielded region suggested a bystander mechanism leading to cell cycle arrest and reduction of cell number.

ATP was identified as a potential candidate for bystander signalling released by irradiated urothelial cells; luciferin-luciferase assays revealed a significantly higher concentration of ATP in the media of irradiated urothelial cells compared with basal levels ($P<0.05$, N=3). Finally, Western blot protocols demonstrated that conditioned medium treatment induced expression of cleaved caspase-3 and Parp proteins in these cells; consistent with a pro-apoptotic mechanism (N=5). These findings were mimicked by ATP (N=5) and abolished with the ATP scavenging enzyme, apyrase.

Interpretation of results

Urothelial cells respond to irradiation by the release of signalling molecules including ATP. This results in a reduction in cell survival via a cascade of events including induction of pro-apoptotic signalling pathways and DNA damage.

Concluding message

Urothelial cells are sensitive to the direct and bystander effects of radiation, which reduce cell survival by inducing apoptosis. The radiation-evoked secretion of ATP in urothelial cells indicates the involvement of purinergic signalling in potentiating toxic bystander effects. This study confirms that radiation bladder toxicity is partly attributable to damage of the urothelium by apoptotic mechanisms.

References

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Disclosures

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