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ATP-evoked P2X7 receptor modulates the activation of pro-inflammatory cytokine IL-1β in urothelial cells

K. Harkin, K. Srivastava, C. Breen, K. D. McCloskey. Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, UNITED KINGDOM.

Introduction The bladder urothelium is negatively-impacted by high intensity γ-radiation during radiation therapy of pelvic malignancies. Radiation therapy induced damage arises from direct irradiation of cells and indirectly, from neighbouring cells through bystander signalling. Radiation cystitis is a common side-effect of pelvic radiotherapy (1), however, knowledge of the radiation-induced inflammatory mechanisms in urothelial cells is limited. The aim of the present study was to elucidate the underlying molecular mechanisms of radiation-induced bystander signalling in urothelial cells.

Methods The immortalized human urothelial cell line SV-HUC was used. Cells were irradiated at 0.5Gy, the medium was conditioned (CM) for 10 minutes, filtered and then transferred to naïve, recipient cells. Control samples received media from non-irradiated cells. Cells were harvested at two time points; 15 minutes or 4 hours post-CM transfer. Cell lysates were processed for qRT-PCR and Western blot experiments. ATP was made up in media and A430879 in DMSO where the final concentration did not exceed 0.1%. Data sets were analysed as mean ± standard error of the mean; n represents the number of independent experiments. ANOVA (post-hoc Tukey and Dunnett tests) was used for significance testing with $P<0.05$ considered as significant.

Results Application of CM increased gene expression of the pro-inflammatory cytokine IL-1β and Caspase-1 (N=2). In Western blot experiments, densitometry analysis of bands corresponding to Caspase-1 showed increased protein expression after CM at both the 15-minute and 4-hour time points (N=3). Application of ATP (10μM) mimicked the effects of CM at gene and protein level (N=3). Inhibition of P2X7 receptors with the antagonist A430879 (10μM) reduced the expression of both Caspase-1 and IL-1β after application of ATP (N=3) demonstrating that P2X7 receptors mediate the bystander signalling response.

Conclusions This project examined the impact of CM and ATP, a signalling factor released from irradiated urothelial cells (1) which was found to activate the P2X7 receptor to induce an intracellular, pro-inflammatory response via caspase-1 and IL-1β signalling. This pro-inflammatory pathway is likely to a major contributor to generation of symptoms of radiation cystitis.

Reference (1) Azzam, E., de Toledo, S. and Little, J. (2004). Current Cancer Drug Targets, 4(1), pp.53-64.