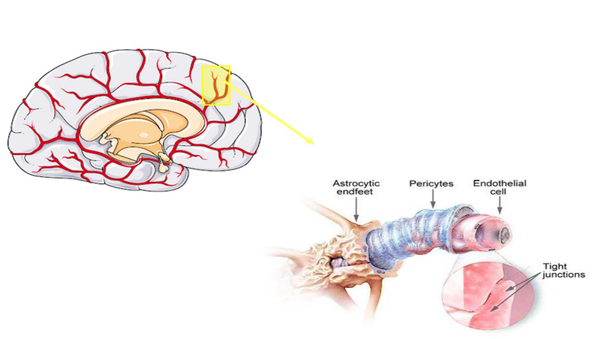
Cx43 channels and signaling via IP_3/Ca^{2+}, ATP and ROS/NO propagate radiation-induced DNA damage to non-irradiated brain microvascular endothelial cells.

Hoorelbeke Delphine, Decrock Elke, De Smet Maarten, De Bock Marijke, Descamps Benedicte, Van Haver Valérie, Delvaeye Tinneke, Krysko Dmitri V., Vanhove Christian, Bultynck Geert, Leybaert Luc

UNIVERSITEIT GENT

Introduction

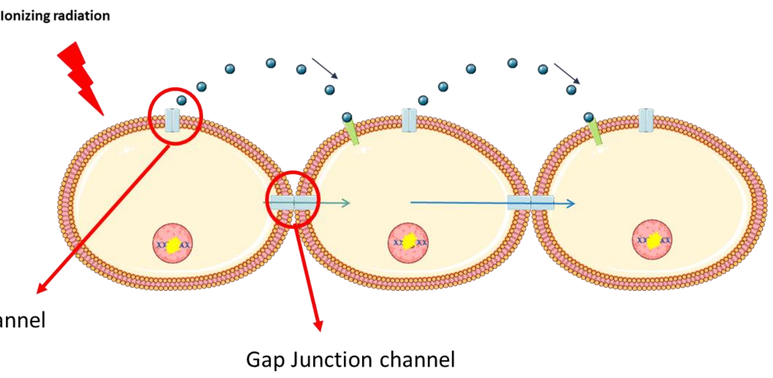


❖ Ionizing radiation induces **endothelial cell** (EC) damage in the brain.

❖ Intercellular communication pathways can propagate radiation-induced effects from directly irradiated cells to non-irradiated bystander cells, a process termed **radiation-induced bystander effects (RIBE).**

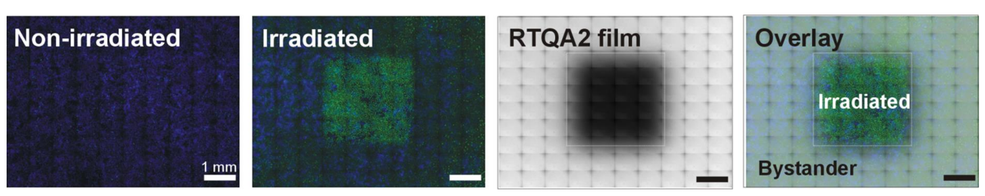
❖ Reactive oxygen species (**ROS**) are frequently proposed to underlie these RIBE. **Intracellular Ca^{2+}** homeostasis and signaling are tightly linked to ROS production.

❖ Changes in intracellular Ca^{2+}-concentration can be propagated between ECs via connexin (Cx) channels, i.e. **gap junction channels** (GJs, direct cell-cell communication) and **hemichannels** (HCs, paracrine communication) which allow diffusion of molecules <1.5 kDa.



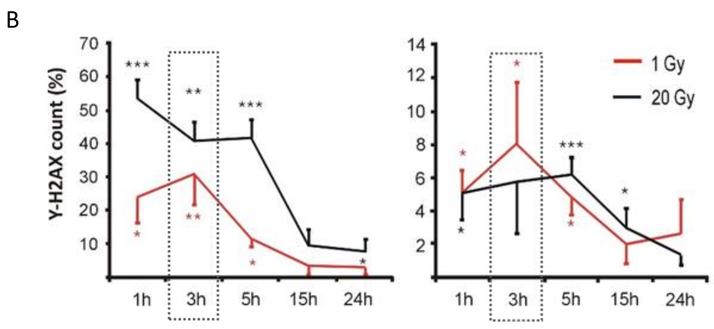
Induction of DNA damage

A

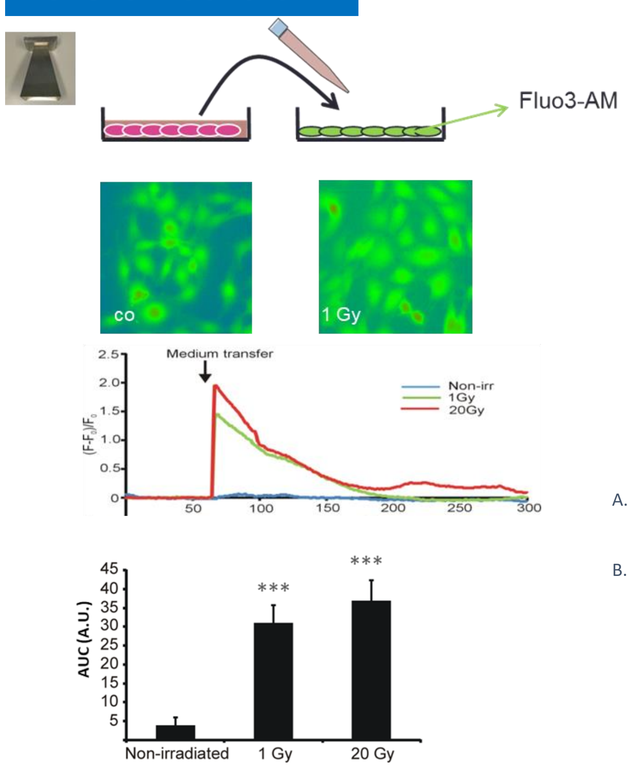


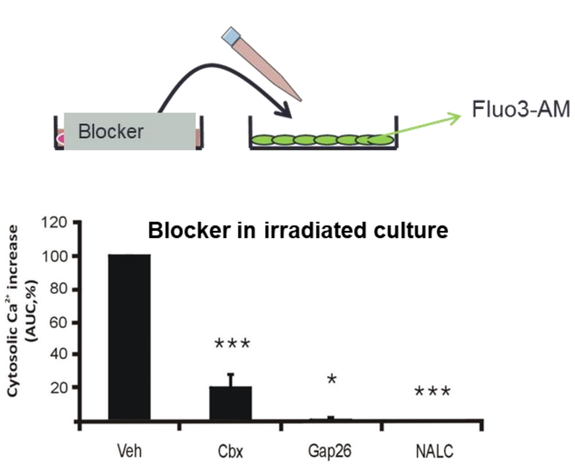
A. γ-H2AX foci staining (green) and DAPI staining (blue; x10 objective) in RBE4 cells 3h following 20 Gy, combined with a transmitted light image of the radiosensitive GafChromic film present underneath the culture during irradiation. B. DNA damage was quantified at several time points post-irradiation (1 Gy - 20 Gy) in the irradiated area and bystander area. The 1 Gy 3h condition was chosen for further investigation of the role of Cx channels and Ca^{2+}-ROS signaling. * vs non-irradiated control ; N=5-11

B



Role of Calcium





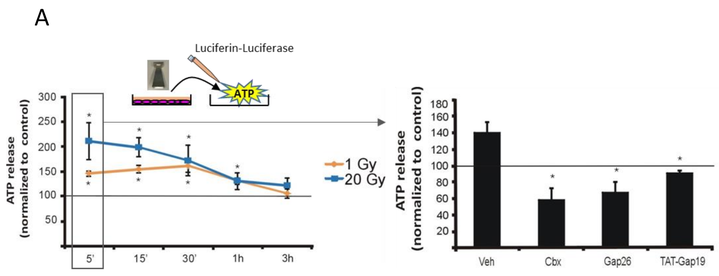
A. The exposure of bystander cells loaded with the Ca^{2+} marker Fluo-3-AM to medium originating from irradiated cells results in the increase of the cytosolic Ca^{2+} concentration in these bystander cells.
B. The addition of blockers (Cbx, Gap26 or NALC) to the irradiated culture results in the inhibition of this increase in cytosolic Ca^{2+} concentration.

Role of Connexin channels

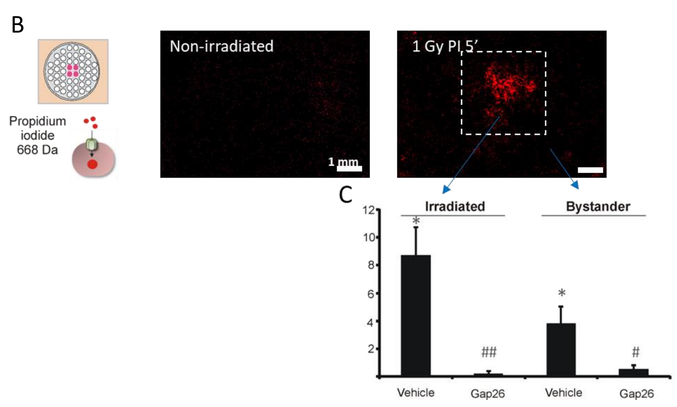
Hemichannels open in response to X-ray exposure

Cx43-hemichannels opening in response to IR exposure shown by two diferent techniques: A. measurement of extracellular ATP after irradiation and normalised to non-irradiated control (set as 100%). Left: extracellular ATP measured at different time points post-irradiation * vs non-irradiated Right: extracellular ATP measured at 5 minutes post-irradiation (1Gy) in the presence of different connexin-channel blockers or a vehicle * vs vehicle B-C: Uptake of the hemichannel-permeable dye at 5 min post-irradiation (1Gy). B. 10x10 montage x10 irradiated zone is indicated with the square . C The effect of Gap26 on the %PI-positive cells, * vs non-irradiated, #vs vehicle

A



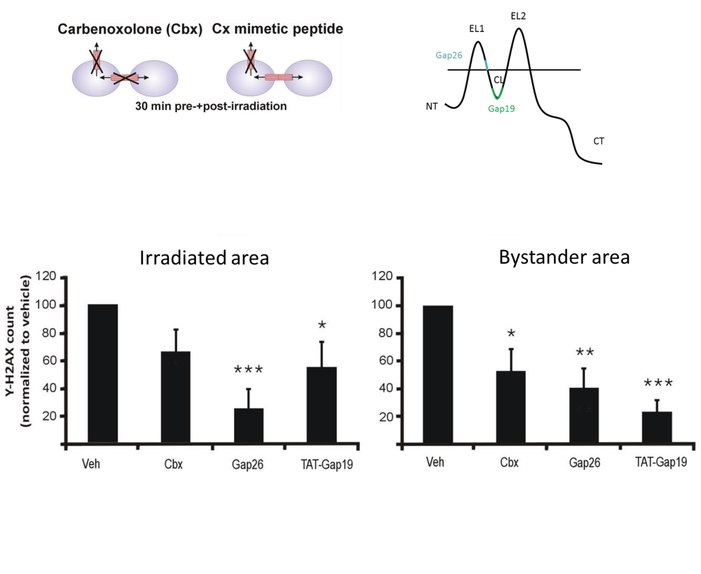
B



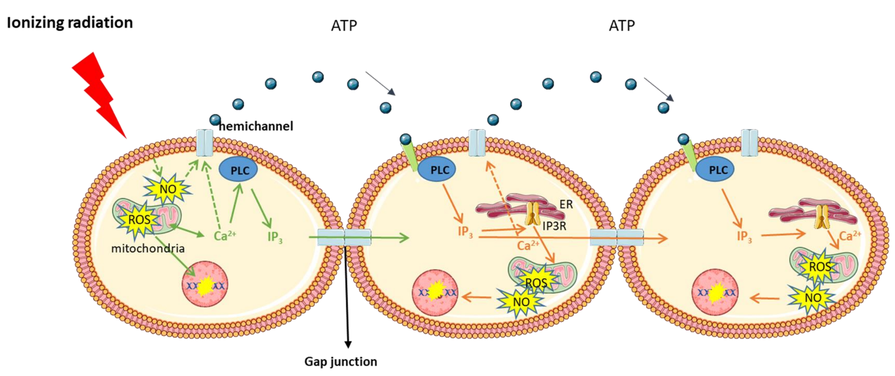
C

Hemichannels contribute to the bystander effect

DNA damage quantification 3h post-irradiation (1 Gy) in cell cultures incubated with the pharmacological blocker Cbx (50 µM) or the Cx mimetic peptides Gap26 (200µM) and TAT-Gap19 (200 µM), Gap26 corresponds to 13 amino acids of the 1st extracellular loop of Cx43 and TAT-Gap19 is composed of 9 amino acids of the cytoplasmic loop of Cx43 coupled to the cell-permeable TAT sequence. Connexin mimetic peptides block HCs under the experimental conditions applied in this study. In contrast, Cbx blocks both Hcs and gap junctions. * vs vehicle, N=8-9.



Conclusion



❖ IR results in the opening of Cx43 HCs

❖ Ca^{2+}/ATP/IP_3 contribute, together with ROS/NO to the spreading of RIBE

Future perspectives

Focus on the later endpoints:

❖ Senescence

❖ EC activation

❖ apoptosis/other cell death modalities