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Exosomes promote survival in squamous head and neck cancer cells after ionizing radiation

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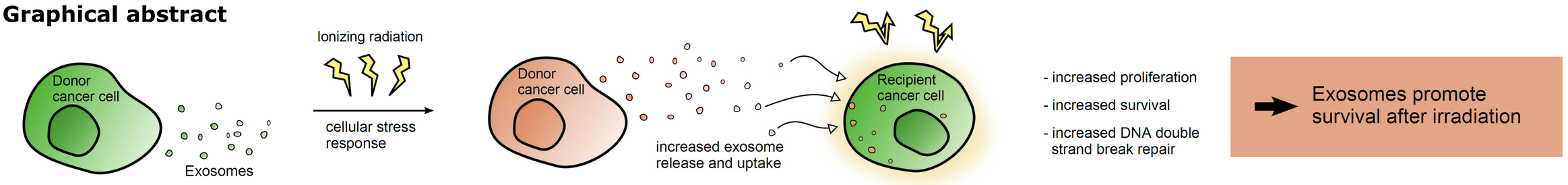
Introduction & Aim: Exosomes are nanometer-sized extracellular vesicles with functions in intercellular communication within tumor tissues and may serve as biomarkers for therapy monitoring during treatment with ionizing radiation. Here, we show that exosomes are able to modify the radiation response of the head and neck cancer cell line BHY.

Material & Methods: Exosomes were isolated from the conditioned medium of irradiated as well as non-irradiated BHY cells by serial ultracentrifugation and quantified by using NanoSight technology. To test whether the released exosomes influence the radiation response, exosomes isolated from non-irradiated and irradiated donor cells were transferred to non-irradiated and irradiated recipient cells.

Graphical abstract



1. Exosome release after ionizing radiation

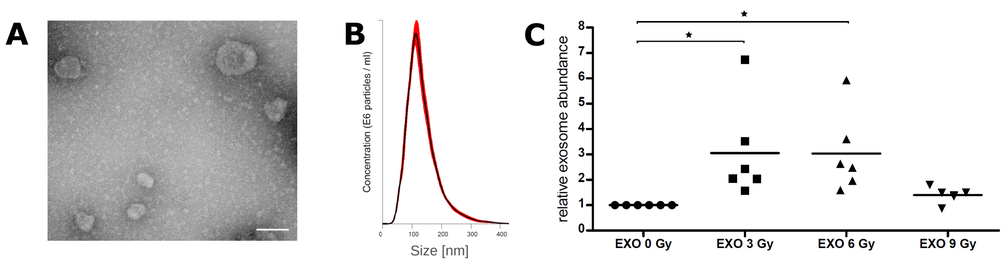


Figure 1. (A) Electron microscopy image of exosomes isolated from the cell culture supernatant of 3 Gy irradiated BHY cells [scale 100 nm]. **(B)** Exosome size in nm. **(C)** Relative exosome abundance after isolation from the culture supernatant of 0, 3, 6 and 9 Gy irradiated BHY cells.

2. Uptake of exosomes by recipient cells after ionizing radiation

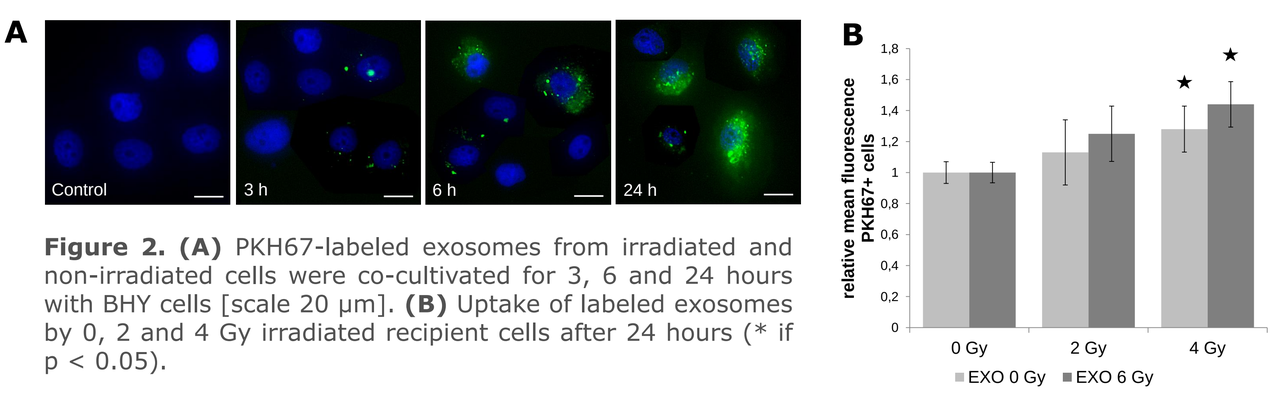
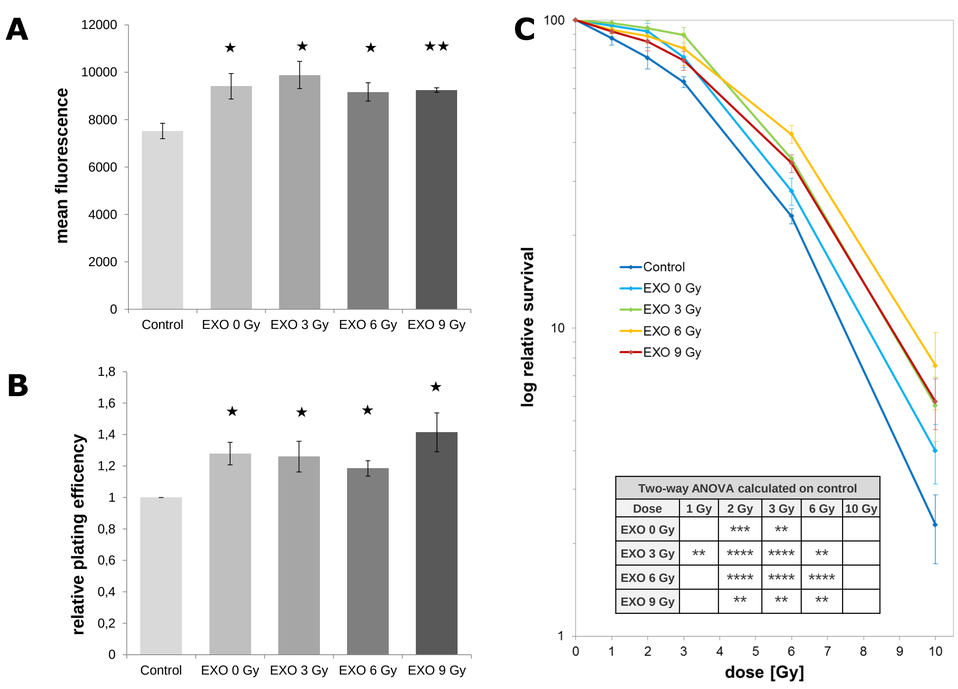


Figure 2. (A) PKH67-labeled exosomes from irradiated and non-irradiated cells were co-cultivated for 3, 6 and 24 hours with BHY cells [scale 20 µm]. **(B)** Uptake of labeled exosomes by 0, 2 and 4 Gy irradiated recipient cells after 24 hours (* if $p < 0.05$).

3. Exosomes sustain proliferation, colony formation and clonogenic survival



Two-way ANOVA calculated on control					
Dose	1 Gy	2 Gy	3 Gy	6 Gy	10 Gy
EXO 0 Gy		***	**		
EXO 3 Gy	**	****	****	**	
EXO 6 Gy		****	****	****	
EXO 9 Gy		**	**	**	

Figure 3. Cultivation of BHY cells with exosomes isolated from irradiated or non-irradiated cells.**(A)** Proliferation **(B)** Plating efficiency **(C)** Clonogenic survival (* if $p < 0.05$, ** if $p < 0.01$, *** if $p < 0.001$ and **** if $p < 0.0001$).

4. Exosomes modulate the repair of DNA double strand breaks in irradiated recipient cells

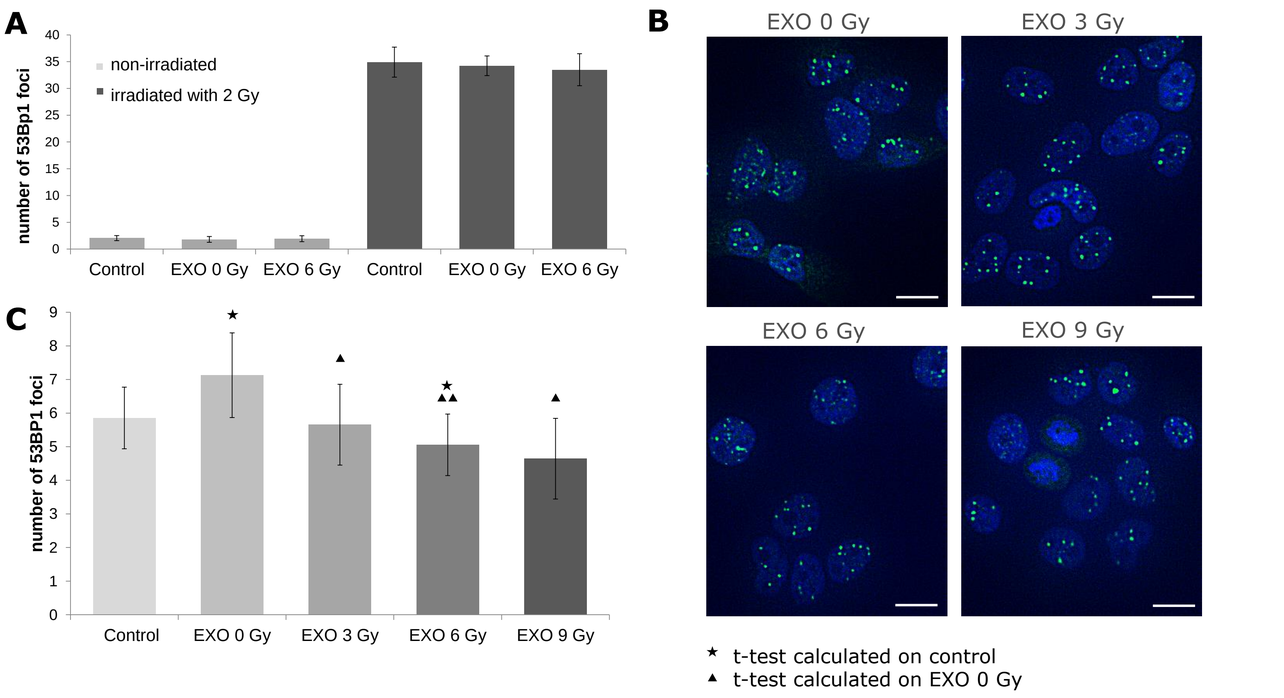


Figure 4. (A) Number of 53BP1 foci in BHY cells 1 hour after irradiation with 0 and 2 Gy and transfer of BHY exosomes isolated after irradiation with 0 and 6 Gy **(B + C)** 53BP1 foci in BHY cells 6 hours after 2 Gy and transfer of BHY exosomes isolated after irradiation with 0, 3, 6 or 9 Gy [scale 20 µm] (*/▲ if $p < 0.05$, **/▲▲ if $p < 0.001$).

Conclusion & Outlook: Our results demonstrate that radiation increases exosome abundance and influences their effect on recipient cells. Exosomes transmit prosurvival effects by promoting the proliferative and radioresistant phenotype of head and neck cancer cells. Future experiments shall analyze if the exosomal effects are caused by a change in exosomal composition after ionizing radiation.
This study indicates a functional role for exosomes in the response of tumor cells to therapeutic radiation exposure, elucidates that radiotherapy influences the cancer progression and encourages that exosomes might be a useful tool to improve therapy strategies.

Reference: Mutschelknaus L, Peters C, Winkler K, Yentrapalli R, Heider T, Atkinson MJ, et al. (2016) Exosomes Derived from Squamous Head and Neck Cancer Promote Cell Survival after Ionizing Radiation. PLoS ONE 11(3): e0152213. doi:10.1371/journal.pone.0152213

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