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Experimental radiotherapy

Comparison of X-ray and alpha particle effects on a human cancer and endothelial cells: Survival curves and gene expression profiles

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ABSTRACT

Background and purpose: Tumours are now considered as complex tissues including endothelial cells of the tumour vasculature, which can decrease radiotherapy efficacy. It is thus important to better characterise the response of both types of cells to irradiation. This study investigated the effects of X-ray and alpha particle irradiation on cancer and endothelial cells.

Materials and methods: A549 non-small-cell lung adenocarcinoma cells and human endothelial cells (EC) were exposed to X-rays or alpha particles. Responses were studied by clonogenic assays and nuclei staining. A gene expression study was performed by using Taqman low density array and the results were validated by qRT-PCR and ELISA.

Results: The relative biological effectiveness of alpha particles was estimated to be 5.5 and 4.6 for 10% survival of A549 cells and EC, respectively. Nuclei staining indicated that mitotic catastrophe was the main type of cell death induced by X-rays and alpha particles. Both ionising radiations induced the overexpression of genes involved in cell growth, inflammation and angiogenesis.

Conclusions: Alpha particle irradiations are more effective than X-rays. The gene expression changes observed in both cell types after alpha particle or X-ray exposure showed possible crosstalk between both cell types that may induce the development of radioresistance.

More than 50% of all cancer patients benefit from a superior clinical outcome with at least one session of radiation therapy during their treatment [1]. However, radiotherapy also causes side effects due to radiation exposure of healthy tissues surrounding the tumour.

One way to improve radiotherapy is to maximise the dose at the tumour location while limiting the damages to healthy tissues. This can be achieved by the use of hadrontherapy instead of conventional X-ray therapy. Hadrontherapy uses particle beams while conventional radiotherapy uses photons. The dose distribution of particles allows a precise targeting of the tumour volume while sparing surrounding normal tissues. Indeed, the photons' energy deposition is maximal close to the entrance in the tissues and decreases exponentially with depth. In contrast, the maximum energy deposition of light or heavy ions occurs within the Bragg peak at a depth depending on the energy of the particle [2]. The dose deposition before this peak is low and almost non-significant after it [3]. Thus, the dose delivered to healthy tissues surrounding the tumour can be minimised.

Another advantage of charged particles over X-rays is their higher linear energy transfer (LET). Compared to sparsely ionising radiation like X-rays, high-LET particles have a higher relative biological effectiveness (RBE) because they produce an intense ionisation along their track and cause severe damages to DNA which are more difficult to repair [4]. Moreover sparsely ionising radiation effectiveness is strongly dependent on the cell cycle position. This variation of cell sensitivity with cell cycle position and proliferative status is considerably reduced with high-LET radiation. Moreover, hypoxia occurs in a lot of solid tumours and leads to radioresistance [5]. As high-LET radiation effectiveness is less dependent upon oxygen, hadrontherapy is more suitable to treat hypoxic tumours. Nonetheless, response of the tumour vasculature to radiation therapy is also playing an important role in the tumour response to radiation. Indeed, as tumours need nutrients and oxygen supply to grow, damaging the tumour vasculature with radiation can lead to an enhanced tumour cell killing [6]. However, tumour cells can actively protect tumour-associated endothelial cells after radiation therapy by secreting growth factors and cytokines that will reduce vasculature damages thereby limiting the treatment response [7].

For these reasons, it is important to understand mechanisms of radioresistance in both tumour and endothelial cells after

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irradiation in order to develop new strategies to increase radiotherapy efficacy. As hadrontherapy seems to be promising, we decided to investigate the radiation responses of human cancer and endothelial cells after irradiation with alpha particles. We compared these results to conventional X-ray irradiation.

Materials and methods

Cell culture

A549 cells, HeLa cells, MCF-7 cells and human endothelial EAhy926 cells were sub-cultured in 75-cm^2 polystyrene flasks (Costar), respectively in modified Eagle's medium, Dulbecco's Modified Eagle Medium (1 g/l D-glucose, Invitrogen) and Dulbecco's Modified Eagle Medium (4.5 g/l D-glucose, Invitrogen) for the two latters, all supplemented with 10% foetal calf serum (Invitrogen). EAhy926 cells are derived from fusion of human umbilical vein endothelial cells (HUVEC) with A549 cells. All cell types were cultured under an atmosphere containing 5% CO_2.

X-ray irradiation

Twenty-four hours before irradiation, cells were plated in 25-cm^2 polystyrene flasks (Costar) to obtain a confluent monolayer for the irradiation. They were irradiated at room temperature using an X-ray generator (RT250, Philips Medical Systems) at a dose rate of 0.855 Gy/min in serum-free CO_2-independent medium (Invitrogen) supplemented with 1 mM L-glutamine (Sigma).

α-particle irradiation

A 2 MV Tandem accelerator (High Voltage Engineering Europa) was used to obtain a $^{2+}$He homogenous broad beam. The experimental set-up and the irradiation procedure are described elsewhere [8]. The linear energy transfer (LET) of the alpha particles was set up to ~100 keV/μm because it corresponds to their maximum relative biological effectiveness (RBE) and the dose rate was 1 Gy/min.

Clonogenic assay

Directly after irradiation, cells were trypsinised, counted and replated for clonogenic survival assay at appropriate cell numbers in 6-well plates. The entire procedure after X-ray and alpha particle irradiations has been previously described [8]. As EC could not grow properly when plated at low densities, we used fresh medium plus 10% serum supplemented with conditioned medium 50% as recommended by Franken et al. [9]. At least three independent experiments were performed for each irradiated cell type and the errors were evaluated as standard deviation. Experimental data were fitted with the linear quadratic model (LQ) given by the formula:

$$SF = e^{-(\alpha D+\beta D^2)}$$

where SF is the surviving fraction, D is the dose and α (Gy^{-1}) and β (Gy^{-2}) are the radiosensitivity parameters.

Cell-based assays

Methods for DAPI staining, for gene expression analysis on TaqMan Low-Density Array and by real-time RT-PCR as well as for ELISAs are described in Supplementary Materials And Methods.

Results

Relative biological effectiveness of alpha particles and radiosensitivity parameters for A549 cells and EC

Conventional clonogenic survival assays were performed on both cell types eleven days after irradiation in order to evaluate the relative biological effectiveness (RBE) of alpha particles. Fig. 1 shows the dose responses of A549 cells and EC to X-rays and high-LET alpha particles. X-rays (250 kV) were used as reference radiation to calculate RBE. We obtained a characteristic shape of sparsely ionising radiation as described by Kogel and Joiner [3]. Alpha particle irradiations from doses ranging from 0.2 Gy to 2 Gy doses were performed on A549 cells and EC to obtain the survival curves. As expected for high-LET irradiations, the experimental data could be fitted with a linear model. The RBE of alpha particles was estimated at 5.5 and 4.6 for 10% survival of A549 cells and EC, respectively. For A549 cells, the average values for α and β parameters were respectively 0.332 ± 0.045 Gy^{-1} and 0.018 ± 0.005 Gy^{-2} for X-rays. The radiosensitivity parameters α and β for X-rays for EC were estimated to be 0.19 ± 0.07 Gy^{-1} and 0.039 ± 0.024 Gy^{-2}, respectively and the α parameter was equal to 1.90 ± 0.26 Gy^{-1} for alpha particles. No significant difference on clonogenic survival curves was observed between the two cell types for each type of irradiation.

Mitotic catastrophe as the main type of cell death induced in A549 cells and EC after X-ray and alpha particle irradiations

Mitotic catastrophe is considered as the major type of cell death induced by ionising radiation in most non-haematopoietic tumour cells [10]. Morphological alterations of the nucleus such as multinucleation and micronucleation can be observed due to problems in cytokinesis and chromosome segregation, which will lead to formation of multinucleated giant cells with several micronuclei [11]. As positive controls of apoptosis and mitotic catastrophe, A549 cells and EC were treated with etoposide or paclitaxel, respectively and nuclei were labelled with DAPI (Fig. 2A). Etoposide induced the formation of condensed nuclei, a typical feature of cells undergoing apoptosis. Paclitaxel treatment induced the formation of micronuclei and giant multinucleated cells (Fig. 2A). Twenty-four hours after the radiation exposure with X-rays or alpha particles,

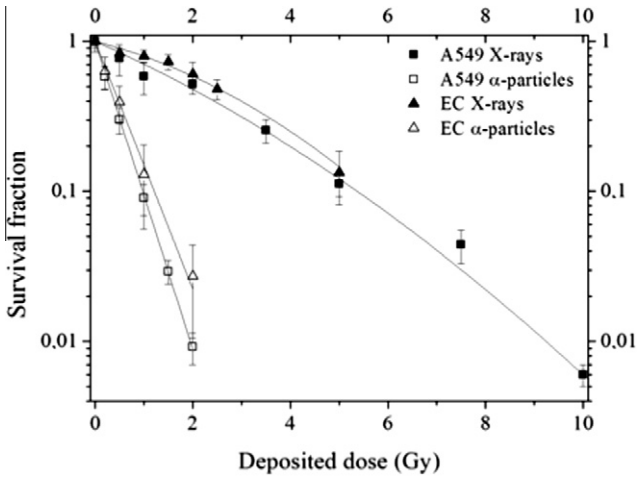

Fig. 1. Survival curves of A549 cells and EC exposed to X-rays or alpha particles. Survival fraction was calculated using conventional clonogenic assay. The LQ model was applied to experimental data (straight lines). Results are presented as means, the error bars represent standard deviation (2 or 3 independent experiments with $n = 3$).