Comparative studies on the effect of x-ray and heavy ion irradiation on ROS signalling and K^+ channel activation in A549 cells*

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Introduction

Previously, we found that irradiation of the epithelial lung cancer cells A549 with photons resulted in a rapid activation of K^+ channels and a concomitant hyperpolarization of these cells [1]. The signaling cascade, includes a rapid generation of H_2O_2 immediately after irradiation. The latter triggers an increase in the concentration of cytosolic Ca^{2+} and the consequent activation of the human intermediate potassium channel hIK [1]. This channel regulates via hyperpolarization of the plasma membrane important functions such as the cell-cycle transition and cell migration [2]. Here we test whether an irradiation of cells with heavy ions triggers the same signal cascade with an eventual activation of hIK channels.

Material and Methods

Membrane currents and changes in H_2O_2 concentration in cells were monitored as described previously [1,2].

Results

The data in Fig. 1 show the fluorescence of A549 cells, which were either exposed to 10 Gy of X-ray (a) or to 10 Gy of carbon ions (b). As a consequence of photon irradiation all the cells revealed a robust increase in the fluorescence ratio $F_{488/405}$ of the H_2O_2 sensor. This confirms that irradiation with x-ray causes a rapid rise in the concentration of H_2O_2 in cells. When cells were irradiated with C-ions, we found only a small fraction of cells (5%) which responded with an increase in the fluorescence ratio (Fig. 1bi). The majority of cells exhibited no effect (Fig. 1bii). The results of these experiments suggest that 10 Gy x-ray irradiation triggers a large increase in ROS production in cells while an administration of the same doses of carbon ions has no effect.

The results of these experiments predict an x-ray induced activation of the hIK channel while an exposure to heavy ions should not. Indeed our experimental data show that the hIK currents in A549 cells are unaffected by irradiation with heavy ions. In the equivalent experiments with x-rays we found that an irradiation with 1 Gy was already increasing this current by a factor of > 2 [1].

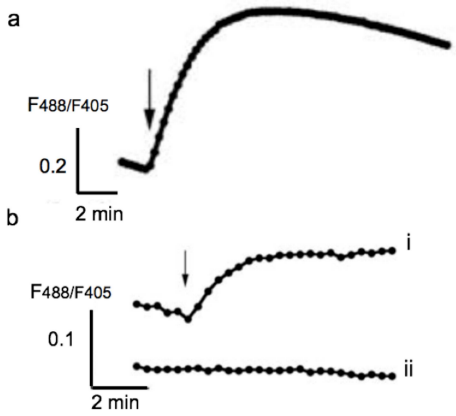

Figure 1: Fluorescence ratio of H_2O_2 reporter HyPer after irradiation of individual cells with 10 Gy X-ray (a) or with 10 Gy C-ions (b). In the latter case only 5% of the cells tested showed a response (trace i) while the majority remained unaffected (trace ii). Cells were irradiated at time indicated by arrow.

Conclusion

The present data show that x-ray irradiation and heavy ion treatment cause different effects in A549 cells. The most significant difference is that heavy ion irradiation has a reduced efficiency in triggering ROS production including the long lasting species H_2O_2. This lack of H_2O_2 production prevents the onset of a signal transduction cascade, which otherwise activates K^+ channels in irradiated cells. As a working hypothesis we assume that the low energy of the heavy ions from the UNILAC presumably generates radicals very close to the ion-track, which may recombine very fast. This could explain the lack of H_2O_2 production after heavy ion irradiation. The heterogenous distribution of radicals, which occurs after x-ray irradiation, may in contrast foster more long lasting ROS species.

References

[1] B. Roth et al. “Low-dose photon irradiation alters cell differentiation via activation of hIK channels”, Pflügers Arch.- Eur. J. Physiol. 2014 (in press).

[2] C. Gibhardt et al. “X-ray irradiation activates K+ channels via ROS signaling”, JGP 2015 (submitted)

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