

Ionizing irradiation causes rapid activation of the human intermediate K^+ channel in A549 cells: link to cell migration and proliferation

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Introduction

In previous experiments it was shown that radiation with photons and high-energy ions results in a rapid activation of K^+ channels in the epithelial lung cancer cell line A549 [1]. Among the responsive channels is the human intermediate potassium channel hIK [1]. This channel is known to regulate in other types of cells the cell-cycle transition and cell migration via hyperpolarization of the plasma membrane [2]. To further test the contribution of hIK channels to the irradiation induced rise in conductance we exposed responsive cells after irradiation to the specific inhibitor 10 μ M Clotrimazole. Since this treatment was effective to block the hIK channel in A549 cells we tested whether an inhibition of the hIK channel in A549 cells is able to influence proliferation and migration.

Material and Methods

We examined the conductance of ion-channels in A549 cells by a planar patch-clamp technique combined with an external perfusion system [3]. Migration and motility was observed under a microscope with a scratch assay. Proliferation rates were determined in a particle counter and cell-cycle analysis was performed by flow cytometer after DAPI staining.

Results

The data in figure 1 show that the high conductance of A549 cells, which were obtained by irradiation, were restored back to the control level by treating cells with Clotrimazole. The results of these experiments underscore the view that irradiation augments the conductance of hIK type channels. To monitor the proliferation behavior of the A549 cells in the presence of Clotrimazole the cell density was counted over a period of 96 hours. The data show a 5times lower proliferation rate in the presence of the channel blocker compared to the control.

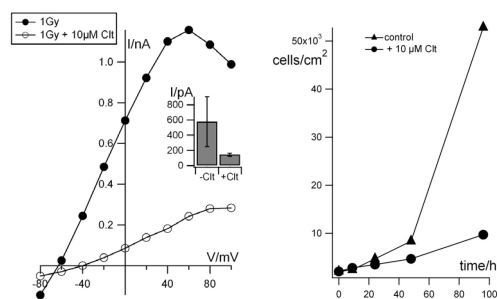


Figure 1: Current response of an A549 cell to a standard pulse-protocol (a). The irradiation augmented conductance can be inhibited by 10 μ M Clotrimazole, a specific blocker of Ca^{2+} -

activated K^+ channels (hIK). Blocking the same ion-channel slowed down the proliferation-rate dramatically (b).

The scratch wound healing assay was used to measure two-dimensional movement of the A549 cells (Fig 2). The result of this assay showed that the specific hIK channel blocker Tram-34 decreases the motility of the cells and their ability to migrate.

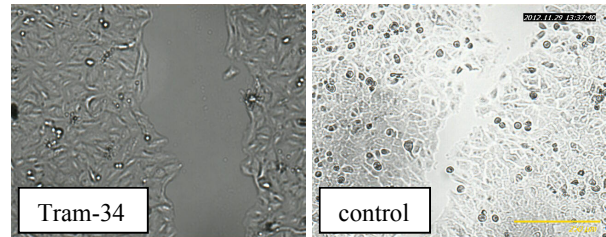
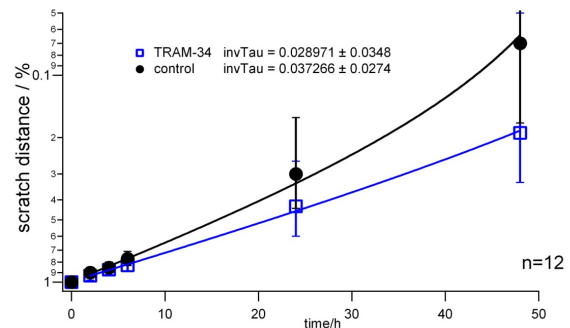


Figure 2: Effects of hIK ion-channel blocker (Tram-34) on migration of A549 cell line. Scratch wound healing assay was performed to compare the migratory capabilities of cells. The images were acquired 12h after scratch.

Discussion

The present data verify and extend a previous report, which has shown that ionizing irradiation increases already at low doses the conductance of the human intermediate conductance potassium channel hIK. On the other hand the hIK channel has been identified as a key player in regulation of proliferation and migration in A549 cells. Hence it is reasonable to speculate that an irradiation-induced activation of the hIK channel can stimulate the proliferation and motility via hyperpolarization of the plasma membrane.

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References

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