INTRODUCTION

Most mammalian cells express a wide range of potassium (K⁺) channels, such as large conductance K⁺ (BK) and voltage gated K⁺ (Kv) channels. In non-excitable cells, K⁺ channels are involved in volume regulation, hormonal secretion, cell proliferation, migration and apoptosis [1,2]. The ability of ion channels to act as modulators of conductance is due to their innate property of rapid conformational alterations to initiate or respond to signal transduction [3].

Several lines of evidence from molecular, biochemical, and biological studies outline that different pathways are operating in cells and organisms at low and high radiation doses leading to a non linearity in the dose–response increase.

A previous study performed in human cell lines showed that both normal and cancer cells exhibit an increase in K⁺ currents after treatment with low dose ionizing radiation (LDIR), suggesting that K⁺ current (I_K) modifications are acting as a component of a signal transduction pathway(s) in response to stress, such as amplification of reactive oxygen species (ROS) signaling cascade [4]. Moreover, it has been shown that ionizing radiation (IR) might directly modify BK channel activity and therefore modulate cytosolic free Ca²⁺ concentration and CAMKII pathway [5]. In this work, we studied the effects of proton (H⁺) irradiation at different doses (0.25 to 4 Gy) on whole-cell I_K and gene expression in a rodent and a human cell system: Chinese hamster lung fibroblasts (V79) and glioblastoma multiform cell line (T98G) respectively.

MATERIALS AND METHODS

V79 and T98G cells were maintained in standard conditions and, 48 hours before irradiation, plated as a monolayer on specially designed stainless-steel Petri dishes for irradiation [6]. Proton (H⁺) irradiations have been performed at the Radiobiology facility at the INFN-LNL 7MV Van de Graaff CN accelerator.

Irradiation facility, beam dosimetry and irradiation modalities have been described in detail elsewhere [6]. H⁺ of 3.0 MeV (0.8 MeV energy at the cell entrance surface, corresponding to LET values of 28.5 keV/μm) have been used. Sham irradiated cells were used in all the experiments as control (un-irradiated) cells.

Patch clamp recording. The cells were detached 2 hours after irradiation using trypsin-EDTA (0.02%), resuspended in culture medium, transferred (within 6 h) to a Petri dish and allowed to attach to the bottom for 30 min.

Culture medium was substituted with the bath solution (pH 7.4) contained (in mM): 133 NaCl, 4 KCl, 2 MgCl₂, 2 CaCl₂, 10 HEPES and 10 glucose. The V79 electrode solution (pH 7.2) contained (in mM): 145 KCl, 1 MgCl₂, 1.8 CaCl₂ and 10 HEPES; the T98G electrode solution (pH 7.2) contained (in mM): 10 NaCl, 120 Kasp, 2 MgCl₂, 4 CaCl₂, 10 HEPES, 10 EGTA, 3 Mg-ATP, 0.2 GTP tris (Sigma Chemical Company, St. Louis, MO).

Membrane currents were measured in the whole-cell configuration of the patch clamp technique at room temperature (22-24°C) with a EPC-10 amplifier (HEKA Instruments, Darmstadt, Germany).

Current traces were acquired at digitizing rates of 5-10 kHz and filtered at 2.9 kHz with an eight-pole low-pass Bessel filter. Voltage steps (20 mV, 100 ms) from -30 to 110/130 mV were delivered at intervals of 1 s; holding potential V_H was set to 0 mV. The patch micropipette tip resistances ranged between 4 and 10 MΩ, when filled with electrode solution.

Gene expression. After the exposure at H⁺ irradiations, dry pellets of T98G cells was frozen at -80 °C. From control and irradiated T98G cells, RNA were extracted with miRNeasy Mini Kit, as recommended by the manufacturer (Qiagen). The samples were profiled using GeneChip Human Exon 1.0 ST Arrays. 100 ng total RNA were used for labeling protocol.

The Human Gene 1.0 ST Array is a focused gene-level expression array that represents 28,869 well annotated full-length genes from RefSeq, Ensembl and putative complete CDS GenBank transcripts. Probes are designed across the whole transcript to provide a more accurate representation of total transcription activity for the gene locus.

Data are expressed as mean ± S.E.M.. Student’s t test was used to compute p values. A threshold of 0.05 was considered for statistical significance.

RESULTS AND DISCUSSION

The amplitude of I_K currents recorded from V79 and T98G cells irradiated by H⁺ showed, as a function of the applied voltages (I-V curves), a general decrease compared
to un-irradiated cells (figures 1 and 2). In particular, we observed that in the rodent cells (figure 1 and 2 top) the \( I_K \) mean amplitude linearly decreased as a function of dose until 1 Gy of irradiation dose. Interestingly, increasing the dose (2-4 Gy) the linear decrement is not maintained. We also observed that in the human cells (figures 1 and 2 bottom) only the 0.25 and the 2 Gy are effective, revealing a strong hypersensibility to \( H^+ \) irradiation.

In conclusion the amplitude of \( I_K \) is a good biological endpoint to assess the radiobiological effect of \( H^+ \) irradiation. A first attempt to explain the mechanism underlying this effect is based on superoxide anion (\( O_2^- \)) production by the mitochondrial electron transport chain. As illustrated in figure 3, this free radical anion might modulate the Kv1.1 channel conductance by the \( \beta 1 \) subunit oxido-reductive sensor, the BK channel conductance by the interaction with the tyrosine kinase and regulate the Mn superoxide dismutase (SOD2) gene expression.

This last pathway is central in the regulation of \( O_2^- \) homeostasis. The modulation of BK channel might exert an action, by changing the Ca\(^{2+}\) concentration and CAMKII activity, on cell migration and cell cycle progression.

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**References**