Role of intracellular RONS in plasma-based cancer treatment

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We describe an in-vitro study aimed at elucidating the role of Reactive Oxygen and Nitrogen Species (RONS) in promoting a selective killing of cancer cells, and the possibility of emphasizing the selectivity towards cancer cells by combining the plasma treatment with the effect of a molecule known to enhance intracellular ROS production. Lung carcinoma cell lines and cultured primary cells isolated from surgical samples of laryngeal and lung cancers as well as healthy tissue counterparts were treated with an RF plasma source. An increase in the level of endogenous Reactive Oxygen Species (ROS) and of NO was observed, but it was markedly higher in cancer cells than in healthy ones. Incubating the cells with antimycin A (AMA), a molecule known to increase ROS production, the effect could be amplified, An increased expression of hypoxia-inducible factor (HIF)-α amd a higher apoptosis in cancer cells than in healthy ones was observed.

1. Introduction

The mechanism underlying the beneficial effects of a low-power, atmospheric pressure plasmas as a tool for cancer treatment has been traced by several authors to the formation of intracellular Reactive Oxygen and Nitrogen Species (RONS). In this contribution we describe an in-vitro study aimed at elucidating the role of RONS in promoting a selective killing of cancer cells.

2. Experimental procedure

The plasma treatment applied for this study is performed with an indirect plasma source, which uses a RF voltage to ionize a helium flow mixed with ambient air in the region between two brass grids [1]. The helium flow enriched with active chemical species is then sent to the substrate to be treated.

H460, MCF7, A549 lung carcinoma cell lines were grown in DMEM medium + 10% fetal bovine serum (Gibco). Primary cell cultures were established from surgical samples of laryngeal cancer and healthy counterparts. Tissue samples were dissociated with collagenase type IV (Sigma) at 37°C for 15 min. Cells were cultured in DMEM medium + 10% fetal bovine serum, and then exposed to the plasma for 2 min. Reactive oxygen species (ROS) and nitric oxide (NO) were detected 30 min, 4hours, and 24 hours later plasma treatment by flow cytometry. Cell death was assessed 24 hours later plasma treatment using Annexin V-FITC Apoptosis Detection Kit (eBioscience).

3. Results

It has already been reported that this kind of treatment induces an increase in the level of endogenous Reactive Oxygen Species (ROS) in eukaryotic human cells [2]. In the present study, ROS generation was confirmed, but the increase was markedly higher in cancer cells than in healthy ones. The same effect was observed for intracellular nitric oxide (NO). Furthermore, incubating the cells for 15 min. with antimycin A (10ng/mL, AMA), a molecule known to increase ROS production [3], the effect could be amplified, both for ROS and NO. The selective increase in endogenous RONS was associated to increased expression of hypoxia-inducible factor (HIF)-α, an oxygen-sensitive transcriptional activator, and to a higher apoptosis in cancer cells than of their healthy counterparts. Again, these effects were emphasized by incubating with AMA. Overall, these results point to confirm the important role played by RONS in plasma-based cancer treatment, and to the possible combination with chemotherapeutic drugs to better tailor the selective effect induced by the plasma treatment.

5. References

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