The ATPase inhibitor protein IF_1 in cancer cells exposed to anoxia mimicking condition favors survival and proliferation of re-oxygenated cells.

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INTRODUCTION

In eukaryotes the ATP synthase complex is regulated by the endogenous inhibitor protein, IF_1, an 81-residue protein in human mitochondria. It binds the enzyme, inhibiting its hydrolytic activity when the mitochondrial membrane potential ($\Delta\mu_H+$) falls, as it occurs in ischemic tissues [1-2]. In the last decades it has been reported that cancer cells overexpress IF_1, suggesting that it can play other critical roles in metabolic reprogramming [3-5]. The molecular mechanisms at the basis of these roles are still under debate and warrant in-depth investigations. We examined the role exerted by IF_1 in human 143B osteosarcoma cells exposed to an anoxia-mimicking condition as it occurs *in vivo* in solid tumors, by collapsing $\Delta\mu_H+$ with the uncoupler FCCP.

Metabolism of controls and IF_1-silenced cells in anoxia-mimicking (FCCP)

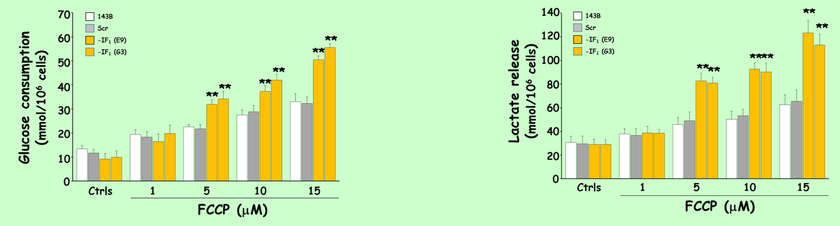

Fig. 1 IF_1 diminishes the effect of OXPHOS uncoupling on cell metabolism. Parental and osteosarcoma-derived cells were cultured for 24h either in absence or in presence of increasing FCCP concentrations. Glucose consumption (left panel), lactate release (middle panel) measured in all types of osteosarcoma cells were reported. **, $p<0.01$ indicates the statistical significance of data compared to controls.

Bioenergetics parameters and viability in uncoupled controls and IF_1-silenced cells

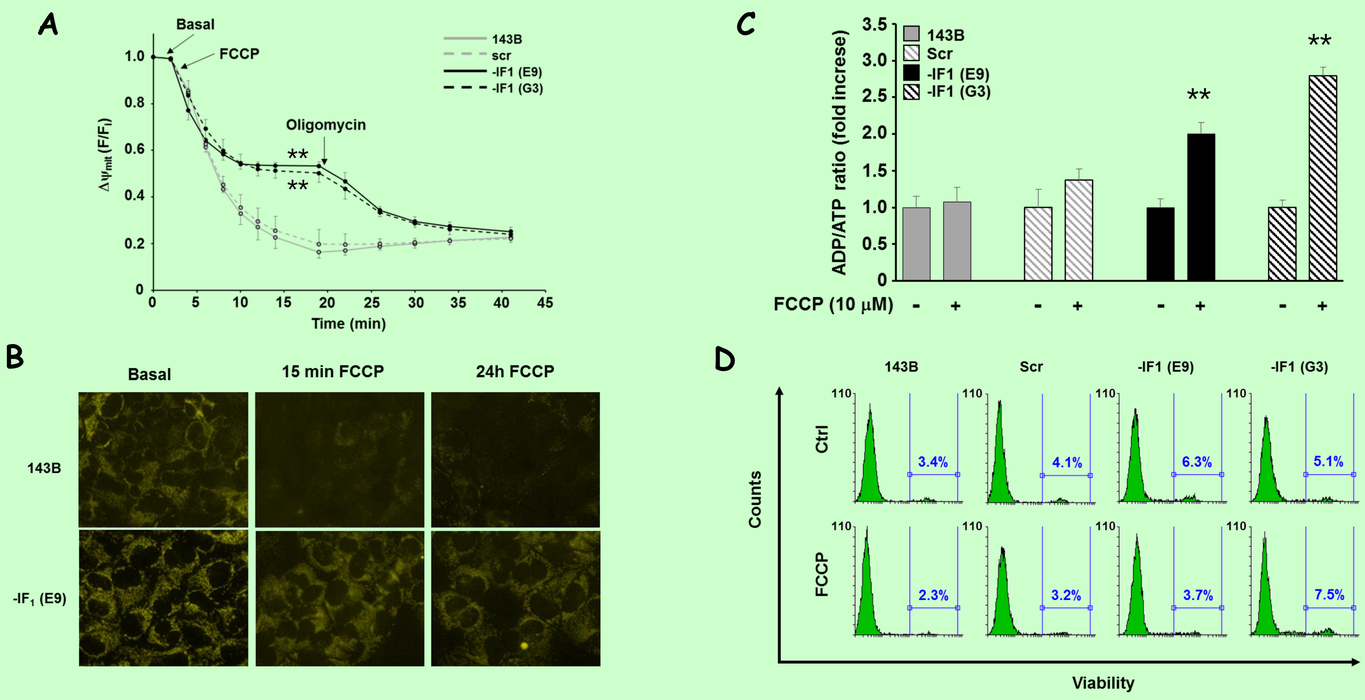

Fig. 2 The F_1F_O-ATPase activity sustains $\Delta\mu_H+$ hydrolyzing ATP in FCCP-treated IF_1-silenced 143B clones only. Flow cytometry kinetic analysis of $\Delta\psi_m$ of TMRM-loaded controls and IF1-silenced clones under uncoupling (1 μM FFCP) conditions (panel A). Fluorescence microscopy images of TMRM-loaded cells under basal conditions and upon 10 μM FCCP addition up to 24h (panel B). ADP/ATP ratio of both control and IF_1-silenced cells cultured under uncoupling conditions for 24h (panel C). Flow cytometry analysis of controls and IF_1-silenced cells viability upon 10 μM FCCP incubation for 24h (panel D). **, $p<0.01$ indicates the statistical significance of data compared to controls.

SUMMARY OF MAIN RESULTS

- Under OXPHOS uncoupling conditions control cells showed a milder increase of both glucose consumption and lactate release compared to IF_1-silenced cells. (Fig. 1).
- FCCP treatment induced a complete $\Delta\Psi_m$ collapse in control cells, whereas IF_1-silenced cells partially sustained the mitochondrial membrane potential, activating the hydrolytic activity of the F_1F_o-ATPase at the expense of cytoplasmic ATP. Concurrently, cells showed higher energy charge compared to IF_1-silenced cells (Fig. 2).
- Mitochondrial mass was conserved under anoxia mimicking conditions both in controls and IF_1-silenced cells (Fig. 3).
- Activation of both mitophagy and mitochondrial biogenesis was observed in control cells only (Fig. 4 and 5).
- IF_1-expressing cancer cells showed both apoptosis resistance and proliferation rescue after re-oxygenation compared to IF_1-silenced cells (Fig. 6 and 7)

Mitochondrial mass in controls and IF_1-silenced cells under anoxia-mimiking conditions

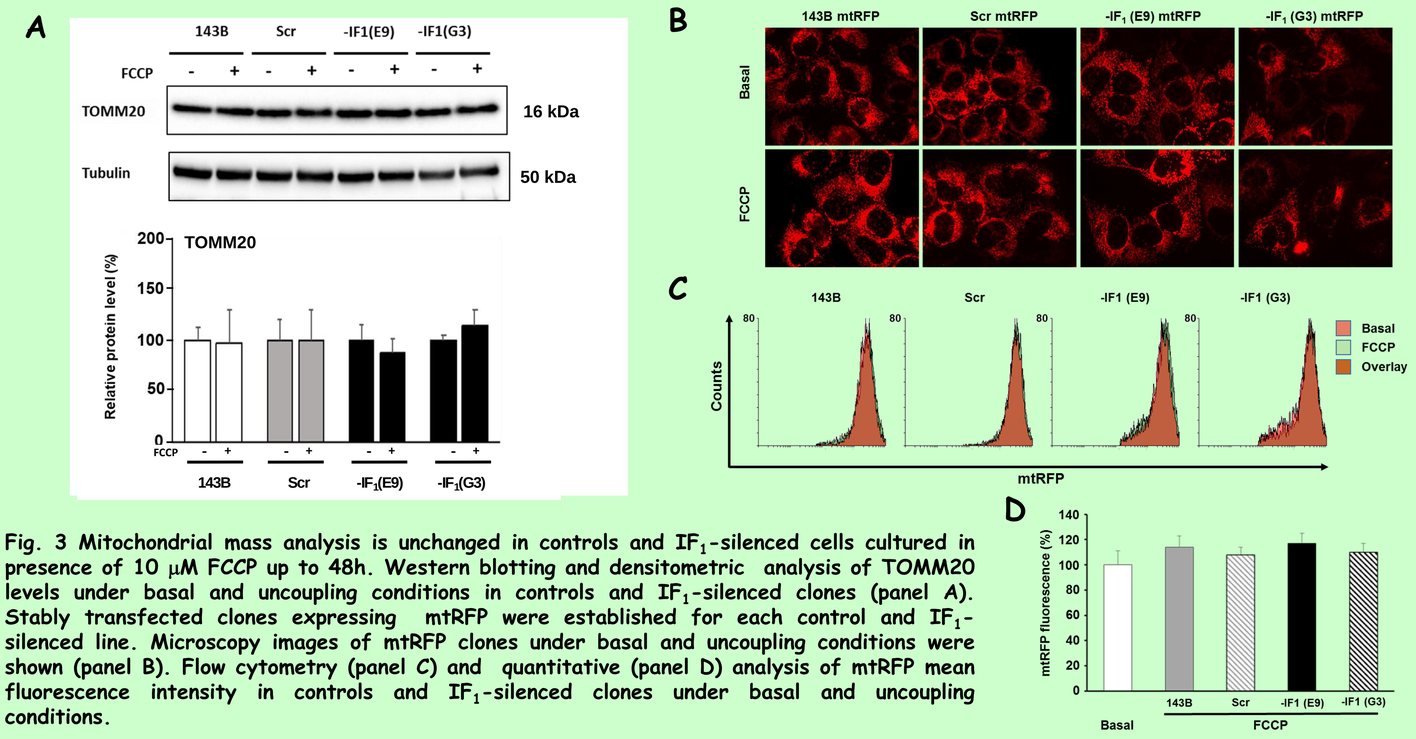

Fig. 3 Mitochondrial mass analysis is unchanged in controls and IF_1-silenced cells cultured in presence of 10 μM FCCP up to 48h. Western blotting and densitometric analysis of TOMM20 levels under basal and uncoupling conditions in controls and IF_1-silenced clones (panel A). Stably transfected clones expressing mtRFP were established for each control and IF_1-silenced line. Microscopy images of mtRFP clones under basal and uncoupling conditions were shown (panel B). Flow cytometry (panel C) and quantitative (panel D) analysis of mtRFP mean fluorescence intensity in controls and IF_1-silenced clones under basal and uncoupling conditions.

Mitophagy and mitochondrial biogenesis in controls and IF_1-silenced cells under anoxia-mimiking conditions

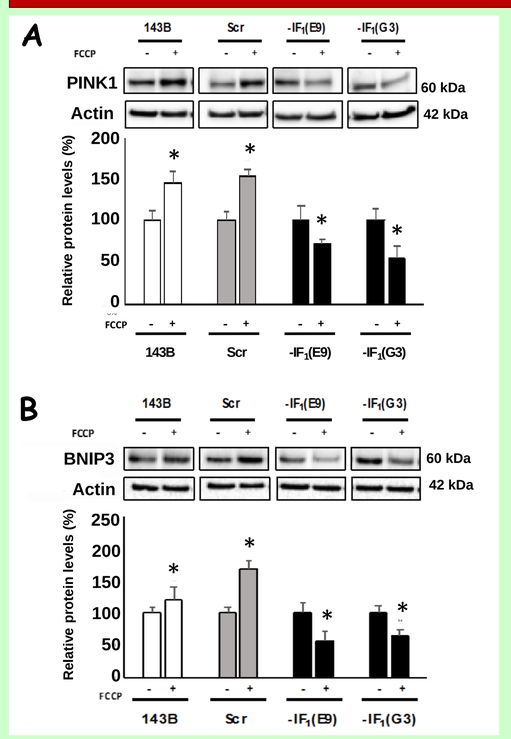

Fig. 4 Mitophagy marker are increased in controls only. PINK (panel A) and dimeric BNIP3 (panel B) levels analyzed upon 24h exposure to FCCP by western blotting and densitometric quantification of the protein bands. *, $p<0.05$ indicates the statistical significance of data compared to control.

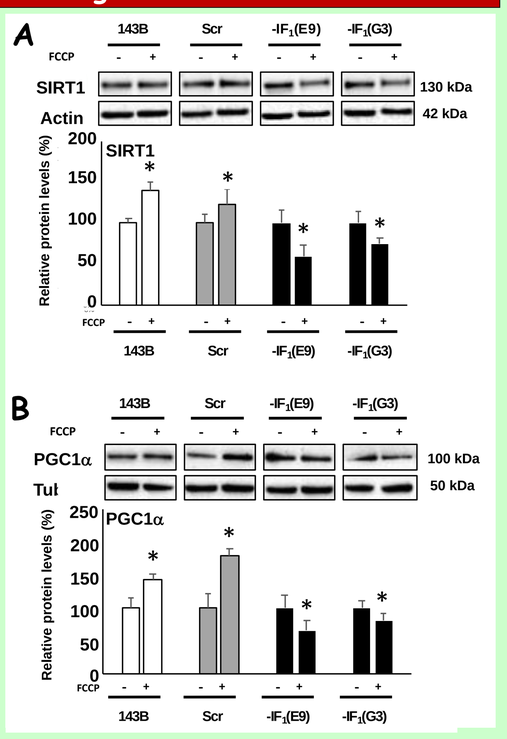

Fig. 5 Mitochondrial biogenesis marker are increased in controls only. SIRT1 (panel A) and PGC1α (panel B) levels analyzed upon 24h exposure to FCCP by western blotting and densitometric quantification of the protein bands. *, $p<0.05$ indicates the statistical significance of data compared to control.

CONCLUSIONS

- IF_1 fully inhibits the ATPase activity under anoxia-mimicking conditions preserving the cellular energy charge.
- IF_1 induces activation of both mitophagy and mitochondrial biogenesis in control cells, promoting renewal of dysfunctional mitochondria and conferring apoptosis resistance.
- IF_1 favors tumor cell survival and promotes cell proliferation recovery after re-oxygenation through energy charge preservation and apoptosis resistance.

Apoptosis in controls and IF_1-silenced cells under anoxia-mimiking conditions

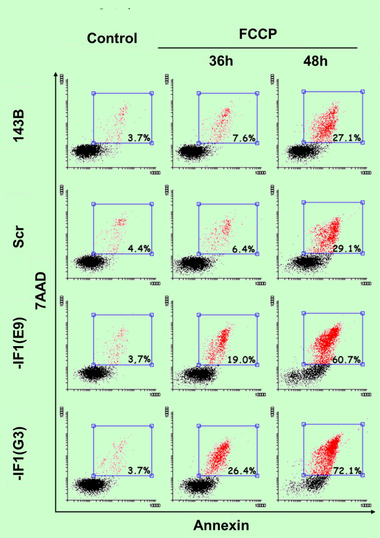

Fig. 6 Apoptosis is much higher in IF_1-silenced cells cultured in presence of 10 μM FCCP up to 48h. Phosphatidylserine externalization were assayed by means of MUSE Annexin V kit. Percentage of cells positive to annexin V was reported.

Proliferation rate after reoxygenation in controls and IF_1-silenced cells

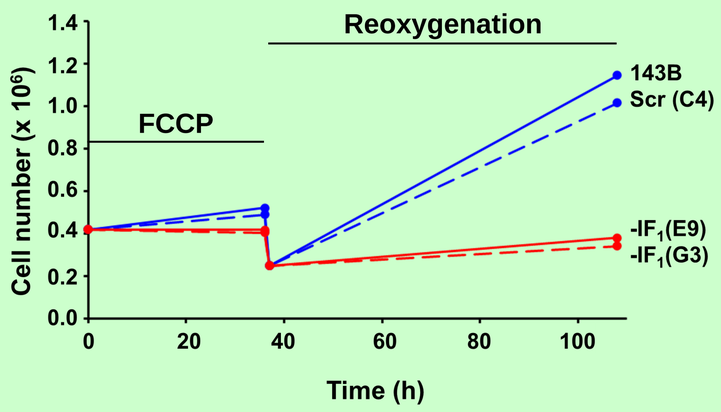

Fig. 7 Cell growth is rescued after re-oxygenation in IF_1-silenced cells only. Cells were cultured in presence of 10 μM FCCP for 48h and then the cells were detached and re-seeded at the same number of live cells in fresh medium without FCCP (re-oxygenation) and cultured for 72h more. **, $p<0.01$ indicates the statistical significance of data compared to control.

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