The IF1 mitochondrial inhibitory protein of ATP synthase modulates the permeability transition pore in a human cancer cell model

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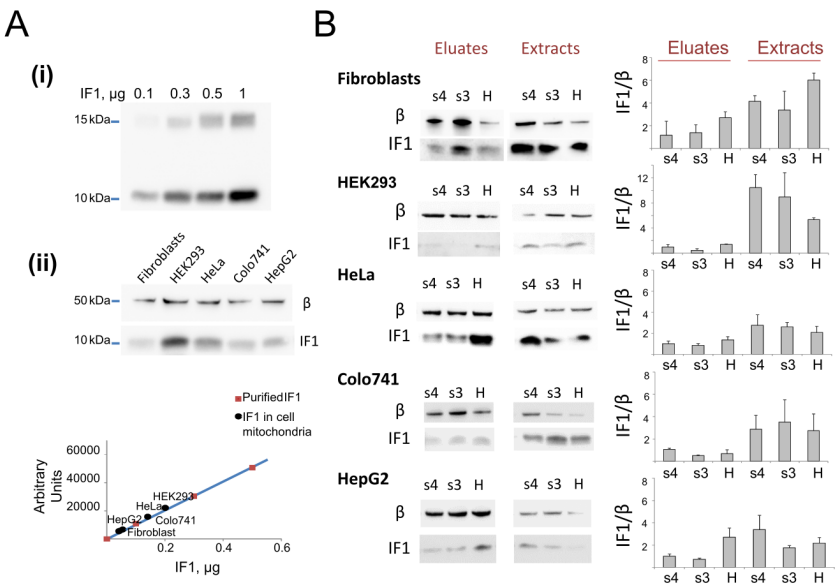
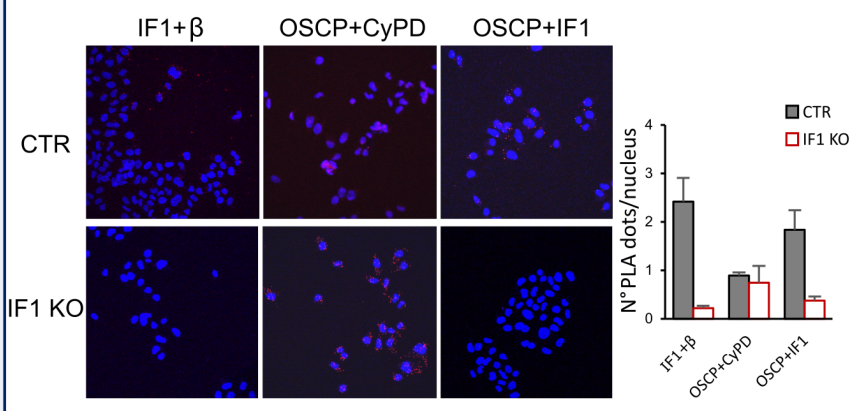
Introduction. The mitochondrial protein IF1 is the natural inhibitor of ATP synthase. It binds to the catalytic F1 domain of the enzyme and inhibits ATP hydrolysis, preventing ATP dissipation in pathophysiological events such as ischemia/reperfusion. As recently reported IF1 can also play a relevant role in promoting cancer development, although the mechanism(s) is still debated. We have characterized human cell lines obtained from different tumors (cervix, colon or lung adenocarcinomas and liver carcinoma). *Results*. Independently of their source these cancer cell lines show higher IF1 mitochondrial content compared to human fibroblasts, but comparable to the highly proliferative model HEK293. In mitochondrial extracts from the above tumor models maintained under different conditions (stimulated ATP hydrolysis, State 3 and State 4 steady state respiratory conditions) IF1 immunoprecipitates with ATP synthase. Since ATP hydrolysis is necessary to allow IF1 binding to the catalytic F1 domain, our finding suggests an additional binding site which anchors IF1 to ATP synthase during oxidative phosphorylation. Moreover, gene disruption (or downregulation of the protein level) of IF1 in these cells significantly reduces colonies formation in soft agar, underlying its important role during cancer development. Importantly, the lack of IF1 does not affect cell proliferation, mitochondrial respiration or ATP synthesis, but sensitizes to the permeability transition pore opening in cells subjected to glucose deprivation or reactive oxygen species.

Figure 1: IF1 quantification and immunoprecipitation



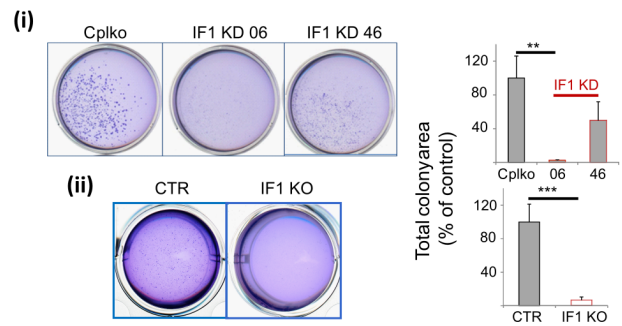
(A) Titration of IF1 purified from bovine mitochondria is shown by Western blotting (i). Western blotting of β subunit and IF1 in mitochondria (10 µg/lane) of the indicated human cell lines, and their IF1 quantification based on the IF1 bands measured in arbitrary units by densitometry (ii), are shown. **(B)** Western blotting of β subunit and IF1 in fractions eluted from ATP synthase immunoprecipitation (eluates) and in the corresponding extracts. Mitochondria are incubated in a buffer promoting State 4 (s4), State3 (s3) respiration or ATP hydrolysis (H). IF1/β ratio (normalized to each s4 eluate) is represented on the right.

Figure 2: IF1 interaction with ATP synthase complex in situ



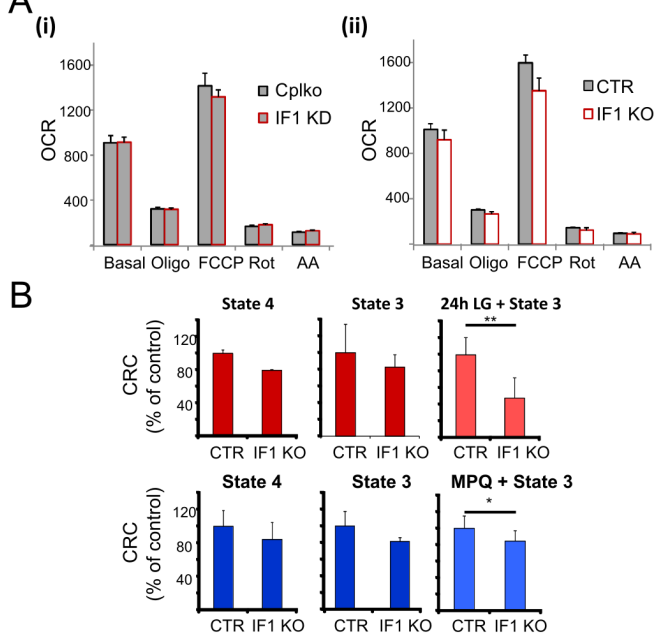
HeLa cells obtained by a CRISP/Cas9 approach with scramble (CTR) or IF1 gene targeting sequences (IF1 KO) are processed using the Proximity Ligation Assay (PLA) to assess protein-protein interactions. The antibodies that are used in PLA to reveal the interactions between the mitochondrial proteins are indicated on the top of the panels and detect the inhibitor protein IF1 (IF1), cyclophilin D (CyPD) and the subunits of ATP synthase β (β) and OSCP (OSCP). The PLA signal is visualized in red, while DAPI-stained nuclei are in blue. Images are acquired with a Leica TCS SP5 confocal microscope equipped with a CCD camera and a 40x objective. Quantification on the right represents the number of PLA dots per nucleus ± SEM.

Figure 3: Effect of IF1 on colony forming capacity



Soft agar in vitro assay showing colony forming capacity of control (C plko, CTR), IF1 KD 06 and 46 (i) or IF1 KO (ii) HeLa cells, grown for 15 days in a phenol-free DMEM with 1% serum. Colonies are stained with 0.005% (w/v) crystal violet in water. Quantification on the right represents the mean total colony area per well (% of control) ± SEM.

Figure 4: Effects of IF1 on mitochondrial respiration and permeability transition



(A) Oxygen consumption rate (OCR) of control (C plko) and IF1 KD 06 (i) or control (CTR) and IF1 KO cells (ii). Mean OCR ± SEM is shown before (basal) or after treatment with oligomycin (oligo); carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) and antimycin A (AA). **(B)** Calcium retention capacity (CRC, % of control) in control (CTR) and IF1 KO permeabilized cells is shown. Measurements are taken during state 4 or state 3 respiration in the presence of succinate (upper panels, red) or glutamate/malate (lower panels, blue) to energize mitochondria. Where indicated, cells are grown in 2.5 mM glucose 24 h before measurements (24h LG) or treated with 5 µM MitoParaquat (MPQ). Calcium Green-5N is used to monitor mitochondrial Ca^{2+} uptake.

Conclusions

This study shows that the mitochondrial inhibitor IF1 might interact with an additional site on ATP synthase in HeLa tumor cells. Moreover, we demonstrate that IF1 is responsible for the desensitization of the permeability transition pore in this cell model, thereby avoiding cell death and allowing proliferation under stress conditions.