activity (14.4 ± 0.9 nmol/min.mg protein) sustained by reversed electron flow of the respiratory chain when succinate and ATP were added. This activity was inhibited by the addition of rotenone (88%), oligomycin (98%), antimycin (77%) and m-CCCP (93%). NO generation by ETPH-Mg^{2+} resulted 0.62 ± 0.03 nmol/min.mg protein in optimal experimental conditions (0.5 mM $MgCl_2$, 0.3 mM KCN). The mtNOS activity was still detectable (99%) in the absence of an exogenous electron donor (NADPH) suggesting that NO production could be supported by electrons derived from the respiratory chain. Rotenone inhibited mtNOS activity (86%) supported by reversed electron flow, but it did not inhibit the activity of isolated nNOS indicating that its inhibitory effect on NO production by ETPH-Mg^{2+} is due to an electron flow inhibition and not to a direct action on mtNOS structure. The dependence of mtNOS activity on metabolic state and membrane potential, and the physical interaction among mtNOS, cytochrome oxidase and complex I proteins, support the hypothesis that mtNOS could interact with complex I proteins using electrons derived from the respiratory chain for its enzymatic activity.

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6P10

Inhibition of complex I regulates the mitochondrial permeability transition through a phosphate-sensitive inhibitory site masked by cyclophilin D

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Inhibition of the mitochondrial permeability transition pore (PTP) has proved to be an effective strategy for preventing oxidative stress-induced cell death, and the pore represents a viable cellular target for drugs. Here, we report that inhibition of complex I by rotenone is more effective at PTP inhibition than cyclosporin A (CsA) in tissues that express low levels of the CsA mitochondrial target, cyclophilin D (CypD); and, conversely, that tissues in which rotenone does not affect the PTP are characterized by high levels of expression of CypD and sensitivity to CsA. Consistent with a regulatory role of complex I in the PTP-inhibiting effects of rotenone, the concentrations of the latter required for PTP inhibition precisely match those required to inhibit respiration; and a similar effect is seen with the antidiabetic drug metformin, which partially inhibits complex I. Remarkably (i) genetic ablation of CypD or its displacement with CsA restored PTP inhibition by rotenone in tissues that are otherwise resistant to its effects; and (ii) rotenone did not inhibit the PTP unless phosphate was present, in striking analogy with the phosphate requirement for the inhibitory effects of CsA. These results indicate that inhibition of complex I by rotenone or metformin and displacement of CypD by CsA affect the PTP through a common mechanism; and that cells can modulate their PTP response to complex I inhibition by modifying the expression of CypD, a finding that has major implications for pore modulation in vivo.

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6P11

The protease inhibitor SERPINB3 inhibits permeability transition pore through the regulation of mitochondrial Complex I ROS production

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SERPINB3 (SB3) is a member of the family of ov-serin-protease inhibitors overexpressed in epithelial and hepatic tumors. We have found that a fraction of SB3 is located in mitochondria of hepatoma cells. In order to understand whether the mitochondrial location of SB3 is somehow connected to biological traits relevant to the neoplastic phenotype, we have addressed the possibility that SB3 is part of the anti-apoptotic machinery that hallmarks most tumor types. In particular, inhibition of the mitochondrial permeability transition pore (PTP) constitutes a point of no-return in cell commitment to death, and its inhibition is believed to play a primary role in tumor cell survival and resistance to anticancer drugs.

We have found that stable transfection with either the human wild type SB3 or the protease-dead DH-SB3 confers protection to death induced in hepatoma cells either with the first-line chemotherapeutic Cisplatin or with the PTP inducer EM20-25. Treatment with the antioxidant compound N-acetyl cysteine could abrogate death induction, and SB3-expressing cells show lower ROS levels both in basal conditions and after EM20-25 treatment. Notably, we observe that SB3 co-immunoprecipitates with respiratory chain Complex I, one of the major sites of superoxide production in cells, and that this interaction results in inhibition of Complex I activity.

Taken together, these results indicate that SB3 protects hepatoma cells from death induced by oxidative stress through down-modulation of Complex I and the ensuing PTP desensitization, paving the way for the identification of novel anti-neoplastic strategies.

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6P12

Complex I deficiency associated to mitochondrial DNA mutations in type I endometrial carcinoma

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Endometrial carcinoma (EC) is a common neoplasia of the female genital tract. This cancer may be grouped into two subsets, namely