Busta n. 1

1. Purificazione di proteine ricombinanti da E. Coli
2. Il ruolo di Opa1 nelle criste mitocondriali
3. Il candidato indichi come ordinare in MS Excel i dati in ordine decrescente
4. Il candidato legga e traduca in Italiano il primo paragrafo del seguente abstract

JOURNAL

Phosphorylation of BCL-2 regulates ER Ca^{2+} homeostasis and apoptosis

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Phosphorylation of BCL-2 within an unstructured loop inhibits its antiapoptotic effect. We found that phosphorylated BCL-2 predominantly localized to the endoplasmic reticulum (ER) and tested whether phosphorylation would control its activity at this organelle, where Ca^{2+} dynamics serve as a critical control point for apoptosis. Phosphorylation greatly inhibits the ability of BCL-2 to lower $[Ca^{2+}]_{er}$ and protect against Ca^{2+}-dependent death stimuli. Cells expressing nonphosphorylatable BCL-2^{AAA} exhibited increased leak of Ca^{2+} from the ER and further diminished steady-state $[Ca^{2+}]_{er}$ stores when compared to cells expressing BCL-2^{wt}. Consequently, when BCL-2 is phosphorylated, Ca^{2+} discharge from the ER is increased, with a secondary increase in mitochondrial Ca^{2+} uptake. We also demonstrate that phosphorylation of BCL-2 inhibits its binding to proapoptotic family members. This inhibitory mechanism manifested at the ER, where phosphorylated BCL-2 was unable to bind proapoptotic members. $[Ca^{2+}]_{er}$ proved coordinate with the capacity of BCL-2 to bind proapoptotic BH3-only members, further integrating the apoptotic pathway and Ca^{2+} modulation. Unexpectedly, the regulation of ER Ca^{2+} dynamics is a principal avenue whereby BCL-2 phosphorylation alters susceptibility to apoptosis.

The EMBO Journal advance online publication, 4 March 2004; doi:10.1038/sj.emboj.7600104

Subject Categories: differentiation & death

Keywords: apoptosis; BCL-2; calcium; endoplasmic reticulum; phosphorylation

possess sequence conservation within four domains (BH1–4), and bind and sequester proapoptotic proteins. Proapoptotic BCL-2 family proteins can be subdivided into 'multidomain' proteins such as BAX and BAK, which display homology within BH1–3 domains, and the 'BH3-only' proteins, which only possess sequence homology within this amphipathic α-helix, which serves as the critical death domain. The ratio between the antiapoptotic and proapoptotic BCL-2 family members helps determine the susceptibility of cells to a death signal (Oltvai *et al*, 1993). Genetic and biochemical studies indicate that multidomain BAX and BAK function as a gateway to the intrinsic death pathway operating at both the mitochondria and endoplasmic reticulum (ER). The upstream BH3-only members respond to select death signals and subsequently trigger the conformational activation of BAX and BAK, inducing their intramembranous homo-oligomerization and permeabilization of the mitochondrial outer membrane (Wei *et al*, 2001). Released intermembranous proteins include cytochrome *c*, which complexes with Apaf 1 and caspase-9 to form a postmitochondrial apoptosome that activates effector caspases (Li *et al*, 1997). Conversely, BCL-2 can sequester activated BH3-only proteins, and thus inhibit the activation of BAX and BAK (Cheng *et al*, 2001).

In the absence of BAX and BAK, cells are resistant to a wide variety of death signals including agents that release Ca^{2+} from intracellular stores, such as the lipid second messengers arachidonic acid and C_2-ceramide as well as oxidative stress. BAX and BAK also localize to the ER, and cells deficient in BAX, BAK have reduced resting $[Ca^{2+}]_{er}$, which accounts for their resistance to Ca^{2+}-dependent death stimuli (Scorrano *et al*, 2003). Reciprocally, expression of the antiapoptotic protein BCL-2 protects cells from death by thapsigargin, an irreversible inhibitor of the sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase (SERCA) responsible for uptake of Ca^{2+} from the cytosol into the ER lumen (Lam *et al*, 1994). BCL-2 is also found at the ER (in addition to mitochondria and nuclear membrane), and its overexpression resulted in reduced $[Ca^{2+}]$ accompanied by an increased

Busta n. 2

5. Induzione di proteine ricombinanti in colture di E. coli trasformate
6. Le principali proteine di fusione mitocondriale.
7. Il candidato indichi come impostare una funzione di addizione in una cella di MS Excel
8. Il candidato legga e traduca in Italiano il primo paragrafo del seguente abstract

Research Article
PINK1 affects Complex I activity in mitochondria

EMBO
Molecular Medicine

Parkinson's disease mutations in PINK1 result in decreased Complex I activity and deficient synaptic function

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Keywords: Complex I; mitochondrial dysfunction; Parkinson's disease; reserve pool deficit

DOI: 10.1002/emmm.200900006

Received: January 7, 2009
Accepted: January 14, 2009

Mutations of the mitochondrial PTEN (phosphatase and tensin homologue)-induced kinase1 (PINK1) are important causes of recessive Parkinson disease (PD). Studies on loss of function and overexpression implicate PINK1 in apoptosis, abnormal mitochondrial morphology, impaired dopamine release and motor deficits. However, the fundamental mechanism underlying these various phenotypes remains to be clarified. Using fruit fly and mouse models we show that PINK1 deficiency or clinical mutations impact on the function of Complex I of the mitochondrial respiratory chain, resulting in mitochondrial depolarization and increased sensitivity to apoptotic stress in mammalian cells and tissues. In *Drosophila* neurons, PINK1 deficiency affects synaptic function, as the reserve pool of synaptic vesicles is not mobilized during rapid stimulation. The fundamental importance of PINK1 for energy maintenance under increased demand is further corroborated as this deficit can be rescued by adding ATP to the synapse. The clinical relevance of our observations is demonstrated by the fact that human wild type PINK1, but not PINK1 containing clinical mutations, can rescue Complex I deficiency. Our work suggests that Complex I deficiency underlies, at least partially, the pathogenesis of this hereditary form of PD. As Complex I dysfunction is also implicated in sporadic PD, a convergence of genetic and environmental causes of PD on a similar mitochondrial molecular mechanism appears to emerge.