Mitochondria in Cell Life and Death

3363-Pos Board B518

A Step Forward in Understanding the Mechanism of VDAC Voltage-Gating

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The voltage-dependent anion channel (VDAC) governs the exchange of ions and metabolites between the mitochondria and the rest of the cell. In its open state VDAC exhibits high conductance and selectivity for anions that facilitates the passage of ADP, ATP, and other metabolites. At increased voltages (>30mV) VDAC switches to lower conducting states, termed as ''closed'' states. Closed states are cation-selective and impermeable for ATP. The voltage-induced transition from the open to closed states is referred to as voltage-gating. Although it is well established that VDAC voltage-gating involves large structural rearrangements, the precise molecular mechanism of this process is still under debate. We investigated VDAC voltage-gating by systematically titrating VDAC charge residues and by using thermodynamic and kinetic approaches to study opening and closing of the channel. All the models proposed so far agree that N-terminal region plays a key role in VDAC voltage-gating. According to the original idea, the N-terminal region is a part of a mobile voltage sensor domain, which slides in and out of the channel lumen in response to the applied voltage. The alternative models consider independent movement of the N-terminal region upon gating. In order to test the role of VDAC N-terminal region in voltage-gating, we engineered a double Cys mutant of murine VDAC1 that cross-links the α-helix to the β-strand 11 of the pore wall. The cross-linked VDAC1 reconstituted into planar lipid membranes exhibited typical voltage gating, which suggests that the N-terminal α-helix is located inside the pore of VDAC in the open state and remains associated with the pore wall during voltage gating. Our findings support a model where β-barrel is not rigid but undergoes a conformational change that leads to a partial constriction upon transition to the closed states.

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Novel Mechanism of Mitochondrial Respiration Control through Competition between Hexokinase-2 and Tubulin for VDAC Binding

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The voltage dependent anion channel (VDAC) is involved in regulation of metabolite flux across the mitochondrial outer membrane (MOM). Hexokinse II (HK2) is known to bind the MOM where it phosphorylates glucose into glucose-6-phosphate (G6P). High expression of HXK2 is a common phenotype of many cancers, where its concentration can be 200 times of that in noncancerous cells, and is implicated in the Warburg effect. It is believed that VDAC serves as a HXK2 binding site in the MOM. The 15 amino acid N-terminal sequence of HXK2 is responsible for mitochondrial binding and, when conjugated to TAT (TAT-HK2), binds to mitochondria with higher affinity than native HXK2, causing HXK2 detachment. We have previously found that dimeric tubulin reversibly binds and partially blocks VDAC inhibiting metabolite flux across the MOM. Now we show that this binding can be attenuated by TAT-HXK2 peptide as well as by full length HXK2. We have found that TAT-HXK2 and recombinant full length HXK2 inhibit tubulin blockage of VDAC reconstituted into planar lipid bilayers without altering characteristic channel properties such as single channel conductance and selectivity. Binding of HXK2 to VDAC is verified by the generation of high-frequency excess current noise without channel closure. HXK2 bound to VDAC prevents subsequent tubulin binding, but only when added before tubulin, and inhibits tubulin-induced VDAC blockage in a dose dependent manner. Moreover, G6P, which is known to cause HXK2 detachment from the MOM, fully reverses the inhibition of tubulin-VDAC binding. This suggests that HXK2 detachment from VDAC (and hence the MOM) is caused by a HXK2 conformational change upon G6P binding. Thus we propose a novel mechanism of mitochondrial respiration control in cancer cells through the competition between HXK2 and tubulin for VDAC binding.

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Reprogramming of Mitochondrial Ca^{2+} Handling in MICU1-Deficient HeLa Cells

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Recent studies have revealed MCU as the pore forming domain and MICU1 as a critical Ca^{2+}-sensitive regulator of the mitochondrial Ca^{2+} uniporter. However, the exact role of MICU1 in Ca^{2+} transport remains to be addressed. Our previous studies showed that prolonged down-regulation of MICU1 in HeLa cells (shMICU1) promotes mitochondrial Ca^{2+} uptake at low $[Ca^{2+}]$, which unexpectedly, fails to effectively increase matrix $[Ca^{2+}]$. To determine the source of discrepancy between the mitochondrial Ca^{2+} uptake and the matrix $[Ca^{2+}]$ phenotypes, first we simultaneously monitored ruthenium red-sensitive clearance of added Ca^{2+} from the cytoplasm and the corresponding matrix $[Ca^{2+}]$ response in permeabilized shMICU1 cells. Under conditions of similar cytoplasmic Ca^{2+} clearance, shMICU1 cells showed a smaller matrix $[Ca^{2+}]$ increase than the control, indicating enhanced buffering of Ca^{2+} in the matrix. Enhanced Ca^{2+} binding in the matrix likely reflects alkalinization and enhanced phosphate transport. To test if upregulation of Ca^{2+} buffering is directly linked to MICU1 depletion, we also assessed mitochondrial Ca^{2+} handling after 72hr silencing of MICU1 (siMICU1). In siMICU1cells both mitochondrial Ca^{2+} uptake and the matrix $[Ca^{2+}]$ rise were effectively stimulated at low Ca^{2+} levels. Thus, upregulation of matrix Ca^{2+} buffering seems to be a component of an adaptive response to sensitization of mitochondrial Ca^{2+} uptake in shMICU1. The adaptive response is likely to be important to attenuate some MICU1-depletion induced cellular impairments that we found to manifest as attenuated mitochondrial ATP production and cell proliferation.

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MICU1-dependent Threshold and Cooperativity of Mitochondrial Ca^{2+} Uptake in the Liver

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Recent studies have revealed MCU as the pore forming domain and MICU1 as a critical Ca^{2+}-sensitive regulator of the mitochondrial Ca^{2+} uniporter. However, the mechanism of the complex Ca^{2+} dependence of the uniporter activity remains elusive. Our previous studies showed that prolonged down-regulation of MICU1 in HeLa cells causes lower threshold and decreased cooperativity of mitochondrial Ca^{2+} uptake. To study the functional significance of the effects of MICU1 we used hepatocytes harvested from the liver of mice exposed to in vivo silencing (4 weeks). Silencing of MICU1 or MCU resulted in >80% decrease in their respective mRNA levels. Silencing of MICU1 caused a leftward-shifted dose response and decreased cooperativity of mitochondrial Ca^{2+} uptake in both permeabilized and intact hepatocytes. By contrast, silencing of MCU resulted in slower Ca^{2+} uptake in the entire range of Ca^{2+} concentrations without change in threshold. Mitochondrial respiration and cellular ATP content were unaffected in media containing both glycolytic and mitochondrial fuels in either MICU1 or MCU-deficient hepatocytes. However, silencing of MICU1 caused an augmented loss of ATP when the cells were confined to oxidative metabolism and an enhanced sensitivity to mitochondrial Ca^{2+} overload and permeabilization. During stimulation with vasopressin, a Ca^{2+} mobilizing hormone, both MICU1 and MCU-deficient cells displayed an attenuated mitochondrial matrix $[Ca^{2+}]$ increase and stimulation of respiration. Collectively, these results show that keeping the gate of MCU closed by MICU1 at low $[Ca^{2+}]$ is required to maintain healthy mitochondria, and MICU1-mediated control of MCU (cooperativity?) is required to support the propagation of short-lasting calcium spikes and oscillations to the mitochondria and the ensuing physiological stimulation of oxidative metabolism.

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Targeting Mcl-1 and Bak as a Therapeutic Tool to Selectively Induce Apoptosis in Hepaptocellualr Carcinoma

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In this study we seek to identify novel drug targets to induce apoptosis in hepatocellular carcinoma (HCC) cells thus providing opportunities to develop novel treatments to improve the prognosis of liver cancer patients. Several apoptotic pathways are mediated through cleavage of Bid (a BH3 domain-only, pro-apoptotic protein) to produce truncated Bid (tBid). tBid induces apoptosis through induction of outer mitochondrial membrane (OMM) permeabilization by activation of pro-apoptotic Bak that resides in the OMM or cytoplasmic Bax. Due to its localization, Bak can mediate the early phase of the response to tBid. We have recently demonstrated that OMM targeting of Bak and the sensitivity to tBid-induced OMM permeabilization is dependent on the expression of