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| • 計畫中文名稱 | 以蛋白質體學分析 GSK-3beta 結合蛋白---探索粒線體內的訊息傳遞機制(I) | | |
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| • 英文關鍵字 | GSK-3?, intramitochondrial signal transduction, electron transport chain, apoptosis. | | |
| • 中文摘要 | 粒線體主管細胞的代謝及能量的產生、並和細胞的生長、凋亡息息相關。肝醣合成.激.-3 芻洶(GSK-3 芻胝可傳遞多種訊息並影響粒線體的代謝及細胞之凋亡，但 GSK-3 芻如何調控、整合粒線體內蛋白質的活性仍不清楚。由於粒線體內大部份蛋白質均以複合體存在，很可能傳遞訊息的.也和相關的蛋白形成訊息複合體(signalsome)以整合其功能。本研究以免疫沉澱/二維電泳 /MALDI-TOF 分析 GSK-3 芻洶結合蛋白之本質及其胺基酸序列，初步發現 GSK-3 芻洶可和調控 TCA cycle、電子傳遞鏈、ATP 產生及細胞凋亡的關鍵蛋白結合。本計畫擬進一步探討 GSK-3 芻洶如何影響粒線體的功能。我們的研究重點如下： (一) 以免疫沉澱法探討 GSK-3 芻洶是否與粒線體蛋白形成複合體，並以蛋白質體學技術分析這些 GSK-3 芻結合蛋白的胺基酸序列,以及粒線體內之 GSK-3 芻是否受細胞外訊息的調控。(二) 以[32P]正磷酸鹽標定或以 Pro-Q Diamond 進行二維電泳膠之螢光染色，再配合 MALDI-TOF 探討粒線體內之 GSK-3 芻是否可以磷酸化粒線體內之受質，並決定 GSK-3 芻磷酸化洶 substrates 之磷酸化位置的胺基酸順序。 (三) 探討 GSK-3 芻的活性是否透過 PDH 影響 glucose oxidation、並調控後續之 TCA cycle、電子傳遞鏈複合體的活性、粒線體膜電位(ΔΨm)、及 ATP 的產生。 (四) 探討 GSK-3 芻的活性是否影響 VDAC、prohibitin 及 cofilin 的磷酸化並近一步影響 Bcl-2 家族蛋白質的表現和磷酸化、細胞週期之進行及後續的凋亡分子之釋出和 caspase cascade。 | | |

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| • 英文摘要 | Mitochondria are involved in metabolism, energy production, redox balance, and apoptosis. Because most of the mitochondrial enzymes reside in the matrix of mitochondria, regulation of these mitochondrial proteins requires an integrated system of signals that enter mitochondria according to external stimuli. Recent evidence suggests that protein kinases /phosphatases can be translocated to mitochondria in response to external signals. Glycogen synthase kinase 3-芻 (GSK-3 芻胝 has been shown to be located in mitochondria and represents a key regulator of cell metabolism and apoptosis. Several lines of evidence suggest that signals of cytoprotective agents are convergent on GSK-3 芻貞洶洶 However, very little is known regarding the mechanisms by which GSK-3 芻 regulates these mitochondrial proteins across the outer and inner membranes. Because GSK-3 芻 may play a pivotal role in the coordination of mitochondrial functions which allows adaptations to the environment and responses to external stimuli, detail understanding of intra-mitochondrial signaling mechanisms of GSK-3 芻 is an important and timely issue. We hypothesized that like most other mitochondrial proteins, mitochondrial GSK-3 芻 exists as part of a complex. To molecularly identify the GSK-3 芻 associated complex and define their roles in mitochondrial function, GSK-3 芻洶 associated proteins undergo immuno-pull down processes and their identities are characterized by proteomic approaches including 2D PAGE and MALDI-TOF MS analysis. Our preliminary data revealed that GSK-3 芻洶 is associated with key elements that regulate mitochondrial metabolism and cell fates. These results suggest that GSK-3 芻 may play a regulatory role in coordinating glucose oxidation, TCA cycle, electron transport, ATP production, and apoptosis in mitochondria. Data obtain from the present proposal should provide more insights into the mechanisms by which intra-mitochondrial signals are regulated. Our specific aims are: Specific Aim (I): To investigate whether GSK-3 芻 and its associated proteins form a signal complex in mitochondria, and to determine whether the GSK-3 芻洶 containing complex is regulated by PI 3-kinase/PDK/Akt pathway. Specific Aim (II): To examine whether GSK-3 芻 would phosphorylate mitochondrial proteins by the [32P]orthophosphate incorporation assay or by phosphoproteomic staining of 2D PAGE or 2D BN-PAGE with ProQ Diamond/Sypro Ruby followed by MALDI-TOF MS analysis, and identify possible phosphorylation sites. Specific Aim (III): To determine whether GSK-3 芻洶 would regulate cell metabolism, and bioenergetics through pyruvate dehydrogenase, electron transport chain, and ATP synthase. Specific Aim (IV): To determine whether GSK-3 芻洶 would regulate cell cycle progression and apoptosis through VDAC, prohibitin, and cofilin. To investigate whether these GSK-3 芻 associated proteins regulate cell cycle regulatory proteins, Bcl-2 family proteins, release of apoptogenic factors and caspase cascade. |