視網酸 X 受體在人類星狀膠質瘤細胞之粒線體生合成角色之研究

Role of RXR-α in Mitochondrial Biogenesis of Human U87-MG Astrocytoma Cells

中文摘要

視網酸 X 受體(RXR-α)是核接受器超級家族的其中一員，會與其它的接受器，如 peroxisome proliferator activated receptor (PPAR), retinoic acid receptor (RAR), thyroid receptor, and vitamin D receptor (VDR)等形成異質雙體而結合到基因上進行轉錄調節。2003 年 Casas 等學者研究發現在粒線體基質中發現一個 44 kDa 截短形式的 RXR-α (mtRXR-α)；本實驗利用 RXR-α表現載體(RXR-α expression vectors)爲了要更明顯的觀察 RXR-α 進入粒線體的現象。目前 9-cis retinoic acid 已被鑑定爲 RXR 之配體(ligand)，有許多研究指出 retinoids 可以抑制細胞的增生及促進細胞的分化；A23187 爲一種鈣離子載體(calcium ionophore)，在先前的研究發現可促進細胞內鈣離子增加，當細胞內鈣離子增加就會誘導鈣激蛋白酶(calpain)活化，在 1996 年 Nishiwaki 等學者發現 calpain 可以將 RXR-α 截成 44kDa。因此，本實驗之目的爲探討 RXR-α蛋白質過度表現(RXR-α overexpression)後對於粒線體生合成的影響爲何。首先利用細胞內生性的 calpain 將 RXR-α截短後送入粒線體，投予 A23187 發現會增加細胞內的鈣離子以及 calpain 活性。利用 Real-Time PCR 及西方點墨法分別分析細胞中粒線體 mRNA 和蛋白質的表現量以評估粒線體生合成之狀況，結果顯示轉染 RXR-α再投予 A23187 以及 9-cis retinoic acid 並不影響細胞轉譯及轉錄作用。以共軛焦顯微鏡觀察 RXR-α進入粒線體的情形，結果發現 RXR-α過量表現後投予 A23187 以及投予 1 μM, 5 μM 以及 25 μM 的 9-cis retinoic acid 可使 RXR-α進入粒線體。使用流式分析儀分析粒線體膜電位(ΔΨ)和細胞凋亡程度，結果顯示 A23187 也會促進粒線體膜電位滲漏及細胞凋亡的程度。因此由本實驗推論 RXR-α並不參與細胞粒線體轉譯及轉錄作用，但投予 9-cis retinoic acid 在氧化磷酸化系統上(OXPHOS)的蛋白質都有降低的趨勢，由此推論當細胞內 RXR-α過量表現(overexpression)時，9-cis retinoic acid 可能會促進配體活化反應(ligand-activation)，這個機制在未來的研究也將深入探討。

英文摘要

The retinoid X receptor alpha (RXR-α) is one of the nuclear receptor superfamily members that regulates transcription of target genes through heterodimerization with several partners, including peroxisome proliferator activated receptor (PPAR), retinoic acid receptor (RAR), thyroid receptor, and vitamin D receptor (VDR). In 2003, Casas discovered truncated RXR-α in mitochondria and called mtRXR-α. 9-cis

retinoic acid has been identified to be the ligand of RXR and more studies have shown that retinoids inhibit cell proliferation but increase cell differentiation. A23187, a calcium ionophore, be discovered could increase cellular calcium concentration and induce calpain activation. In order to study the mechanism of RXR-α translocation and regulation of mitochondrial biogenesis and to investigate the effects of mitochondrial biogenesis on neuron degeneration, we created a cellular studied model with RXR-α overexpression on U87-MG astrocytoma cells followed by A23187 treatment. The results showed RXR-α protein expression 2.5 folds higher than control when RXR-α overexpression in U87-MG cells. The levels of mRNA and protein of respiratory enzyme subunits from both of mtDNA-encoded genes and nuclear DNA-encoded genes were determined by real-time PCR and western blot, respectively. There were no differences of transcription and translation levels of cells giving A23187 and 9-cis retinoic acid after RXR-α overexpression. However, cells with RXR-α overexpression followed by 1 μM and 5 μM 9-cis retinoic acid treatment showed lower mtDNA encoded protein ND6 compare to control. The proportions are 0.798±0.239 and 0.42±0.141 when compared to control, respectively. The leves of RXR-α translocation into mitochondria in cells given A23187 and 1 μM, 5 μM and 25 μM 9-cis retinoic acid after transfect RXR-α were observed by confocal microscope. The mitochondrial membrane protential and apoptosis level analyzed by flow cytometry and showed A23187 treatment could induce mitochondrial membrane protential loss. The ratios are 21.07±1.544, 6.81±0.327, 9.53±0.036 in the control, in the Mock+A23187, and RXR-α +A23187 groups, respectively. The A23187 also induced cell apoptosis, cell early apoptosis ratios are 3.16±0.28%, 15.66±4.86%, 11.49±0.16%, 12.17±0.92% in control, control+A23187, Mock+A23187 and RXR-α+A23187 groups, respectivtly. In this study, results indicated that RXR-α may not participate in mitochondrial transcription and translation. However, given 9-cis retinoic acid could decreased the protein levels of oxidative phosphorylation system including complex V alpha, COX I and the COX II. These studies suggested that cellular RXR-α overexpression followed given 9-cis retinoic acid could induce ligand-activation where RXR-α translocated into mitochondria. The same results are determined in cells incubated with A23187.