摘要

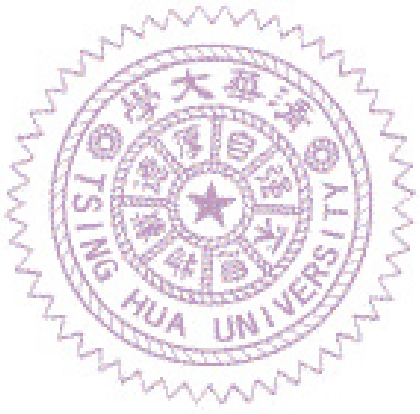
人類粒線體(mitochondria)第一蛋白複合體(complex I)次單元的缺陷會引發多種嚴重疾病，例如賴博氏遺傳性視覺神經症(Leber hereditary optic neuropathy)和萊氏症候群(Leigh syndrome)。由於目前的傳統療法對於大多數遺傳性粒線體疾病僅能達到緩和病情的效果，有鑑於此，發展更確實與便利的新療法是迫切需要的。

先前研究指出，將蛋白和 HIV 之穿膜胜肽 TAT 連接後之重組蛋白能夠不受細胞膜的限制進入細胞內，並保持原蛋白的生化活性。本研究旨在結合 TAT 與粒線體領導序列(leader sequence)之功能，期望此結合能夠成功將粒線體蛋白由細胞外運輸至粒線體內以彌補原先蛋白之缺陷。由於在人類粒線體第一蛋白複合體次單元的缺陷中，最早被發現與引發萊氏症候群有直接關係的是 NADH dehydrogenase (ubiquinone) Fe-S protein 8 (NDUFS8)，因此在本篇論文中，我們以 NDUFS8 為範例進行研究，目標是開發用於人類粒線體第一蛋白複合體缺陷的新治療方法。

目前在本論文的研究結果中顯示，不論將 TAT 放在 NDUFS8 的 N 或 C 端(TAT-NDUFS8 or NDUFS8-TAT)都能夠成功的將蛋白從細胞外運送到粒線體中，此外這兩種蛋白也都能夠被轉化成為成熟蛋白(mature protein)。研究中也發現 TAT-NDUFS8 和 NDUFS8-TAT 進入粒線體的方式不是經由已知的粒線體外膜轉移蛋白(translocase of the outer membrane)/粒線體內膜轉移蛋白(translocase of the inner membrane)所調控的途徑。另外，為了模擬 NDUFS8 蛋白缺陷以及加入 TAT-NDUFS8 後的回復狀況，我們利用本實驗室所建構之大量抑制 NDUFS8 表現量的細胞株(shRNA-C3)進行功能分析實驗。結果顯示加入 TAT-NDUFS8 後能夠完全回復 shRNA-C3 細胞株之人類粒線體第一蛋白複合體中 NDUFS8 的蛋白組成。此外，shRNA-C3 細胞株在粒線體第一蛋白複合體活性和耗氧量實驗中分別提升 30%和 79%的功能活性。另一方面，我們發現 TAT-NDUFS8 進入細胞之

後，能夠促使核內體(endosome)靠近粒線體並形成 endosomes-mitochondria juxtaposition 的現象，我們推測此現象是 TAT-NDUFS8 進入粒線體的途徑，此現象發生的同時，TAT-NDUFS8 能夠從核內體被傳送到粒線體之中。

總結來說，在本論文研究中，我們提出了 TAT-NDUFS8 從細胞外到細胞內進入粒線體的可能途徑，此外，歸因於功能分析回復的實驗結果，本論文研究也能夠作為一個成功的範例以應用於未來粒線體疾病治療方法上。



Abstract

Defects in subunits of mitochondrial complex I are associated with severe diseases, including Leber hereditary optic neuropathy and Leigh syndrome. However, to date, conventional treatment for the majority of genetic-based mitochondrial diseases can only be palliative. Therefore, developing a reliable and convenient treatment approach is in an urgent need.

Fusion of the protein transduction domain (PTD) of HIV-1 transactivator of transcription (TAT) with proteins has been demonstrated to bring proteins into cells by crossing plasma membranes while retaining the biological activity of proteins. In this study, we tried to apply the protein transduction concept of TAT with the mitochondrial-targeting capability of the specific leader sequence to generate a therapeutic protein delivery system which can specifically carry target proteins into mitochondria. Here, NADH dehydrogenase (ubiquinone) Fe-S protein 8 (NDUFS8), the first complex I subunit linked to Leigh syndrome, was used as the model subunit to test our specific aims, with a hope that this newly developed method could become a novel treatment for complex I deficiency.

Currently, our findings showed that both exogenously produced TAT-NDUFS8 and NDUFS8-TAT could be delivered into mitochondria and processed into the mature forms of NDUFS8. We also showed that the mechanism of TAT-NDUFS8 and NDUFS8-TAT entering mitochondria is not through the well-recognized translocase of the outer membrane (Tom) /translocase of the inner membrane (Tim) mitochondrial import pathway. Furthermore, in order to mimic the rescue of complex I deficiency, a NDUFS8 expression knockdown cell line (shRNA-C3) was used in functional analyses as the therapeutic model. Treating with TAT-NDUFS8 could completely restore the assembly of complex I in shRNA-C3 cells, and the respiratory rate of these

NDUFS8 knockdown cells was also increased about 31% and 79% in the in-gel activity assay and oxygen consumption assay, respectively. Moreover, we demonstrated that when cells were cultured with TAT-NDUFS8, endosomes were found to be retrieved in close proximity to mitochondria, indicating that TAT-NDUFS8 may enter mitochondria via the endosomes-mitochondria juxtaposition.

In conclusion, our findings provide both the possible mechanism of TAT-NDUFS8 entering mitochondria and the model for therapeutic treatment of mitochondrial disorders.