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• 計畫中文名稱	活化的 Notch 受體結合蛋白及其下游基因調控之研究
• 計畫英文名稱	Study of the Activated Notch receptor-Associating Proteins and Their Regulation on Target Genes
• 主管機關	行政院國家科學委員會
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• 研究人員	葉添順; 曾銘仁 Yeh, Tien-Shun; Tseng, Min-Jen
• 中文關鍵字	紫杉醇
• 英文關鍵字	Taxol; N1IC; Notch1; .beta.II-tubulin; CBF1
• 中文摘要	Notch 接受子對於生物的發育過程扮演很重要的調控角色。Notch1 接受子的活化是經由 Notch1 接受子細胞外區域與鄰近細胞的 ligand 結合而引發，造成 Notch1 接受子經過蛋白斷而釋放出 Notch1 接受子細胞內區域 (Notch1 intracellular domain, N1IC)。N1IC 移動至細胞核中與轉因子 CBF1 結合，活化 Notch 訊息傳遞的下游基因。本實驗室經由 yeast two-hybrid 方法尋找與 N1IC 結合的細胞內蛋白，發現 βII-tubulin 與 N1IC 在細胞內結合。本研究工作先在 K562 以及 HeLa 細胞株的細胞核偵測到 βII-tubulin 蛋白，並且進一步證實在 HeLa 細胞株的細胞核中，細胞核內的 βII-tubulin 會與 N1IC 結合。以 taxol 處 K562 以及 HeLa 細胞株後，經由 CBF1 媒介的 Notch1 訊息傳遞會被活化；以 colchicine 處細胞則影響經由 CBF1 媒介的 Notch1 訊息傳遞。此外，以 taxol 處 K562 以及 HeLa 細胞株，會造成細胞核內 βII-tubulin 及 α-tubulin 蛋白的上升，但是 以 colchicine 處細胞則影響細胞核中的 βII-tubulin 及 α-tubulin 蛋白含。而且經由 taxol 處而進入細胞核的 βII-tubulin 及 α-tubulin，48 小時後仍穩定存在細胞核中。以上結果顯示細胞核內 βII-tubulin 與 Notch-1 接受子在癌細胞細胞核內有結合關係，並且可以調控 Notch1 訊息傳遞。
• 英文摘要	The Notch signal pathway plays important roles in proliferation, apoptosis, and differentiation. Abnormalities in Notch signaling are linked to many human diseases. After ligand binding, Notch signaling is activated through the cleavage of Notch receptors to release and translocate the Notch intracellular domain into the nucleus. The Notch1 receptor intracellular domain (N1IC), the activated form of the Notch1 receptor, can modulate downstream target genes via both CBF1-dependent and -independent pathways. To further dissect the Notch1 signaling pathway, we screened the N1IC-associated proteins using a yeast two-hybrid system and identified nuclear .beta.II-tubulin as a candidate for the N1IC-associated proteins. It was suggested that the presence

of .beta.II-tubulin in nuclei might be correlated with the cancerous state of cells. However, the function of .beta.II-tubulin locating in the nucleus is still unknown. Herein, we show that the complex of .alpha.- and .beta.II-tubulin is associated with N1IC in cancer cells by a co-immunoprecipitation analysis. The ankyrin (ANK) domain of the Notch1 receptor alone was sufficient to associate with .beta.II-tubulin. Furthermore, .alpha.- and .beta.II-tubulin were localized in the nucleus and formed a complex with N1IC. Treatment with taxol increased the amounts of nuclear .alpha.- and .beta.II-tubulin in K562 and HeLa cells and promoted the CBF1-dependent transactivation activity of N1IC. We also demonstrate that nuclear .beta.II-tubulin was bound on the CBF1-response elements via the association with N1IC. These results suggest that nuclear .beta.II-tubulin can modulate Notch signaling through interaction with N1IC in cancer cells.