探討活化型態的 Notch1 受體與其結合因子之結合生物功能

Study on the biological function of the interaction between activated Notch1 receptor and its binding factors

中文摘要

Notch 是個演化上高度保留，穿過細胞膜一次的受體蛋白，最早在果蠅中被發現。已知哺乳類的 Notch 受體蛋白分爲 Notch1?4，位於細胞表面，被認爲可調控基因的轉錄、細胞的增生、分化及早期細胞命運的決定，而 Notch 受體蛋白異常的活化會造成某些疾病的發生。當相鄰細胞表現的 ligand 結合在 Notch 受體蛋白的細胞外區域時，Notch 訊號傳遞系統就被活化，使得整個細胞內區域在靠近內膜處斷裂，而形成活化型 Notch (Notch IC, 細胞內區域)。此活化型 Notch 會轉移至細胞核中，並且和一些可與 DNA 結合的轉錄因子，如：RBP-Jκ/CBF1，以及其他細胞因子結合，進一步調控其標的基因的表現。由於這些參與的因子，對於 Notch 訊息傳遞路徑影響甚巨。因此，爲了進一步探討 Notch 訊息傳遞路徑，本論文首要目標著重於探索參與 Notch1 下游訊息傳遞路徑仍未被發現的結合因子，及初步分析因結合所產生的生物功能。

在實驗的第一部分，我們利用 K562、Jurkat、SUP-T1 等人類細胞株證實了一個轉錄因子 YY1 與 Notch1 IC (N1IC)之間的結合關係，同時也利用 GST pull-down assay，找出 N1IC 和 YY1 相互之間的結合區域，並藉由 Sucrose gradient analysis 證實細胞核內 N1IC 及 YY1 以大的 complex 結合形式存在，本論文也證實 YY1 藉由 N1IC 與 CBF1 的直接作用結合在 Wild type CBF1-response elements 上，YY1 會抑制 CBF1 媒介之 Notch1 訊息傳遞。同時，應用 ChIP 嘗試尋找未知受人類 Notch1 訊息傳遞所調控的下游基因。綜觀上述實驗結果，我們得知 YY1 藉由與 N1IC 結合，形成高分子量的複合體調控 Notch 1 訊息傳遞。

在實驗的第二部分，延續之前本實驗室已證實 N1IC 與 b2-tubulin 之間相互結合的結果，應用上述細胞分析 N1IC 與 b2-tubulin 之間的結合生物功能。實驗結果證實 N1IC 與 b2-tubulin 之間的結合是同時發生於細胞核及細胞質的，而應用 RNA interference 分析及細胞處理 taxol 的實驗結果更進一步證實 b2-tubulin 可能抑制經 CBF1 媒介之 Notch1 訊息傳遞。

英文摘要

Notch receptors are evolutionally highly conserved and play important roles in modulating cell fate decisions throughout the development from invertebrate to vertebrate species. Four homologues of Notch receptors have been identified in human and aberrant Notch signaling is associated with a number of human diseases. There is a consensus model of Notch signaling pathway containing multiple proteolytic steps. After ligand binding, Notch receptors are cleaved to release the

intracellular domains of Notch receptors. The intracellular domains, the activated form of Notch receptors, are then translocated into nuclei and interact with other transcriptional machineries to regulate the expression of cellular genes.

To dissect the molecular mechanisms of Notch signaling, the cellular targets interact with Notch 1 receptor intracellular domain (N1IC) were screened. In this study, we found that endogenous transcription factor Ying Yang 1 (YY1) was associated with exogenous N1IC in human K562 erythroleukemic cells. The ankyrin domain of N1IC and zinc finger domains of YY1 were essential for the association of N1IC and YY1 by the pull-down assay of GST fusion proteins. Furthermore, both YY1 and N1IC were present in a large complex of the nucleus to suppress the luciferase reporter activity trans-activated by Notch signaling. The transcription factor YY1 indirectly regulated the transcriptional activity of the wild-type CBF1-response elements via the direct interaction of N1IC and CBF1. We also demonstrated the association between endogenous N1IC and intrinsic YY1 in human Jurkat cells and T-cell acute lymphoblastic leukemia cells. Additionally, we identified the cellular target genes modulated by the high-molecular-weight Notch complex by chromatin immunoprecipitation. Taken together, these results indicate transcription factor YY1 may modulate Notch signaling via association with the high-molecular-weight Notch complex.

We also demonstrated that the microtubule component 2-tubulin was associated with exogenous and endogenous N1IC in cells, Further study showed that N1IC and 2-tubulin associated in both nucleus and cytosol. The expression of 2-tubulin was inhibited by RNA interference and the nuclear 2-tubulin was depleted by the treatment of taxol to investigate the roles of 2-tubulin in Notch1 signaling pathway. The results suggested that 2-tubulin suppresses the luciferase reporter activity trans-activated by Notch1 signaling.