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• 中文關鍵字	Notch 受體
• 英文關鍵字	Notch receptor
• 中文摘要	大量表現 Notch1 接受體細胞內區域會抑制 K562 細胞的增生。 表現 HA-N1IC 融合蛋白的 K562 細胞株累積的細胞數量，低於控制組的細胞數量，因此 HA-N1IC 融合蛋白的表現會抑制 K562 細胞的增生。 Notch1 接受體細胞內區域增加 K562 細胞在 G1 時期的比率，而且經由 Taxol 處理之後，細胞週期停止在 G2/M 時期。 N1IC 增加在 G1 時期的細胞比率，從 37%增加到 44%，並且減少在 G2/M 時期的細胞比例，從 63%降到 56%，因此，這些資料可以證明出的確可以輕微的將 K562 細胞停止在 G1 時期。細胞數目會因爲 N1IC 的存在而受到抑制。而這樣的結果與上述的實驗所得到的結果有一致。除此之外，當給予 3.5 mM Taxol 之後，無論 N1IC 是否存在，大多數的 K562 細胞會停止在 G2/M 時期。 在生物體內，Notch 接受體細胞內區域及 Taxol 會抑制 K562 腫瘤細胞的生長 在接種腫瘤細胞後第 15 天，可以觀察到表現 HA-N1IC 融合蛋白之 K562 細胞株，其腫瘤大小跟對照組相較之下減少了 19%。在以 3.5 mM Taxol 處理 24 小時後，表現 HA-N1IC 融合蛋白之 K562 細胞株與對照組細胞皆不再生長。如同先前報導指出，Taxol 會使細胞核內的 bII-tubulin 的量增加，並驅使 N1IC 的 CBF1-dependent 之轉活化能力提高。然而，由 Taxol 所誘導核內 bII-tubulin 量之增加，在此並不會驅使裸鼠的腫瘤形成。在相同濃度(3.5 mM)處理下，Taxol 也可使細胞停留在 G2/M 時期並抑制腫瘤生長。這些結果顯示：在生物體內 N1IC 與 Taxol 均可制止 K562 細胞週期，並且抑制腫瘤細胞的生長。 使用 small interfering RNA (siRNA)方法，降低 bII-tubulin 蛋白質的表現後，藉由 Notch1 細胞內區域所活化的 CBF1 dependent 報導基因活性會被抑制。 在轉染了 bII-tubulin 蛋白 siRNA 表現質體 (pSilencer 2.1-U6-1431) 的細胞萃取液中，觀察到 FLAG-NLS-bII-tubulin 融合蛋白的表現量減少的情形，然而在同時轉染了另一個 siRNA 表現質體 (pSliencer 3.1-H1-105) 或是對照組質體 (pSliencer 2.1-U6 neo) 的情形下，皆不影響 bII-tubulin 蛋白的表現。由於細胞骨架相關蛋白質含量非常豐富，所以內生性的 bII-tubulin 蛋白的數量無法利用暫時性轉染 bII-tubulin 蛋白的 siRNA 表現質體 pSilencer 2.1-U6-1431 的方法，將內生性的 bII-tubulin 蛋白之表現全部抑制(data not shown)。 在 HeLa 細胞株中，共同轉染了 bII-tubulin 蛋白 siRNA 表現質體 (pSilencer 2.1-U6-1431 與 pSliencer 3.1-H1-1431)

後，觀察到由 N1IC 所活化的 CBF1-dependent 報導基因活性減少，但在共同轉移感染了 siRNA 表現質體 (pSliencer 3.1-H1-105) 或是對照組質體 (pSliencer 2.1-U6 neo) 卻無法觀察到此結果。除了在 HeLa 細胞之外，同樣在 K562 細胞株當中可以觀察到 CBF1-dependent 報導基因活性的抑制現象。根據上述的實驗結果推測，當 bII-tubulin 蛋白的表現受到 siRNA 的降低時，CBF1 所仲介的 N1IC 轉錄活性會受到抑制，這些結果與之前發表的研究 : Taxol 會藉由 N1IC 來增加 CBF1 dependent 導基因活性之結果是一致的。 在 K562 細胞株中，細胞週期相關蛋白的表現會受到活化型式 Notch1 接受體調控 相對於對照組細胞，於表現 HA-N1IC 的融合蛋白之 K562 細胞中，有較高的 ppRb 及 Rb 蛋白表現量。此外，在有 N1IC 存在下，E2F1 蛋白之表現量有減少的現象。過去認爲 Rb 蛋白之磷酸化可被 cyclin D/CDK4 及 cyclin E/CDK2 所調控，進而使細胞由 G1 時期進入 S 時期，本計劃接著更進一步去探討在 N1IC 存在之下，是否可調控 CDK4 及 CDK2 之表現。與對照組細胞相比較，於表現 HA-N1IC 的融合蛋白之 K562 細胞中，CDK4 及 CDK2 之表現會被抑制，同時 CDK4 及 CDK2 之活性也有被抑制的情況。這些結果指出：經由 Rb、E2F1、CDK4 及 CDK2 等蛋白的作用，Notch 接受體訊息傳遞路徑對於由 G1 時期進入 S 時期的調控，扮演舉足輕重的角色。

- 英文摘要

Overexpression of the Notch1 receptor intracellular domain inhibits the proliferation of K562 cells. All of the cumulative cell numbers of the HA-N1IC fusion protein-expressing K562 stable cell lines were lower than their control cells (K562/pcDNA3). These results suggest that the expression of the HA-N1IC fusion protein suppressed the proliferation of K562 cells. Notch1 receptor intracellular domain increases the proportion of K562 cells in the G1 phase, while Taxol arrests cells in the G2/M phase. Results showed that N1IC increased the proportion of cells in the G1 phase of the cell cycle from 37% to 44%, and decreased the proportion of S-G2/M-phase cells from 63% to 56%. Therefore, these data demonstrate that N1IC slightly arrested K562 cells in the G1 phase. Notch1 receptor intracellular domain and Taxol suppress tumor growth of K562 cells in vivo. Tumors of HA-N1IC fusion protein-expressing K562/HA-N1IC cells were reduced to 19% of the sizes observed in the control K562/pcDNA3 cells on day 15 after tumor inoculation. After treatment with 3.5 ?gM Taxol for 24 hours, tumors of neither K562/HA-N1IC nor K562/pcDNA3 cells exhibited any growth. As described previously, Taxol increased the nuclear content of bII-tubulin to promote the CBF1-dependent transactivation of N1IC. However, this enhancement of nuclear bII-tubulin induced by Taxol did not promote the tumorigenesis in nude mice here. At the same concentration (3.5 mM), Taxol also arrested the cells in G2/M phase to inhibit the tumor growth. These results suggest that N1IC and Taxol can arrest K562 cells, thus suppressing tumor growth in vivo. The CBF1-dependent luciferase reporter activity transactivated by the Notch1 receptor intracellular domain is suppressed by small interfering RNAs (siRNAs) targeting bII-tubulin. A decrease in the expression of the FLAG-NLS-bII-tubulin fusion protein was observed in the lysates of cells transfected with the bII-tubulin siRNA expression construct (pSilencer 2.1-U6-1431), whereas neither another siRNA expression construct (pSilencer 3.1-H1-105) nor the control vector (pSilencer 2.1-U6 neo) affected bII-tubulin expression. A decrease in the CBF1-dependent luciferase reporter activity transactivated by N1IC was shown in HeLa cells co-transfected with bII-tubulin siRNA expression constructs (pSilencer 2.1-U6-1431 and pSilencer 3.1-H1-1431), but not in those co-transfected with another siRNA expression constructs (pSilencer 3.1-H1-105) or the control vector (pSilencer 2.1-U6 neo). In addition to HeLa cells, this suppression of CBF1-dependent luciferase reporter activity was also observed in K562 cells. The above data suggest that CBF1-mediated transactivation activity of N1IC was inhibited when bII-tubulin was knocked down by siRNA. These results are consistent with a previous study in which Taxol augmented the CBF1-dependent luciferase reporter activity transactivated by N1IC. Expressions of cell cycle-related proteins in K562 cells are regulated by the activated Notch1 receptor. In HA-N1IC fusion protein-expressing K562 cells, the expressions of phospho-RB (ppRB) and Rb were higher than those of the control cells, whereas E2F1 expression was decreased in the presence of

N1IC. Since phosphorylation of the Rb protein is regulated by both cyclin D/CDK4 and cyclin E/CDK2 which allows cells to progress from the G1 to the S phase, we next investigated whether modulation of CDK4 and CDK2 expressions occurs in the presence of N1IC. The expressions of both CDK4 and CDK2 were suppressed in HA-N1IC fusion protein-expressing K562 cells, compared to control cells. The activities of both CDK4 and CDK2 were also suppressed in K562/HA-N1IC cells as compared with K562/pcDNA3 cells. These data indicate that the Notch signal pathway pla