探討 Notch 訊號傳遞路徑對 c-Myc 啓動子之調控作用

Study on the control of c-Myc promoter by Notch signal pathway

中文摘要

Notch 受體蛋白在細胞增生，凋亡，以及分化過程中，皆扮演著很重要的角色。本實驗室先前的研究顯示，Notch1 細胞內區域（也就是活化形式具有功能之 Notch1 受體蛋白）被證實會和具多功能之轉錄因子 YY1 結合，並且抑制 CBF1-dependent 之 Notch 訊息傳導路徑。轉錄因子 YY1 已知與許多基因表現之調控有關。因此本論文擬定，欲以不同角度探討 N1IC 與 YY1 所形成之 N1IC-YY1 複合體，是否可藉由結合到 DNA 序列上之 YY1-response element，進一步調控其下游基因之表現。研究結果發現，N1IC 可活化 YY1-response element 之活性，且此活化作用是經由 CBF1-independent 之訊息傳導路徑。

研究報導指出，c-Myc 致癌基因與一些惡性腫瘤之致病性有關，如乳癌、卵巢癌、前列腺癌、肝細胞癌、直腸癌，淋巴癌以及血癌。在人類以及老鼠的腫瘤模式中，過度表現的 YY1 蛋白可活化內生性以及外生性的 c-Myc 啓動子之活性。另外，本實驗室也發現 c-Myc 基因之表現會受到 Notch 訊息傳導路徑所調控，

搜尋人類全長 c-Myc 基因之啓動子序列，可找到許多可能的 YY1 結合位置，並且利用報導基因分析證實 N1IC 與 YY1 所形成之 N1IC-YY1 複合體，可藉由 YY1-response element 之 DNA 序列，結合至 c-Myc 啓動子區域上，活化 c-Myc 啓動子之活性，同時此活化作用亦可受共同外送轉染表現之 YY1 蛋白所大量提升，因此推測 c-Myc 啓動子活性之提升與 N1IC-YY1 複合體之形成有關，並且是經由 CBF1-independent 之訊息傳導路徑。

另外，利用與持續表現 Notch ligand - 分泌型 Jagged-1 蛋白質（Jagged-1ext）之細胞株共同培養（co-culture）或收集其條件培養基（conditional medium）的方式誘導內生性 Notch 之活化，結果也顯示由 Jagged-1ext 所誘導之內生性 Notch 訊息傳遞路徑確實也可以調控 c-Myc 啓動子之表現。本論文也藉由 Real-Time PCR 以及西方墨點法探討，可觀察到在持續性表現 N1IC 之細胞株中，其 c-Myc 之 RNA 以及蛋白質表現量皆明顯增加。

本實驗室先前 in vivo 以及 in vitro 的實驗結果顯示，持續表現 N1IC 之 K562 細胞株，其生長速率與腫瘤形成速度皆較慢。本論文藉由流式細胞儀分析，觀察到可持續表現 N1IC 細胞株之細胞週期分佈，與對照組細胞相比，趨向於停留在 G0/G1 時期，且其 Rb（Retinoblastoma）以及 ppRb（Hyperphosphorylated retinoblastoma）蛋白質之表現量，皆明顯較對照組高出許多。

綜合本論文研究顯示，Notch 訊息傳遞路徑與 YY1 以及 c-Myc 啓動子之間有著密切的關係，且同時參與了細胞週期進行分佈之調控。

英文摘要

The Notch proteins play important roles in cell functions including proliferation, apoptosis, and differentiation. In the previous studies, the Notch1 intracellular domain (N1IC, the functionally active form of Notch1 receptor) was demonstrated to associate with multifunctional transcription factor YY1 to suppress the CBF1-dependent Notch signal pathway. YY1 was shown to regulate the expression of many genes. In this study, we investigate whether N1IC-YY1 complex regulates the expression of YY1 target gene through the YY1-response element. We found that N1IC increased the luciferase activity of reporter gene with YY1-response element via a CBF1-independent pathway.

The activity of proto-oncogene c-Myc has been implicated in the pathogenesis of malignancies such as breast, ovarian, prostate, hepatocellular, and colorectal carcinoma as well as lymphoma and plasma cell tumors. In murine and human tumor models, overexpressed YY1 activates both endogenous and exogenous c-Myc promoters. Furthermore, the c-Myc expression can be regulated by Notch signaling. There are many putative YY1-binding sites in the human c-Myc promoter. To check whether N1IC can bind on the YY1-binding sites to regulate c-Myc expression, the reporter gene assay were performed. N1IC enhanced the activity of reporter gene containing c-Myc promoter through the CBF1-independent pathway. This increment of the reporter gene activity was further promoted by exogenous YY1. This activation by YY1 was dependent on the formation of N1IC-YY1 complex.

Alternatively, the endogenous Notch signaling was induced by co-culturing with the stable cell lines constitutively expressing the secreted form of Notch ligand Jagged-1 or treating with their conditional medium. The c-Myc promoter activity was also up-regulated after the activation of endogenous Notch signaling by secreted Jagged-1. In addition, both the mRNA and protein expressions of c-Myc were up-regulated in the presence of N1IC by the Real-Time PCR and Western blot methods.

Previously, both in vivo and in vitro experiments demonstrated that the cell growth and tumorigenesis of K562 cells were delayed in the presence of N1IC. Compared with control cells, the cell cycle analysis of N1IC-expressing K562 cells revealed G0/G1 arrest. The N1IC increased expression of the cell cycle related proteins such as Rb（Retinoblastoma） and hyperphosphorylated Rb (ppRb).

Taken together, these results suggest the Notch signal pathway cross-talks with YY1 and c-Myc proteins, and this interaction is involved in the cell cycle regulation.