I. JAK kinases 與 IL-3 受體 α 和 β c 次單位結合之探討

II. p38 訊息傳遞於細胞分化與生長抑制之角色探討

I. Determinants for the interactions between the cytoplasmic regions of the Interleukin-3 receptor subunits α and βc and JAK kinases

and JAK kinases
II.Roles of the p38 pathway in cell differentiation and growth inhibition

中文摘要

IL-3 爲細胞激素家族的一員，調控著細胞的生長、增生、分化與凋亡。IL-3 受體上沒有酪胺酸激?，必須藉由 JAK 蛋白激?傳遞訊息。我們實驗室過去以免疫沉澱法證實 JAK1 會與 IL-3 受體 β 次單位結合，而 JAK2 則與 IL-3 受體 α 次單位結合。而 JAK1 和 JAK2 與 IL-3 受體的結合是不受細胞激素刺激影響的。JAK 蛋白上有七個同源的區域，分別命名 JH1 至 JH7。目前已知 JH1 具有酵素活性，而 JH2 調控 JAK 的活性，而 JH3 至 JH7 片段則與受體的結合有關。但 JAK1 和 JAK2 究竟以哪個區域與 IL-3 受體結合？我利用實驗室先前建構好的 GST-IL-3 受體融合質體及一系列的 JAK1 和 JAK2 缺失質體，以 GST-pull down 方法分析。實驗結果顯示：JAK1 的 JH3 至 JH7 片段與 IL-3 受體 β 次單位結合，與 α 次單位有微弱的結合。JAK2 的 JH7 或 JH6 至 JH7 區域缺失的情況下，皆不影響與 IL-3 受體 β 次單位結合的能力，與 α 次單位有微弱的結合。

中文摘要

p38 pathway 對於調控細胞增生、分化及細胞凋亡扮演重要的角色。本實驗室過去研究發現，Activin A 誘導 globin gene promoter 活性及抑制細胞生長的作用會被 p38 抑制劑 SB203580 所抑制。SB203580 是專一性抑制 p38α 和 p38β。爲了想更進一步了解 p38α 或 p38β 參與細胞激素調控細胞增生與分化的情形，首先我利用 RT-PCR 分析在 K562 及 Jurkat 細胞內四個 p38 isoforms 表現的情形。在 K562 及 Jurkat 細胞中 p38α,p38β,p38γ 和 p38δ 皆有表現。我們的實驗結果發現 Activin A 所誘導的 globin 和 globin gene promoters 活性會被 p38 dominant negative mutants p38αAF 和 p38βAF 所抑制，顯示 Activin A 誘導 ˜globin 和 ˜globin gene promoters 活性的作用是透過 p38α 和 p38β。另外，c-Jun，紅血球分化的抑制者，其蛋白表現量於 K562 細胞中會受 SB203580 正向調控。最後，我利用 Interferon alpha（IFN-α）刺激 Jurkat 細胞，發現 IFN-α 會透過 p38 pathway 抑制 Jurkat 細胞生長。綜合這些結果，於 K562 和 Jurkat 細胞中 p38 扮演調控細胞生長和分化的重要角色。

英文摘要

IL-3, a member of cytokine family, is involved in the cell growth, proliferation, differentiation and apoptosis. IL-3 stimulates its biological effects by binding to a heterodimeric receptor composed of α and β subunits. JAK1 and JAK2 are rapidly activated after binding of IL-3 to their receptors, and are essential for the initiation of intracellular signaling. Previously, we reported that JAK1 and JAK2 were associated with the IL-3 receptor β and α subunits, respectively. In addition to the JH1 domain（kinase domain）, the JAK family contains the JH2-JH7 domains. JH2 has been shown to have a negative regulatory effect on JAK2 kinase activity. JH3-JH7 domains have been implicated in receptor association. However, which regions of JAKs interact with IL-3 receptors is now unclear. In this study, I demonstrated that the JH3-JH7 domains of JAK1 interacted with βc subunit, but had weak interaction with IL-3 receptor α subunit by GST-pull down assay. The deletion of JH7 domain or JH6-JH7 domains of JAK2 did not affect the interaction with IL-3 receptors.

Abstract

p38 play a central role in cellular responses such as cell proliferation, differentiation and apoptosis. We have previously reported that Activin A-induced globin promoters and cell growth inhibition were inhibited by p38 inhibitor SB203580. The p38 inhibitor SB203580 targets both p38α and p38β. To further investigate that p38α, p38β or both kinases are involved in cytokine-mediated cell proliferation and differentiation, we performed the RT-PCR to analyze the expression of four p38 isoforms on K562 and Jurkat cells. We showed that p38α, p38β, p38γ and p38δ transcripts are expressed in K562 and Jurkat cells. Expression of the exogenous p38 dominant negative mutants, p38αAF and p38βAF, reduced Activin A-induced α- and ζ-globin promoters activity, indicating that Activin A activated α- and ζ-globin gene promoters through p38α and p38β. In addition, the expression of c-Jun, a blocker of erythroid differentiation, was up-regulated by SB203580 in K562 cells. At last IFN-α inhibited Jurkat cell growth through p38 pathway. Together, these results suggest that p38 plays an important role in regulating cell proliferation and differentiation.