# TNF-$\alpha$抑制 Activin A 誘導之紅血球系分化於 CML 細胞中之分子機制研究

# The molecular mechanism of TNF-α inhibited ActivinA-mediated erythropoiesis in CML cells


## 中文摘要

TNF-$\alpha$爲 pro-inflammatory cytokine。TNF-$\alpha$不只參與發炎及感染反應，也與抑制紅血球分化有關。Activin A 爲 transforming growth factor (TGF)-$\beta$ superfamily 其中的一個成員，會促進紅血球的分化。過去本實驗室利用 PDTC (NF-$\kappa$B 抑制劑) 證明在 CML 病人建立的 K562 細胞中 TNF-$\alpha$可透過 NF-$\kappa$B 路徑增加 c-Jun 表現而抑制 Activin A 誘導的紅血球分化。在本論文中，利用 transient transfection 或穩定細胞株大量表現 NF-$\kappa$B p50 或 NF-$\kappa$B p65 更進一步證明於 K562 細胞中 NF-$\kappa$B 於 TNF-$\alpha$誘導 c-Jun 表現所扮演的角色。實驗結果顯示在 K562 細胞中，NF-$\kappa$B p65 會活化 c-Jun promoter activity，NF-$\kappa$B p50 會抑制 c-Jun promoter activity。c-Jun 或 NF-$\kappa$B p65 透過 AP-1 binding site 調控其 c-Jun promoter，因此推測 NF-$\kappa$B p65 透過 c-Jun 作用於 AP-1 binding site，這可能爲 TNF-$\alpha$抑制紅血球分化的原因之一。JNK 抑制劑 SP600125 可抑制因 TNF-$\alpha$活化的 c-Jun promoter 活性，此結果表示 TNF-$\alpha$也可以透過 JNK 路徑調控 c-Jun 的表現。此外，NF-$\kappa$B p65 活化 c-Jun promoter 的活性部分可被 JNK 抑制劑所抑制。所以推測 TNF-$\alpha$是分別透過 JNK 以及 NF-$\kappa$B 兩個個別路徑調控 c-Jun 的表現。RT-PCT 的結果顯示 Activin A 可誘導 K562 細胞中 erythroid genes($\alpha$-globin, $\zeta$-globin, NF-E2p45 and GATA-1)的表現；TNF-$\alpha$則抑制這些 genes 的表現。當 NF-E2 大量表現時會增加 Activin A 誘導$\zeta$-globin reporter 的活性，而 c-Jun 大量表現時，會抑制此現象。由這些實驗結果顯示 TNF-$\alpha$透過 NF-$\kappa$B 增加的 c-Jun 表現可抑制 NF-E2 增加 Activin A 誘導紅血球的分化。未來，將利用 ChIP assay 研究 NF-$\kappa$B p65 是否結合於 c-Jun promoter 上 AP-1 binding site 的 c-Jun 而增加 c-Jun promoter 活性。



## 英文摘要

The pro-inflammatory cytokine, tumor necrosis factor (TNF)-α, is linked to erythropoietic inhibition and may contribute to different forms of anemia. Activin A, a member of the transforming growth factor (TGF)-β superfamily, is an erythroid differentiation factor. In our previous study, we demonstrated that TNF-α up-regulated c-Jun expression through the NF-κB pathway to inhibit Activin A-mediated erythropoiesis with NF-κB inhibitor PDTC in chronic myeloid leukemia (CML)-derived K562 cells. In this study, we carry out the over-expression of different

NF-κB family members, p50 and p65, by transient transfection or stable expression in K562 cells to further explore the role of NF-κB on TNF-α-induced c-Jun expression. Our data show that p65 activates c-Jun promoter; however, p50 represses c-Jun promoter. The c-Jun or NF-κB p65 activates c-Jun promoter through AP-1 binding site, suggesting NF-κB p65 act on AP-1 binding site through c-Jun which may lead to erythropoietic inhibition in TNF-α signaling. The JNK inhibitor SP600125 represses the TNF-α-activated c-Jun promoter, indicating that TNF-α?n also exerts its effect through JNK activation. Furthermore, p65-activated c-Jun promoter is partly inhibited by JNK inhibitor, suggesting TNF-α ?nregulates the c-Jun promoter through both NF-κB and JNK pathways. The results of RT-PCR show that activin A up-regulates the expression of erythroid genes (α-globin, ζ-globin, NF-E2p45 and GATA-1) and TNF-α?n down-regulates these genes. The over-expression of NF-E2 enhances Activin A-induced ζ-globin reporter activity, co-expression of c-Jun and NF-E2 can inhibit these effects. These results indicate that TNF-α up-regulates c-Jun through NF-κB pathway to antagonize the NF-E2 transcriptional activity by Activin A in erythroid differentiation. In the future work, whether NF-κB p65 binding to c-Jun on the AP-1 binding site of c-Jun promoter to enhance the c-Jun promoter activity will be investigated by ChIP assay.