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The Functional Role and Signal Transduction of Synergistic Activation of Janus Kinases in Hematopoietic Progenitor Cells

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^ ` ¡GJAK¡A† › ŁMEfŁA¤ ŁF‹／¡˘S €˛¡A„ T i » ˜C›»˜A／†›ŁMEfl T„„

Abstract

The proliferation, differentiation, and apoptosis prevention of hematopoietic cells are regulated by multiple cytokines. Cytokine receptors possess no kinase activity, the cytoplasmic Janus kinase (JAK) play a very important role for cytokine signaling pathways. Most cytokines can activate two or more than two JAKs; the role of only one JAK has concentrated among cytokine signaling on many researches. However, the mechanism of simultaneous activated-JAKs how to affect the signaling and cell activity are not clear. The present study used a conditional activated system, CD16/CD7/JAK (CDJAK), for JAK activation in IL-3 dependent cells. The fusion proteins consist of CD16 external domain, CD7 transmembrane region, and JAK1 (CD16/7/JAK1; CDJAK1) or JAK2 (CD16/7/JAK2; CDJAK2) cDNA as cytoplasmic domain. We established several stable cell lines: CDJAK1 and CDJAK2 (CDJAK1+2) co-expression, CDJAK and kinase mutant CDJAKKE (CDJAK1KE+2 or CDJAK1+2KE) co-expression, and CDJAK1 or CDJAK2 alone expression in Ba/F3 cells. Anti-CD16 antibody cross-linking induced CDJAK tyrosine phosphorylation of Ba/F3-CDJAK1 or Ba/F3-CDJAK2 cells, but no signal transduction and cell activity. Antibody cross-linking induced signaling of tyrosine phosphorylation and phosphorylation of STAT5, MAPK and Akt in the Ba/F3-CDJAK1+2 cells clustered the chimerical proteins of stable cell lines. These results suggested that synergistic activation of signal transduction need to cluster JAK1 and JAK2. The mutant of kinase activity, CDJAKKE, was further proved the normal kinase activity was essential for JAK1 and JAK2 to activate the synergistic effect of signal transduction. In addition, synergistic effect of both JAK1 and JAK2 activate βc phosphorylation. Furthermore, CDJAK1 and CDJAK2 are constitutively associated with βc and IL-3Rα, respectively. Therefore, the data suggest that synergistic activation effect of CDJAK1 and CDJAK2 may be through cluster two complexes, CDJAK1-βc and CDJAK2-IL-3Rα to form big complexes, and further activate βc tyrosine phosophorylation and signal transduction.

Keywords: JAK, Cytokine, Synergistic Effect, Tyrosine Phosphorylation, Signal Transduction

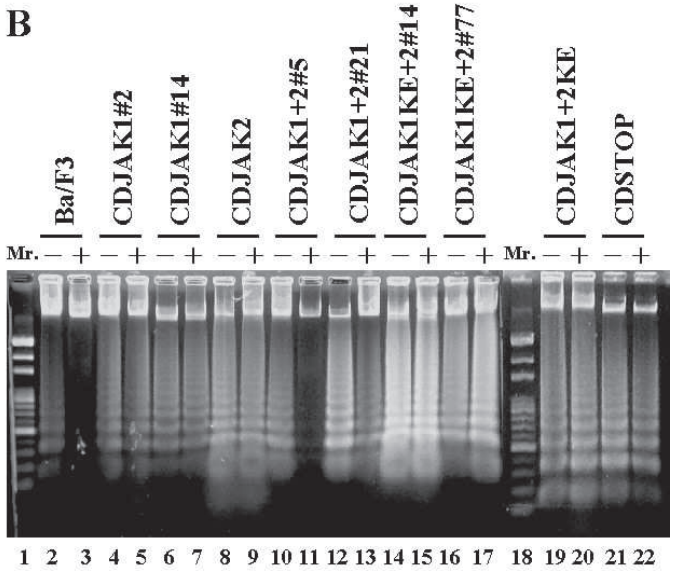
Introduction

Janus tyrosine kinase (JAK)fi a −¥JAK1 ¡AJAK2 ¡AJAK3 'MTyk2' †ƒ¤A¥ ƒ › ' † ›M¿E fl " " T fi S ¶ · » ... ⁄ ⁄ S Œ t" EPO(erythropoietin)¡pAIL-3 (interleukin-3) ¡AIL-5 'M GM-CSF (granulocyte macrophage-colony stimulating factor) [1-4]¡ C ⊕¤kinases » ‰ ›-« n"" † \ ƒ h ¥ ˝ " ¥\fl »¥M.‹WA ¡A⁄ ⁄˘¡A„ w ¤ › ⁄ ' ⁄¿SK[4-7]¡JAK‹ †⁄˘"" ¤ ' EPO signaling ƒ † , ‰M•"[ªs[8] ¡ŒEPO receptor‹ single chain""homodimer ¡A• EPO»Preceptor""dimer ƒX † ¥¤receptor †" c S ¯ ¤›M⁄ " ˘ S ¡A"ªre-associated JAK2fl † „L"æ¶ Z ""– †¶i ƒ cross phosphorylation¡Œ ¡⁄˘""JAK2¶i⁄ @B¶ · » „T „ ¤ ˆ£¤ M"⁄'¡'[9-11]¡Œƒ ¥~¡A' jak2-/- knockout mouse"‹ ªs⁄ F JAK2' IL-3 ¡AL-5 'MGM-CSF ¡ATPO⁄ ¿IFN-γ " " T fi S ¶ »...⁄]S Œ"t › « › ‰"¤¡Œƒ

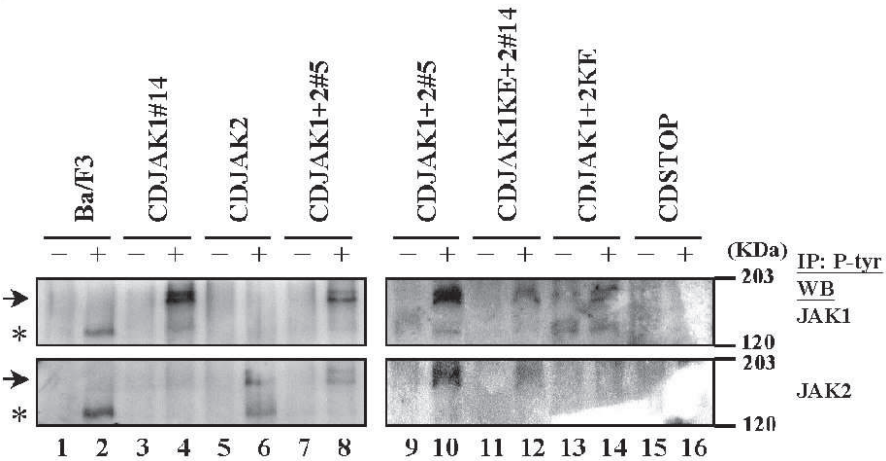
† \ ƒ h † › M ¿ E fl ¥ i ¥ H ‹⁄˘"¤JAKs'¡Œ¥ ›«æ‰P‰ ‹˘""JAKs ƒ p ƒ...v ¯T T fi ¶ ·›M† M‹'¡°""S @ ¥ ¸ ´ ¤ˆ¡Œ¥†M‰-3 subfamily (¥ $t IL-3 ¡AIL-5 'M GM-CSF)‹ ¤ ¡ŒIL-3 ¡AIL-5 'MGM-CSF • |‹ ‹˘JAK1 'MJAK2[14-19]¡ŒIL-3 ¡AIL-5 'MGM-CSF ""receptor‹ heterodimer¥ $t⁄@› α chain¡A fl S ' w ' ¤ C @ ¡A†M›‰¿Œfimmon β (βc) chain¡A‹ ƒ „T†› ›M Efl ‰¸[13]¡ C ⁄ j † ¡ ¥ ""¥„Œ¥ ˝S Q « ⁄ ˘ · ⁄ ‰k¥AK2n"¤⁄Q ƒ ¡C† ¤·„ Œ ¯S¥¸ conditional activation system ‹ ¡⁄˘JAK2 ¤ ˆ ¥ B ‹¥¡⁄˘¤ Ø·"" proteins¡ŒThe chimeric molecule, β/JAK2, ¥ $tβc extracellular 'Mtransmembrane regions »P JAK2 ¿ ƒX¡ A„¿ ˜ ƒ X † J ¥ ' †›M"⁄•Œ|˜«ø[20]¡C M ¡Aβ/JAK2-overexpressed cells »P IL-3 dependent parental cells ‹ , ß ⁄ S ⁄ Ø † proliferated activity 'M apoptotic activity¡Cƒ ¥~¡ACD16¿ ƒXJAK2 ""† J ¥ '»Œ¶ ¥ ⁄U¡A` Mƒ† T fi ¶ ·›A« o ‰k·£ ¤ † › M ¥ƒS[21]fi[22]¡Œ o ¤ · † G ¯ƒª¥JAK2‹ ¡ ⁄ ˘ ‹ Ø£¤⁄ «ø† ›M.. W fl ⁄O¡C¤ ¥ ƒ JAK1 ¤ ƒ ""‹ ª ¤ s ƒ–«oƒ ""„ Œ "G¡A' FDCP-1cells " †{αα β chimera¡A extracellur and membrane domains of mIL-5Rα and the cytoplasmic domain of βc ' †ƒ¤A ' mIL-5 ¤ ° ¿ E ⁄ U ¥•JAK1¤ ˆ ¥ B L " k ⁄ «† ‹ ¡M"[16]¡Œ·" æ ‹ ªsJAK1 and JAK2 ""‹ ¡ ›•⁄ ‹ ‹ ...v¯TIL-5 system[23]¡ŒIL-5 ¤ ° E⁄¡Wdominant-negative (DN) mutants of JAK2 ⁄ j ¶ Q "†{" † M L›kœ¤JAK1 and βc "" tyrosine phosphorylation¡A' DN-JAK1 " †{""† ›M¡A¥ i »œ‰JAK2 and βc "" tyrosine phosphorylation¡Aƒ ‹ O T „ †¡Q› MS¡A ¤ ¥ « e ‹ ⁄¤JAK1 and JAK2 †QIL-3 ƒ P fi ‹ ¡›" ¤ ‹ OS _ † z L„ˆ EPOR-JAK2 system. ƒ ¥~¡ A£ƒP""JAKs ƒ P fi ‹ ¡⁄OS„ T„¶ · » ... ⁄†‹ ¡M‹ O¥ ‰ ""¡C ⁄ F ƒ " † o › ¡ASS › ƒP... ¸ ƒ SQ€CD16/CD7 molecule ‹ conditional activation system ¤ ‹ ¡ ⁄˘JAKs¡C†o¤ · chimeric proteins ¥ $t CD16 extracellular domain¡ACD7 transmembrane domain, ' MHJAK1 (designated as CDJAK1)¡AJAK2 (designated as CDJAK2)¡AJAK1KE (designated as CDJAK1KE)¡Aor JAK2KE (designated as CDJAK2KE) ""cDNA ‹ cytoplasmic domain¡C «¥"^ ›w† ›Mi‡¡Aƒ † † W"¡A†¿ƒ Fi " †{wild type ""CDJAK1 and CDJAK2¡A ¿ƒ Fi " †{a wild type ""CDJAK and a dominant negative mutant CDJAKKE; ¤^⁄ "R†o¤ · chimeric proteins „ T„⁄ ¸ † ›M¡'°""... v̄T in Ba/F3 cells¡CS › "",Œ ¯ u ¯ ¥ ¡AJAK1 ' ¸JAK2 ‹ ¥i¥H† ‡Lcross-link † ¥¤transphosphorylation¡Cƒ ‹ O„ ' ⁄«øell proliferation and anti-apoptosis and signal transduction «h» › n ‰¶both wild type JAK1 and JAK2.

Results and Discussion

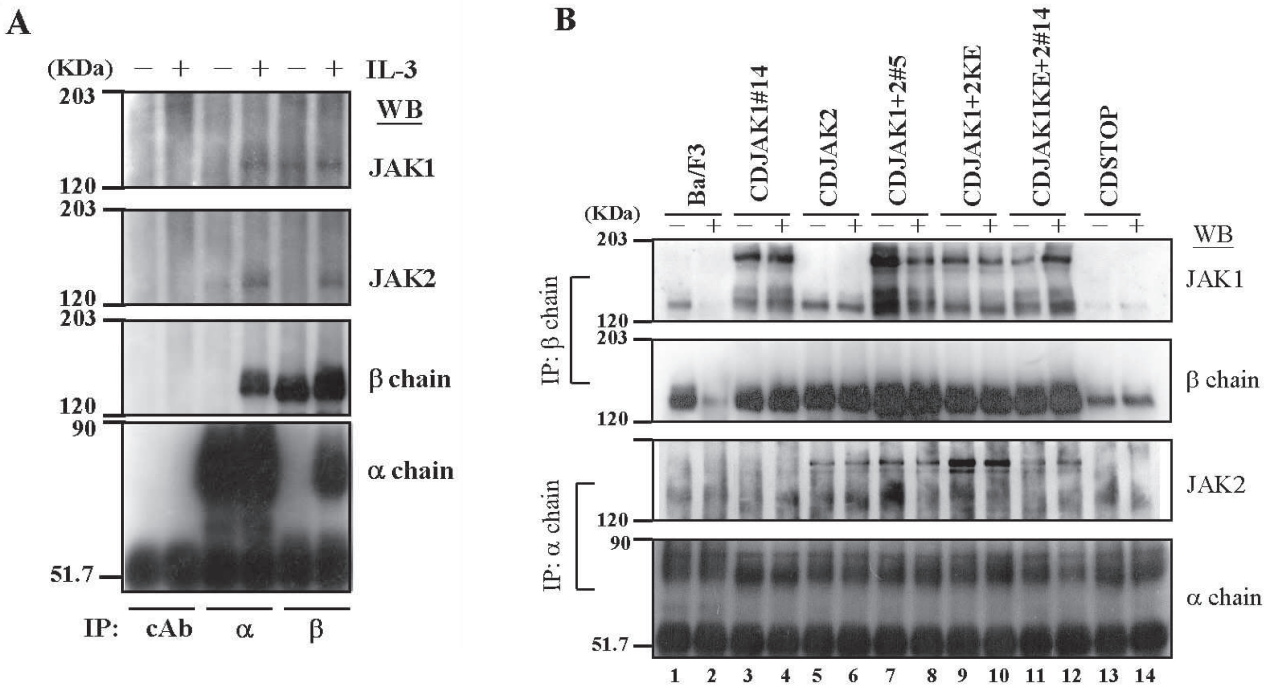
[illegible] CDJAK1 [illegible] CDJAK2 [illegible] (CDJAK1+2) [illegible] tyrosine [illegible] CDJAK1 [illegible] CDJAK1 [illegible] CDJAK2 [illegible] CDJAK1+2 [illegible] CDJAK1+2 [illegible] CDJAK1KE+2 [illegible] CDJAK1KE+2 [illegible] CDJAK1+2KE [illegible] CDSTOP [illegible] Ba/F3 [illegible] Ba/F3-CDJAK1+2 [illegible] (lane 8) [illegible] IL-3 [illegible] Ba/F3 [illegible] (lane 2) [illegible] Ba/F3-CDJAK1+2 [illegible] CDJAK [illegible] Ba/F3 [illegible]



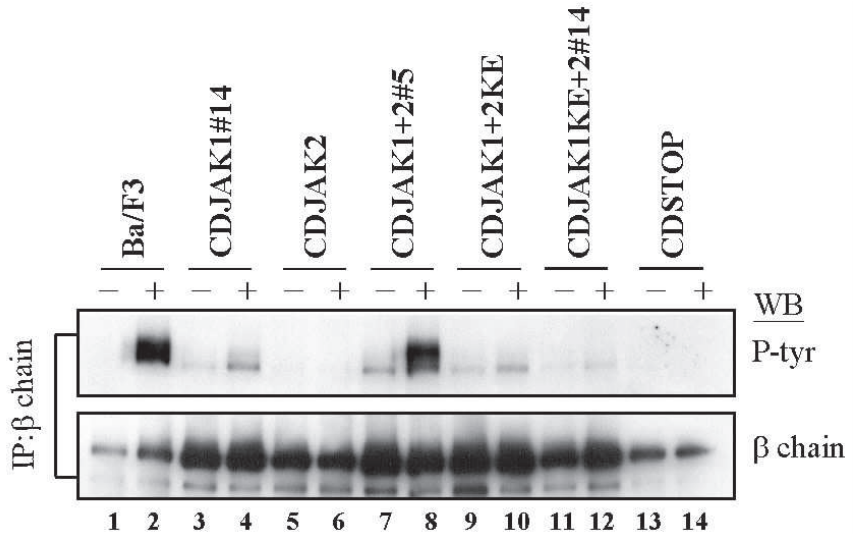
[illegible] Ba/F3 [illegible] anti-phosphotyrosine antobody [illegible] anti-JAK1 antibody [illegible] anti-JAK2 antibody [illegible] western blotting [illegible] CDJAK1 [illegible] CDJAK2 [illegible] (lane 4 and lane 6) [illegible] CDJAK1 [illegible] CDJAK2 [illegible] (lane 8) [illegible] Kinase mutant, CDJAK1KE [illegible] CDJAK2KE, [illegible] wild type CDJAK1 [illegible] CDJAK2 [illegible] KE mutants [illegible] wild type [illegible] (lane 12 and lane 14) [illegible]



„ L ¥ h " " Œ¯, JAK¥pre-associated with cytokine receptor, ƒ] ƒ „ Š › • Q¤Š" ¶i⁄@¤B " „DCDJAK1 and CDJAK2 synergistic effect ‹ O Š»Þ IL-3 receptor α⁄ βc ƒX › " ¥ ⁄ "R⁄"¥˝ ' ª"JAK1 'MJAK2 »Þ IL-3 receptor ƒX"–Š¸ Š Œ¸IP (Š K ‹) "kN †›M""IL-3Rα¡B βcƒ ‹ ¶ MƒAK1¡BJAK2 Š ¯Øwestern blotting ⁄ " R ªG¸JAK1 »Þβc pre-association, JAK2 »Þα chain pre-association. ƒbCDJAK ^ › ' w † ›MƒiŒH‹CD16 Š ¯ Ø ƒ ‡L›ŒŸ¶ŠQ ¥¸IP 'Mwestern blotting ⁄ " R†¡"Y¤ ф{ƒbŠ ¯ ØE›¶ Š_¡ ' †¡"+¡"CDJAK1 ‹ •|'Mβc † ƒX¡CDJAK2 • |Mα chain ƒX ƒ „ ^ › ' w " ª pMfiLPcells ""⁄ " ¥'JAK1 'MJAK2 ‹ ⁄Š»Þβc and α chain interaction¡ C ¥ ¥ H ⁄ W " " Œ¯ CDJAK1¥MCDJAK2 ⁄ Š»Þβc ƒ , ‡ ƒ »Þ , ‡ ƒ – ¤⁄¡L A†ƒX«Œ Š ¸ƒ¤ " " ‰ ˘ ƒ X ¯ Ø ⁄ ~ ¥ i ¥ H ¶»¡.Š„ ª"" T,„⁄ ¸



ƒb † › M ¿ Ë ¡Á¤¿E Ø•U| tyrosine ` C ›⁄¡˜Aƒ ¶ ±@"¤B N ¥"±T„',„⁄ U ¶»¡.. ŒH′ß «ø† M"¥¿ – ¥˝»¤ ⁄ † ›M› ⁄'¡Cƒ ¥H⁄ W „ Œ ¯ ˜[„CDJAK1 'MCDJAK2 'Mreceptor – ƒX ƒ ] ƒ„¤' ... ¯vT"'¤ ƒP‹ ¡⁄˜Š @ ¥ ¸ ‹ Š_¤‡z„Ø¶ · » ...T¤˜„Ba/F3-CDJAK1+2 † ›Mƒ ‡} ƒn¥ "±" ‹'p¡C› " ¥ Š →NSQ¥ ¸βc Š ¯ Ø Š K ‹ ¤ I "k¡–M¤ƒ¥Š¸G¥fosfine ` C ›⁄˜ Š ¯Ø⁄ "R¡ C † G Š›ƒØ»E¶ ¡'+¡ ‹ÆBa/F3-CDJAK1+2 † ›Mβc tyrosine ` C » ˜ ⁄ ˜ " "{« »P¥H IL-3 ¤ ° Œ⁄'U"Ba/F3 † › M ƒC ƒ•JAK1KE ' ¸JAK2KE ƒ s ›fí(Ba/F3-CDJAK1KE+2¡B Ba/F3-CDJAK1+2KE), βc¤ ^ £•tyrosine ` C ›⁄¡˜A † G¯€CDJAK1 and CDJAK2 ‡ J ¥‰Ł ¤ Š ¯Ø‹˜‹« Æ " ª ¡M βcƒ‡tryosine ` C » ˜ ⁄ ""¡Q¶H



ƒ b ‡ o › „Œ¯ §⁄ ⁄ › – o ¤ ¥ HU· X† ›˜@,†‰‡ ¿ WE¶CDJAK1 or CDJAK2 § ¥i ‡ y ƒ ¤ ¤¥tyrosine phosphorylation, ƒ ‹ ƒPƒ›i ¶ CDJAK1 and CDJAK2 ¤ ˆ ⁄ £ ¦…W¥[tyrosine phosphorylation " " {«. †˜⁄G, ¥ ƒi‡ƒ Ƒ » ¶ CDJAK1 and CDJAK2 ¥i¥Hinduce tyrosine phosphorylation ""signal, and activate STAT5, Akt and MAPK; ¤ˆ¥Bprovide cell proliferation and apoptosis suppression. †˜⁄T, ƒ Ƒ » ¶ CDJAK1 and CDJAK2 ¥i¥Hinduce βc tyrosine phoephorylation. † ¥|, CDJAK1 and CDJAK2 are constitutively interacted with IL-3Rα and βc, respectively. ƒ J„„ CDJAK1 and CDJAK2 ¥ i fl ‡z»„Œ ¶ ¤ ›‰CDJAK1-βc 'MCDJAK2-IL-3Rα§ ¸ ƒ ¤ § ⁄ j " " ‰ ˘ ƒ XØβc ˆ„¶iT⁄@¤B»›⁄ ˜ C»˜⁄T˘fiM¶ ˙›C.

JAK1 and JAK2 are activated at the same time that both should be thought playing the important role in cytokine signaling. Although in the recent years the role of JAK2 has been studied to participate in proliferation and in anti-apoptosis activity, little is known about JAK1 function. We purpose that JAK1 enhance the threshold of activated signaling with the JAK2, then integral activated-JAKs pathway can be delivered. Analysis of the synergistic effect of both JAK1 and JAK2 in our system should enable us to achieve following goals, 1) The establishment of central role of JAK1 and JAK2 in signaling. 2) Investigation of regulation mechanism of JAKs pathway. 3) Identification of downstream components of JAKs. JAK/STAT pathway is widely used by members of the cytokine receptor superfamily. These cytokines are central for hematopoietic cell biology and hematopoietic therapy alike, including EPO, GM-CSF, granulocyte colony-stimulating factor (G-CSF), thrombopoietin (TPO), interferons etc. Indeed, to study this pathway has provides wealth of information on hematopoiesis and hematopoietic diseases.

References

1. Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K, Thierfelder WE, Kreider B, Silvennoinen O. Signaling by the cytokine receptor superfamily: JAKs and STATs. Trends Biochem Sci. 1994;19:222-227.

2. Ihle JN, Kerr IM. Jaks and Stats in signaling by the cytokine receptor superfamily. Trends Genet. 1995;11:69-74.

3. Watanabe S, Arai K. Roles of the JAK-STAT system in signal transduction via cytokine receptors. Curr Opin Genet Dev. 1996;6:587-596.

4. Leonard WJ, O'Shea JJ. Jaks and STATs: biological implications. Annu Rev Immunol. 1998;16:293-322.

5. Rane SG, Reddy EP. JAKs, STATs and Src kinases in hematopoiesis. Oncogene. 2002;21:3334-3358.

6. Igaz P, Toth S, Falus A. Biological and clinical significance of the JAK-STAT pathway; lessons from knockout mice. Inflamm Res. 2001;50:435-441.

7. O'Shea JJ, Gadina M, Schreiber RD. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell. 2002;109 Suppl:S121-131.

8. Remy I, Wilson IA, Michnick SW. Erythropoietin receptor activation by a ligand-induced conformation change. Science. 1999;283:990-993.

9. Witthuhn BA, Quelle FW, Silvennoinen O, Yi T, Tang B, Miura O, Ihle JN. JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. Cell. 1993;74:227-236.

10. Zhuang H, Patel SV, He TC, Sonsteby SK, Niu Z, Wojchowski DM. Inhibition of erythropoietin-induced mitogenesis by a kinase-deficient form of Jak2. J Biol Chem. 1994;269:21411-21414.

11. Gouilleux F, Pallard C, Dusanter-Fourt I, Wakao H, Haldosen LA, Norstedt G, Levy D, Groner B. Prolactin, growth hormone, erythropoietin and granulocyte-macrophage colony stimulating factor induce MGF-Stat5 DNA binding activity. EMBO J. 1995;14:2005-2013.

12. Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, Vanin EF, Bodner S, Colamonici OR, van Deursen JM, Grosveld G, Ihle JN. Jak2 is essential for signaling through a variety of cytokine receptors. Cell. 1998;93:385-395.

13. Kitamura T, Sato N, Arai K, Miyajima A. Expression cloning of the human IL-3 receptor cDNA reveals a shared beta subunit for the human IL-3 and GM-CSF receptors. Cell. 1991;66:1165-1174.

14. Callus BA, Mathey-Prevot B. Interleukin-3-induced activation of the JAK/STAT pathway is prolonged by proteasome inhibitors. Blood. 1998;91:3182-3192.

15. Silvennoinen, O., Witthuhn, B. A., Ouelle, F. W., Cleveland, J. L., Yi, T., Ihle, J. N. Structure of the murine Jak2 protein-tyrosine kinase and its role in interleukin 3 signal transduction. Proc. Natl. Acad. Sci. U. S. A. 1993;90:8429-8433.

16. Kouro, T., Kikuchi, Y., Kanazawa, H., Hirokawa, K., Harada, N., Shiiba, M., Wakao, H., Takaki, S., Takatsu, K. Critical proline residues of the cytoplasmic domain of the IL-5 receptor £\ chain and its function in IL-5-mediated activation of the JAK kinases and STAT5. Int. Immunol. 1996;8:237-245.

17. van der Bruggen, T., Caldenhoven, E., Kanters, D., Coffer, P., Raaijmakers J. A. M., Lammers, J. J., Koenderman, L. Interleukin-5 signalling in human eosinophils involves JAK2 tyrosine kinase and STAT1£\. Blood. 1995;85:1442-1448.

18. Huang HM, Huang CJ, Yen JJ. Mcl-1 is a common target of stem cell factor and interleukin-5 for apoptosis prevention activity via MEK/MAPK and PI-3K/Akt pathways. Blood. 2000;96:1764-1771.

19. Quelle FW, Sato N, Witthuhn BA, Inhorn RC, Eder M, Miyajima A, Griffin JD, Ihle JN. JAK2 associates with the beta c chain of the receptor for granulocyte-macrophage colony-stimulating factor, and its activation requires the membrane-proximal region. Mol Cell Biol. 1994;14:4335-4341.

20. Liu, C. B., Itoh, T., Arai, K. I., and Watanabe, S. Constitutive Activation of JAK2 Confers Murine Interleukin-3-independent Survival and Proliferation of BA/F3 Cells. J. Biol. Chem. 1999;274:6342-6349.

21. Sakai, I., Nabell, L., Kraft, A. S. Signal transduction by a CD16/CD7/Jak2 fusion protein. J. Biol. Chem. 1995;270:18420-18427.

22. Sakai, I., Kraft, A. S. The kinase domain of Jak2 mediates induction of Bcl-2 and delays cell death in hematopoietic cells. J. Biol. Chem. 1997;272:12350-12358.

23. Ogata, N., Kouro, T., Yamada, A., Koike, M., Hanai, N., Ishikawa, T., and Takatsu, K. JAK2

and JAK1 Constitutively Associate With an Interleukin (IL-5) Receptor α and βc Subunit, Respectively, and Are Activated Upon IL-5 Stimulation. Blood. 1998;91:2264-2271.