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† ›M¿ E fl » ⁄ ⁄£,ƒP‡"˝ fi | ¤‰ ƒ †y† ›M" " §ƒ¡¤Janus kinases (JAKs)‡ Qo † { ƒ b@¤T,„‡ fi|⁄ ⁄ § Œ " t "›«›ƒ ¡C ƒb⁄j‡ ¥ "˝¤ ⁄l⁄ ⁄ fl'W"† ›M¿ fl ¥ ¥HƒP fi ‹ ¡ ⁄ ˘ @›"¥JAK¡C M ƒPfi ‹¡⁄"" JAK ƒ p ƒ …v† ›M"˝‹ ¡' ¤^⁄£† M¡¡C§ › ⁄ §e"˝„ Œ ¯ ¶GƒPfi¥´ · CDJAK1 'M CDJAK2 " " ƒ†•y F ›¥† ›Mfi Ł ' § ¯Ø»E¶ «Æ¥i » · œ ¤`Ø»˜T,„¥ ‹A STAT5 ¡B MAPK 'MAkt "˝„ Ti» ˜ Ø»˜¡Cƒ b{⁄ "˝ ‹ ¤s¡¶i⁄@¤ B ƒ §Q¥AK1 ' ¸JAK2 inactive mutants (KE)¤ œ ¥¤⁄ ⁄ @›JAK¡A' –o¤ "˝ ¤ › † ›MfiŁ⁄ § O Ba/F3-JAK1KE+2 'M Ba/F3-JAK1+2KE¡C‰T„Œ' § ¯ ØE¶ ¥kE § ¤ ' ¸› §CSTAT5¡AMAPK 'MAkt "˝„ Ti » ˜ Ø»˜ ¥H⁄ ¸ † ›MW¥˝"˝‹ ¡' °¡C‡o¤˙ ¶G ¯ ¥ ¡AJAK1 'MJAK2 ‡ Q ‹¡⁄˘ †£¥˝"˝¤ ƒP §@¥ ¸¥ ¥H· ß T,„¶ ›…'M† ›M..W¥˝"˝‹ ¡ ' °¡C

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Abstract

Cytokines are able to induce various signaling pathways during hematopoiesis. Janus kinases (JAKs) were found to play a very important role in some signaling pathways. In most cases, more than one JAK will be simultaneously activated by specific cytokine. However, it is not clear that how simultaneous activated-JAKs can affect cellular activity. Our previous results have shown that phosphorylation signaling, including tyrosine phosphorylation of STAT5, MAPK and Akt, can be detected after antibody cross-linking in hematopoietic progenitor cells co-transfected with CDJAK1 and CDJAK2. In present study, one of JAKs was replaced by JAK1 or JAK2 inactive mutants (KE). The resulted two cell lines, Ba/F3-JAK1KE+2 and Ba/F3-JAK1+2KE, respectively, indeed produce either inhibited or reduced phosphorylation profiles of STAT5, MAPK and Akt, as well as proliferation activity of cells after antibody cross-linking. These results show that both JAK1 and JAK2 need to be activated to produce synergistic effect for signal transduction and cellular proliferation.

Keywords: JAK, Tyrosine phosphorylation, Signal transduction

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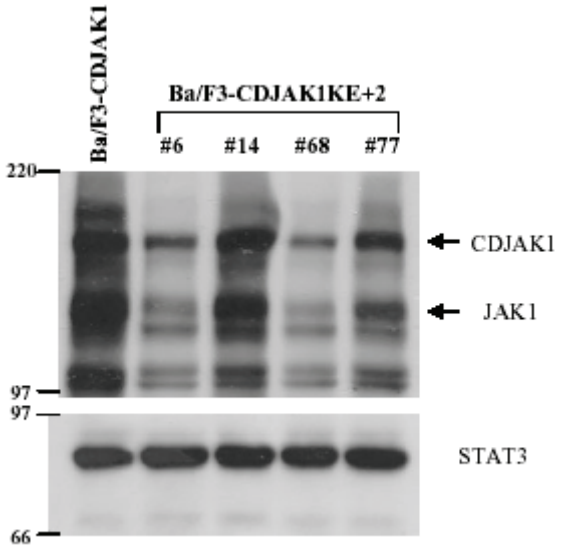
ƒ †y† ›M"˝¥˝ƒ¤» ›n‡ z „ ‡\†ƒh›M¿E fl (cytokine) 'M† ›M¿ fl ¤ ¯Ø(cytokine receptor) "˝ †ƒX¡ A §¥ ¸T,„¶ ›…(Signal transduction)¤ ‹ ¡˘´ ¿ ƒ]⁄l¡ ¶iƒ „Fƒ ‰ , …–ƒa† ›M⁄ ƒPfi ·`"˝‹ ¡' °(1)¡C¤ ⁄⁄¡A JAK-STAT ‡ fi|' † ›M¿Efl ‰–† ›M"˝…W ¥˝¡ B ⁄˘¡B§ ¤ † ›M› ⁄ "˝"‹ ¡' °⁄ W § Œt › «n"˝¤ ƒ¡Gƒb› ¤^† ›M⁄ ⁄ o†4 › JAK (Janus kinase)fi a ƒ¤›ß¡GJAK1¡BJAK2¡BJAK3 'MTYK2¡F⁄ ¸7 › STAT (Signal transducers and activators of transcription) fi a ƒ¤›ß¡G STAT1 ¡BSTAT2 ¡BSTAT3 ¡BSTAT4 ¡B STAT5a¡BSTAT5b 'MSTAT6¡C• † ›M¿ fl 'M¤ ¯Ø †ƒX«Æ¡A‹ ¡˘"˝JAK ‡ J ¥ ‰Ł¶i|⁄@ ¤B"˝‹ ¡˘† ›M¿ fl ¤ ¯Ø¡A¤ ` §˜⁄˘"˝† ›M

¿ E fl ¤ ¯ Ø • | † £ ¥ ˝ ˇ @ ›ŧJ¥˝‰ŒDJ/AK‡ 'ƒMCDJAK2 ¥ i ¥¤ƒ P ¢ˇŧ ›M ˇ/˜˝„ T i ›` C›(tyrosine phosphorylation) (Docking site)¡ A † ¥¤› ŧdocking site ˝ ŧ J ¥‰¥Ł ] Aˇ§STAT ŧ J ¥‰ŧASTAT ŧ ¥ ‰Łˇ O/@ ´ ¿ƒ¡A•l STAT ŧ J ¥‰Ł ƒX ¤ ƒ„ˇ @ ƒ,«ŒE¡A• |ŧQJAK ŧ J ¥‰Ł ¡˜¡A†£ ¥˝` C ›ˇ˝† ¶H¡A¥ B § ¸ ¥ƒP‰˜ø¯Ø (homodimers) ' †§ ‰˜ ƒX ´ø ¯Ø (heterodimers)¡ ¶i/@¤B¤ † › M ¡ˇA †ƒX ¤ fl §w˝ DNA § · ₵/₩A˝ †{fl §w˝ ˝ ¤¥ ‰Ł(2)¡C

„ ¥h˝ˇ ª ¤ ‹{¡Aˇ £ ¥–˜˝ †{ JAK 'MSTAT ŧ J ¥‰Ł ŧyƒ¤ƒ † ¤Ł˝ˇfl ¤lf¡C ¤ ƒp¡GJAK1¡BJAK2¡BSTAT3 'MSTAT5 ˇ£ ¥ ¢ ˜˝ˇ ¡ˇ• |ŧyƒ¤T † ›M˝† O ˇ , ~‰Ł£ ¥ ¢ ˜˝ˇ ¡ˇ STAT1 'MSTAT5 «h• |ŧyƒ¤« ' ° ' ¯Ł ø(3)¡Cƒ„¥~† ›Mił¡ˇJAK ŧ J ¥‰Ł ¤ ƒ P ¢ˇ§@¥¸¡C¤ ˇ/ ˝ ›w† ›Mił Ba/F3-CDJAK1KE+2 ˝¸ ˜ ƒ X ¤ƒ˝ ‰Ł{¶q„ß§C¡A ƒ ƒ„¡A› « • ¤D¸ ›w† ›Mił¡A–N˝ †{ CDJAK1KE ˝ ‰Ł¯Ø´ ·ƒ Ba/F3-CDJAK2 † ›M¡A¥Hwestern blotting ˇ ˝R¡Aƒ p˘' ¥ ¡G #14 'M#77 † ›M»PBa/F3-CDJAK1 † ›M˝ †{ ‹ • ˝ CDJAK1 ¡CSTAT3 ‹ internal control¡Cƒ ƒ„§Q¥¸Ba/F3-CDJAK1KE+2 #14 ' ¸#77 † ›M¶if ¥H/U˝„ Œ ¡C



ˇ£ƒP˝ˆ ›w† ›Mił¥H CD16 § ¯ ØE¶Æ¡A ˇ ˝R¤ tyrosine phosphorylation ŦG„¡C Western blotting ˝Gƒ p˘' ¥ ¡A§ ¯ ØE¶ ¡'+¡˝«Æ Ba/F3-CDJAK1 † › M+•,ßiz˝ tyrosine phosphorylation ŦG„(lane 4) ¡A Ba/F3-CDJAK1+2 † M ƒ# –j˝ ŦG„(lane 8)¡A»PBa/F3 † ›M¥H† ›M¡ ¢ '+¡˝¤°¿E«Æ ˝ˇ` ⊗˜/˘ ŦG„‹ • (lane 2) ¡Cƒ Ba/F3-

¡ E fl ¤ ¯ Ø • | † £ ¥ ˝ ˇ @ ›ŧJ¥˝‰ŒDJ/AK‡ 'ƒMCDJAK2 ¥ i ¥¤ƒ P ¢ˇŧ ›M (Docking site)¡ A † ¥¤› ŧdocking site ˝ ŧ J ¥‰¥Ł] Aˇ§STAT ŧ J ¥‰ŧASTAT ŧ ¥ ‰Łˇ O/@ ´ ¿ƒ¡A•l STAT ŧ J ¥‰Ł ƒX ¤ ƒ„ˇ @ ƒ,«ŒE¡A• |ŧQJAK ŧ J ¥‰Ł ¡˜¡A†£ ¥˝` C ›ˇ˝† ¶H¡A¥ B § ¸ ¥ƒP‰˜ø¯Ø (homodimers) ' †§ ‰˜ ƒX ´ø ¯Ø (heterodimers)¡ ¶i/@¤B¤ † › M ¡ˇA †ƒX ¤ fl §w˝ DNA § · ₵/₩A˝ †{fl §w˝ ˝ ¤¥ ‰Ł(2)¡C

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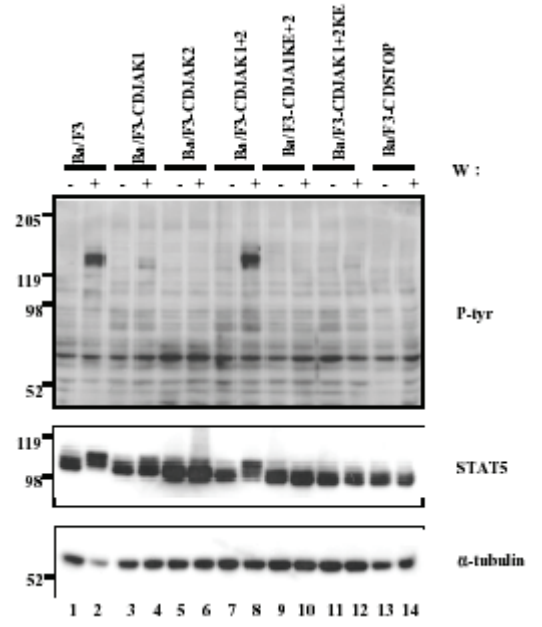
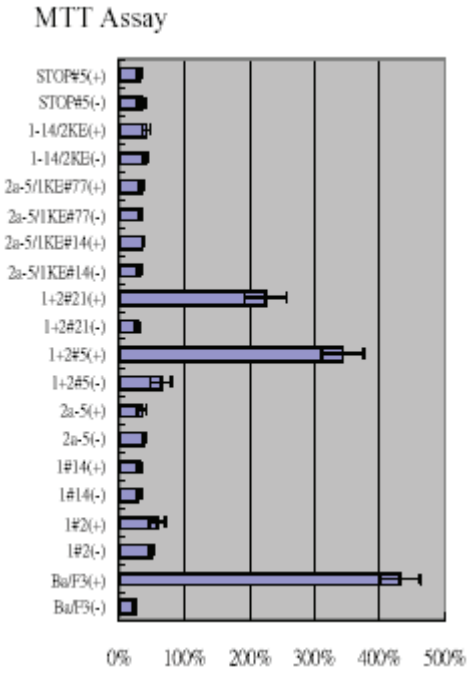
ˇ£ƒP˝ˆ ›w† ›Mił¥H CD16 § ¯ ØE¶Æ¡A ˇ ˝R¤ tyrosine phosphorylation ŦG„¡C Western blotting ˝Gƒ p˘' ¥ ¡A§ ¯ ØE¶ ¡'+¡˝«Æ Ba/F3-CDJAK1 † › M+•,ßiz˝ tyrosine phosphorylation ŦG„(lane 4) ¡A Ba/F3-CDJAK1+2 † M ƒ# –j˝ ŦG„(lane 8)¡A»PBa/F3 † ›M¥H† ›M¡ ¢ '+¡˝¤°¿E«Æ ˝ˇ` ⊗˜/˘ ŦG„‹ • (lane 2) ¡Cƒ Ba/F3-

CDJAK2 ／ Ｓ † › Ｍ ¤ ^ Ｌ¥ityrosine‟” phosphorylation Ｔ„(lane 6)¡Ｃ ƒ „ † Ｇ»Ｐ¥ „ Ｅ ¯«‟” † ‟ Ｇ¡›Ｍ lane 9~12 ф{¡Ａ Ba/F3-CDJAK1KE+2 † ›Ｍ／¸ Ba/F3-CDJAK1+2KE † ›Ｍ' Ｓ Ø»Ｅ¶¡‟¡Ａ¤ tyrosine phosphorylation ‟” Ｔ„'Ｍ Ba/F3-CDJAK1+2 † ›Ｍ‹ ，ß ƒ ≠j‟Ｔ«Ｕ › '¸Ｓ ¤ ¡ＣＳ Ｗ¸ STAT5 Ｓ ¯Ø／ ‟Ｒ ф{ Ba/F3-CDJAK1+2 † Ｍ‟STAT5 ǂ Ｊ ¥‰Ł ǂ' „ Ｗ† °‟”†{¶Ｈ(lane 8)¡Ａ»Ｐ Ba/F3 † ›Ｍ¥Ｈ† ›Ｍ¿Ｅ fl ¤¿Ｅ ≠ ƒＰ‟” ‟Ｇ(lane 2)¡Ｃ† ｏＣ¥ ' `Ｃ » ≥˘‟”STAT5¡Ａ¤˘−ｏƒ ｂ ｄ‰ƒＷ † ，ß Ｌ`Ｃ » ≥˘‟”STAT5 ǂ Ｊ ¥‰Ł ‟”Ｃ¡Ｃα-tubulin Ｓ ¯ Ø／ ‟ Ｒ ‟ ‘／Ｊ ǂ Ｊ ¥‰Ł‟Ｈ‟Ｗ¶Ｋ‘Ｏ／¥¶ｑ ‟Ｒ／Ł‹¡ Ａ ˘ „ Ｓƒｂ Ø» ¶ ¡'+¡ ”Ｕ¡Ａ† ｏ ¤^ › ' ｗ ‟ ｆ／ＧＪＤＪＡＫ ǂ Ｊ ¥ ‰Ł‟” › Ｍ ¤...Ｗ¥´ ‟”‹ ¦°¡Ｃ‹ ／Ｆ ǂ„Ｃ ¤ ^ ›'ｗ › Ｍ fi Ł Ｓ† ›Ｍ ‹ ¦°／£¤ Ｓ ¥ ´ fl ‹ Ｄ ¿„¡Ａ'{／¿ ｚ ¿¥Ｘ／Ｓ fl Ｓ fi † ›¦Ｍ¶Ł ƒ ｈ ¿ ¥t›wŒ › Ｍ Ł¤ ／ ‟Ｒ(Ba/F3-CDJAK1¡›2¡ＢBa/F3-CDJAK1 ¡›14¡ＢBa/F3-CDJAK1¡˘2¡›5¡ＢBa/F3-CDJAK1¡˘2¡›21¡ＢBa/F3-CDJAK1KE¡˘2¡›14¡ＢBa/F3-CDJAK1KE¡˘2¡›77)¡Ｃ„Ｅ † ‟Ｇ ｏ†{ Ba/F3CDJAK1+2 † › ＭＷ¥´ Ø† ƒｎ¡Ｃ† › ＭＷ¥”– ¡ ｐ'¥Ｈ IL-3 ¤ ¿Ｅ／Ｕ‟” Ba/F3 † ›Ｍ‹ ƒ ¡ Ｅ Ｈ／Ｗ ‟Ｇ¯ ¥ ¡Ａ† ›Ｍ‟” tyrosine phosphorylation Ｔ , „ ¿ƒｎ‟”... Ｗ´ fl ／Ｏ» ›nCDJAK1 'ＭCDJAK2 ' ／ _‟”¤ ƒＰ‹¡／ ˘ Ｓ¥(11)¡Ｃƒ ¥~¡Ａƒ„¤ ƒＰ‹¡／ ˘ Ｓ¥¥¿i ¥Ｈ‹ ¡／˘MAPK 'ＭAkt ‟”„ Ｔ i ≈ `Ｃ¥(data not shown)¡Ｃ





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› p ◂e ¥ ‰ — ／ Ｇ~ƒ‚¿ƒ¤s¡Ａƒ ‹Ｏ ¥ ŧq„ Ｌ ‹／ƒ~／Ｓ›p„”¡Ｃ› p ƒe † Ｔ× ¤s ¥ ‟” Specific Aim#1: To identify the synergistic activation effect of both JAK1 and JAK2 is necessary for integral signaling. Specific Aim#2: To explore the synergistic activation effect of both JAK1 and JAK2 is required for the proliferation activity in cytokine-dependent cells. Specific Aim#3: To explore the downstream components of synergistic activation effect of both JAK1 and JAK2. ¥ « e ／ w „ F ƒ ¤ ﾌ ŧ¥ ¥.→ƒ‚Ｗ^` ¥ Ｂ¿ƒbƒ‹¶ † «Ｒ ¤sƒ¤‟Ｇ¡Ａ Ｓ ˘ fl Ｓ ｏ ‟ ' ˙ Ｎ·¡ＧＺ† ‹ ¤sƒ¤‟Ｇ¥i ƒ ” ／h ‰ ／ (11) ¡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.

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