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The Role of MEK Kinase 1 in Bcr-Abl-Induced Self-Renewal Activity of Embryonic Stem Cells

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Abstract BCR-ABL oncogene, the molecular hallmark of chronic myelogenous leukemia, arises in a primitive hematopoietic stem cell that has the capacity for both differentiation and self-renewal. Its product, Bcr-Abl protein, has been shown to activate signal transducers and activators of transcription 3 (STAT3) and to promote self-renewal in embryonic stem (ES) cells, even in the absence of leukemia inhibitory factor (LIF). MEK kinase 1 (MEKK1) is a 196-kDa mitogen-activated protein kinase (MAPK) kinase kinase involved in Bcr-Abl signal transduction. To investigate the role of MEKK1 in Bcr-Abl-induced transformation of stem cells, p210 Bcr-Abl was stably transfected into wild type (WT^{p210}) and MEKK1-/- ($MEKK1\text{-}/\text{-}^{p210}$) ES cells. Bcr-Abl enhanced MEKK1 expression in ES transfectants, as it does in other Bcr-Abl-transformed cells. In the absence of LIF, WT^{p210} cells showed constitutive STAT3 activation and formed rounded, compact colonies having strong alkaline phosphatase activity, a characteristic phenotype of undifferentiated ES cells. $MEKK1\text{-}/\text{-}^{p210}$ cells, by contrast, showed less STAT3 activity than WT^{p210} cells and formed large, flattened colonies having weak alkaline phosphatase activity, a phenotype of differentiated ES cells. These results indicate that MEKK1 plays a key role in Bcr-Abl-induced STAT3 activation and in ES cells' capacity for LIF-independent self-renewal, and may thus be involved in Bcr-Abl-mediated leukemogenesis in stem cells.

Key words: bcr-abl, mek kinase 1, stat3, self renewal activity, embryonic stem cells

Introduction

Chronic myelogenous leukemia (CML) is a neoplasm arising from primitive hematopoietic cells expressing the 210-kDa form of Bcr-Abl, a chimeric oncoprotein generated by the reciprocal translocation t(9;22).[1] Patients with CML have a rare but consistently detectable population of quiescent Bcr-Abl-positive $CD34^+$ cells that possess an ability for self-renewal and contribute to the disease's pathogenesis.[2] One recent study showed that Bcr-Abl constitutively activates signal transducers and activators of transcription 3 (STAT3) and promotes self-renewal of embryonic stem (ES) cells, even in the absence of leukemia inhibitory factor (LIF). Similarly, $CD34^+$ cells purified from CML patients show increased levels of STAT3 activation.[3]

MEK kinase 1 (MEKK1) is a 196-kDa mitogen-activated protein kinase (MAPK) kinase kinase that is activated in response to a variety of stimuli, and has been shown to participate in the regulation of c-Jun N-terminal kinase (JNK) and extracellular signal regulated kinase (ERK) activity and to be involved in the activation of NF-κB.[4] In addition, we previously showed that cell transformation induced by Bcr-Abl leads to increased expression of MEKK1, which then serves as a downstream target of Bcr-Abl, mediating an anti-apoptotic effect.[5]

To investigate the role of MEKK1 in Bcr-Abl-induced transformation of stem cells, we used a stable line of p210 Bcr-Abl-transfected MEKK1-/- ES cells, and examined the effects of disrupting MEKK1 expression on the Bcr-Abl-induced undifferentiated phenotype and constitutive STAT3 activation. Our findings show for the first time that the MEKK1-STAT3 pathway is a downstream target of Bcr-Abl that mediates maintenance of the undifferentiated phenotype of ES cells.[6)]

Bcr-Abl increases MEKK1 expression in ES cells

We began investigating the relationship between Bcr-Abl and MEKK1 by evaluating MEKK1 expression in p210 Bcr-Abl-transformed ES cells. We found that expression of MEKK1 protein was constitutively increased in Bcr-Abl-transformed wild type (WT^{p210}) cells, as compared to green fluorescent protein (GFP)-transformed wild type (WT^{GFP}) cells. Furthermore, treatment of imatinib mesylate (STI571), a selective inhibitor of Bcr-Abl tyrosine kinase activity,[7)] reduced the expression of MEKK1 in WT^{p210} cells. These results indicate that Bcr-Abl increases MEKK1 expression in ES cells and that the effect is largely dependent on Bcr-Abl tyrosine kinase activity.

MEKK1 is required for inhibition of ES cell differentiation by Bcr-Abl

Introduction of the Bcr-Abl oncogene into wild type and MEKK1-/- ES cells did not induce any growth advantage beyond the control. On the other hand, WT^{p210} cells formed compact, rounded colonies, a phenotype characteristic of undifferentiated ES cells on uncoated culture dishes without LIF. By contrast, MEKK1-/-^{p210} cells showed large, flattened colonies, a phenotype characteristic of differentiated ES cells. To confirm the differentiation status of cells, we measured alkaline phosphatase activity[8)] and found that, whereas about 67% of WT^{p210} cells were alkaline phosphatase-positive in the absence of LIF, MEKK1-/-^{p210} cells were almost all alkaline phosphatase-negative. This suggests that Bcr-Abl confers the ability to maintain an undifferentiated state in the absence of LIF, and that this effect is mediated by MEKK1.

MEKK1 is essential for Bcr-Abl-induced STAT3 activation

One recent study showed that Bcr-Abl constitutively activates STAT3, thereby promoting self-renewal in ES cells even in the absence of LIF.[3)] We therefore investigated the extent to which MEKK1 is involved in Bcr-Abl-induced STAT3 activation. Using immunoblot analyses, electrophoretic mobility shift assays and luciferase assays, we found that, in the absence of LIF, STAT3 was more strongly activated in WT^{p210} cells than in WT^{GFP} cells. By contrast, the level of activated STAT3 in MEKK1-/-^{p210} cells was similar to that in MEKK1-/-^{GFP} cells, which suggests that MEKK1 is required for Bcr-Abl-induced STAT3 activation. Furthermore, reconstitution of MEKK1-/- ES cells by transfecting them with wild-type MEKK1 (MEKK1 add-back) restored the ability of Bcr-Abl to activate STAT3, confirming that MEKK1 is required for Bcr-Abl-induced STAT3 activation.

Bcr-Abl-induced MEKK1-STAT3 pathway is not involved with ERK, JNK, JAK2 or Rac1

We next investigated which intracellular signaling protein(s) is involved in the Bcr-Abl-MEKK1-STAT3 pathway. Bcr-Abl enhanced ERK activation in both WT^{p210} and MEKK1-/-^{p210} cells in the absence of LIF. On the other hand, JNK activation was not elevated in either cell type, and MEKK1-/-^{GFP} and MEKK1-/-^{p210} cells, respectively, showed less ERK activity than WT^{GFP} and WT^{p210} cells. Furthermore, the experiment with the MEK1/2 inhibitor U0126 suggested that the reduced ERK activity seen in MEKK1-/-^{p210} cells did not cause a decrease in STAT3 activation. Janus kinase 2 (JAK2) and small GTPase Rac1 are physiological regulators of STAT3 activation,[9)10)] but we found that activation of JAK2 and Rac1 were unaffected by Bcr-Abl. Therefore, our findings suggest that Bcr-Abl activates a MEKK1-STAT3 pathway, though not through ERK, JNK,

JAK2 or Rac1.

Conclusion

During the chronic phase of CML, Bcr-Abl-expressing leukemic stem cells are characterized by their persistent ability to differentiate towards all hematopoietic lineages. The signaling pathway by which the Bcr-Abl-expressing clone expands and undergoes self-renewal remains largely unknown, however. We therefore used murine undifferentiated ES cells to investigate the early molecular events that occur in Bcr-Abl-associated leukemogenesis. Surprisingly, the ability of Bcr-Abl, in the absence of LIF, to induce changes in both the morphology of ES cell colonies and alkaline phosphatase activity that were consistent with an undifferentiated phenotype was MEKK1-dependent, suggesting MEKK1 plays a key role in Bcr-Abl-induced self-renewal of ES cells via a Bcr-Abl-mediated transformation pathway.

The role of STAT3 activation in the promotion of self-renewal in ES cells is well established[11]; indeed, constitutive activation of STAT3 has been shown not only in Bcr-Abl-expressing ES cells but also in primary CML cells.[3] Nevertheless, it was unexpected that MEKK1 would be required for Bcr-Abl-induced STAT3 activation in ES cells. Our findings with genetically deficient cells enable us to report for the first time that not only is MEKK1 involved in STAT3 activation but its action is independent of MAPK family pathways (Fig. 1).

In conclusion, our findings suggest that MEKK1 plays a key role in self-renewal and STAT3 activation in Bcr-Abl-transformed ES cells. Although it remains to be determined precisely how MEKK1 contributes to Bcr-Abl-induced transformation of hematopoietic stem cells, our findings suggest MEKK1 may be a useful therapeutic target for the treatment of CML.

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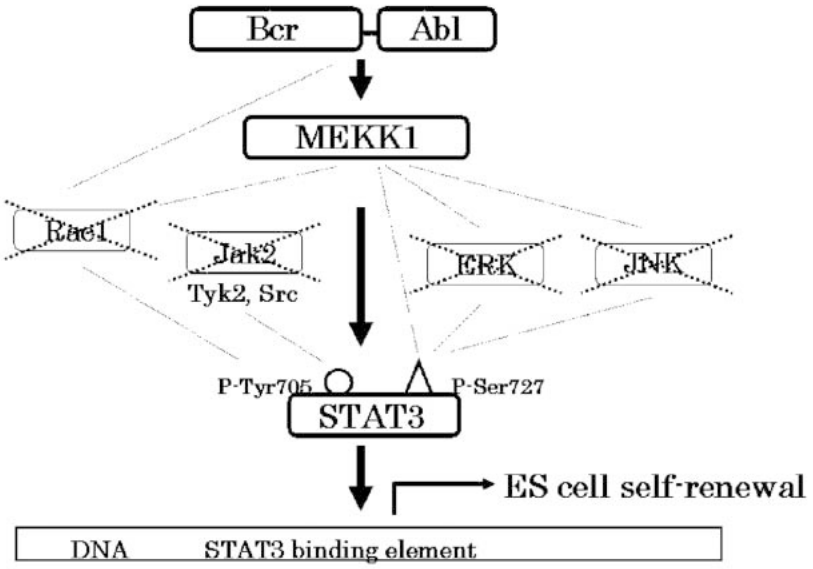

Fig. 1 MEKK1 is essential for Bcr-Abl-induced STAT3 activation and LIF-independent self-renewal activity of embryonic stem cells. ERK, JNK, JAK2 or Rac1 is not involved in this MEKK1-dependent STAT3 activation.

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