Effect of Mutationally Activated Ras on the Ras to MAP Kinase Signaling Pathway and Growth Inhibition of Myeloid Leukemia Cells by Inhibitors of the MAP Kinase Cascade

CH. W. M. REUTER*, M. A. MORGAN and I. BERGMANN

Summary

Ras proteins are small G-proteins that have been shown to play a key role in signal transduction, proliferation and malignant transformation. Mutations in the ras genes have been implicated in a large number of human cancers including myeloid leukemias (AML, CML, CMML, JCML). Transfection of mutationally activated Ras into NIH3T3 fibroblasts resulted in a transformed phenotype and activation of the MAP kinase signal transduction pathway (MAP kinase kinase activators, B-Raf and c-Raf-1 [5 fold and 17 fold], MAP kinase kinases, MEK-1 and MEK-2 [6-8 fold], and MAP kinases, ERK-1 and ERK-2 [3-12 fold]). The activation of the MAP kinase cascade was dependent on both the type and the expression level of the Ras proteins. Enhancement of the MAP kinase cascade was not observed in cells overexpressing wild-type c-H-Ras. Treatment of the cells with inhibitors of MAP kinase signaling (e.g. MEK and FTase inhibitors) resulted in a reversion of the Ras-induced transformed phenotype of these fibroblasts. In addition, 10 myeloid leukemia cell lines were tested for MAP kinase activation and the effect of these inhibitors on leukemia cell growth. In five cell lines MAP kinases ERK-1 and ERK-2 were found to be constitutively activated. Incubation with the MEK inhibitor resulted in a significant inhibition of the colony formation of these cell lines (60-100%). Maximal inhibition of cell growth was observed after 7 days of incubation and hematopoietic growth factor dependent cell lines were most sensitive to PD 098059. Most FPTase inhibitors were less effective in the inhibition of myeloid leukemia growth; the FPTase inhibitor 3 inhibited growth of leukemic cells by 95-100%. These results support the potential role of inhibitors of the Ras to MAP kinase signaling pathway in the treatment of myeloid leukemias.

Introduction

The deregulation of Ras function appears to be a common theme in the molecular pathogenesis of myeloid leukemias. Activating ras mutations were identified in up to 30% of both adult and childhood acute myeloid leukemia (AML) (Bos 1989, Clark & Der 1995). The mutations arise at codons 12, 13 and 61 of N-ras and occasionally K-ras. These mutations lead to the production of constitutively activated Ras proteins that cannot be switched off. Activating mutations of Ras may result in uncontrolled growth-factor-independent proliferation of hematopoietic progenitors and/or accumulation due to reduced levels of apoptosis (Byrne & Marshall, 1998). In addition, over-expression of all three ras genes due to mutations in the promotor region of ras has been implicated in the leukemogenesis of myeloid leukemias.

Deregulation of Ras in myeloid leukemias can also occur by expression of constitutively activated versions of normal proto-oncogenes and tumor suppressor genes. Philadelphia-chromosome positive chronic myelogeneous leukemia (CML) is a myeloproliferative condition characterized by the presence of a balanced translocation between chromosomes 9 and 22, t(9;22), which leads to the
expression of a BCR-Abl fusion tyrosine kinase. The expression of this deregulated chimeric protein results in cellular transformation by abrogation of growth factor dependence, blockade of differentiation and direct inhibition of apoptosis. The involvement of Ras has been demonstrated by the presence of increased levels of GTP-Ras in cells expressing BCR-Abl (Cortez et al. 1995).

Another fusion tyrosine kinase, which is the result of the t(5;12) translocation found in a subset of patients with chronic myelomonocytic leukemia (CMML), consists of Tel, a member of the Ets-family of transcription factors, and the platelet-derived growth factor receptor β (PDGF-R β). The constitutive activation of Ras is thought to be a result of the Tel-PDGF-R β fusion protein dimerization in the absence of ligand (Golub et al. 1994).

Patients with juvenile chronic myeloid leukemia (JCML) commonly show activating ras mutations or loss of the neurofibromin type 1 (NF-1) gene. The NF-1 gene encodes a Ras GTPase activating protein which down-regulates Ras (Xu et al. 1990). This gene is also inactivated in the autosomal dominant condition neurofibromatosis type 1. Deletions of NF-1 lead to moderate but consistent elevation of GTP-Ras levels in leukemic cells from children with neurofibromatosis type 1 (Bollag et al. 1996).

Ras activation may also occur as a result of point mutations of receptor tyrosine kinases which are upstream of Ras. Mutation of c-Fms, the M-CSF receptor or the c-kit receptor activate proliferation of hematopoietic cells (Carlberg et al. 1994, Kitayama et al. 1995).

In the GTP-bound state, Ras interacts with various downstream effectors leading to cellular proliferation, differentiation or protection against apoptosis. These Ras effectors include the Raf protein kinases, phosphatidylinositol 3-OH kinase, PI(3)-K, and Raf-GDS (Marshall 1996, Byrne & Marshall 1998). The interaction of the Raf kinases (A-Raf, B-Raf, and c-Raf-1) with GTP-Ras results in the phosphorylation and activation of the MAP kinase kinases, MEK-1 and MEK-2, which in turn phosphorylate and activate the MAP kinases ERK-1 and ERK-2. These three sequential kinases are the core components of the Ras to MAP kinase signaling cascade (Fig. 1). Activated ERKs translocate into the nucleus where they phosphorylate transcription factors such as Elk-1, c-Myc and CREB and thereby regulate gene expression.

The role of Ras in the pathophysiology of myeloid leukemias has considerable potential implications for a therapeutic approach which targets the Ras to MAP kinase signaling pathway. To address this issue we studied the effect of mutationally activated Ras on MAP kinase activation in NIH3T3 fibroblasts and the potential of inhibitors of the Ras to MAP kinase cascade to revert the Ras-induced, transformed phenotype. Furthermore, we demonstrate the constitutive activation of ERK-1 and ERK-2 in several human myeloid leukemia cell lines as well as growth inhibition of leukemia cells incubated with various inhibitors of the Ras to MAP kinase cascade. These results suggest a potential role of these inhibitors in the treatment of myeloid leukemias.