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ORIGINAL ARTICLE

Frequent loss of RAF kinase inhibitor protein expression in acute myeloid leukemia

A Zebisch[1,2,3], A Wölfler[1], I Fried[1], O Wolf[1], K Lind[1], C Bodner[1], M Haller[4], A Drasche[4], D Pirkebner[4], D Matallanas[2], O Rath[3], K Blyth[3], R Delwel[5], E Taskesen[5], F Quehenberger[6], W Kolch[2,7], J Troppmair[4,7] and H Sill[1,7]

RAF kinase inhibitor protein (RKIP) is a negative regulator of the RAS-mitogen-activated protein kinase/extracellular signal-regulated kinase signaling cascade. We investigated its role in acute myeloid leukemia (AML), an aggressive malignancy arising from hematopoietic stem and progenitor cells (HSPCs). Western blot analysis revealed loss of RKIP expression in 19/103 (18%) primary AML samples and 4/17 (24%) AML cell lines but not in 10 CD34 + HSPC specimens. In *in-vitro* experiments with myeloid cell lines, RKIP overexpression inhibited cellular proliferation and colony formation in soft agar. Analysis of two cohorts with 103 and 285 AML patients, respectively, established a correlation of decreased RKIP expression with monocytic phenotypes. RKIP loss was associated with RAS mutations and in transformation assays, RKIP decreased the oncogenic potential of mutant *RAS*. Loss of RKIP further related to a significantly longer relapse-free survival and overall survival in uni- and multivariate analyses. Our data show that RKIP is frequently lost in AML and correlates with monocytic phenotypes and mutations in *RAS*. RKIP inhibits proliferation and transformation of myeloid cells and decreases transformation induced by mutant *RAS*. Finally, loss of RKIP seems to be a favorable prognostic parameter in patients with AML.

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Keywords: RAF kinase inhibitor protein; acute myeloid leukemia; *RAS* mutation

INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive malignancy caused by transformation of hematopoietic stem and progenitor cells (HSPCs). It is a heterogeneous disorder characterized by differentiation defects and uncontrolled growth of the leukemic clone ultimately leading to bone marrow failure. Classification of AML is based on clinical, morphological, immunological and genetic parameters.[1–4] Despite intensive treatment strategies including stem cell transplantation, the prognosis of patients with AML is still dismal with the majority succumbing to resistant disease.[3]

The RAS-mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway is activated by a spectrum of cytokine receptors in response to ligand binding and plays an important role with respect to proliferation, survival and differentiation of hematopoietic progenitors. Transmission of signals from the cell surface to intracellular effectors within this pathway is mediated by RAS-induced activation of a three-tiered kinase cascade comprising RAF, MEK and MAPK/ERK.[5,6] Oncogenic mutations affecting this pathway are frequently observed in human cancers with *NRAS* or *KRAS* mutations occurring in about 20% of AML patients.[5] Constitutive RAS-MAPK/ERK signaling is also initiated by somatic mutations in genes encoding the upstream *FLT3* and *c-Kit* receptor tyrosine kinases in an additional 25–40% of AML cases.[3,7,8]

RAF kinase inhibitor protein (RKIP)—also known as PEBP1, phosphatidylethanolamine binding protein 1—has been identified as a negative regulator of the RAS-MAPK/ERK signaling cascade. It inhibits the interaction between C-RAF (also termed RAF1) and MEK thereby preventing C-RAF-mediated MEK phosphorylation, which is necessary for signal propagation.[9] More recently, RAS-MAPK/ERK independent functions have been described. RKIP suppresses the activity of the nuclear factor-κB–Snail circuitry and inhibits the epithelial-to-mesenchymal transition program, which is a pivotal step in tumor invasion and the formation of metastasis.[10–12] In several solid neoplasms, RKIP expression is indeed frequently reduced or absent, and while this has no effect on the growth of the primary tumor it correlates with an increased risk of metastatic disease and enhanced invasiveness of cancer cells *in vitro*.[13–16]

We previously described loss of RKIP in patients with therapy-related AML and *C-RAF* germline mutations.[17,18] RKIP silencing was shown to be a somatic, leukemia-specific event and contributed to C-RAF driven malignant transformation. However, it is unknown whether loss of RKIP is restricted to this small subset of cases or whether it is of broader significance for myeloid leukemogenesis.

PATIENTS AND METHODS

Patient samples and cell lines

One hundred three blood and bone marrow samples from patients with AML (AML cohort 1) were collected at the Division of Hematology, Medical University of Graz, Graz, Austria, and processed as described.[18,19] Ninety-

[1]Division of Hematology, Medical University of Graz, Graz, Austria; [2]Systems Biology Ireland, University College Dublin, Dublin, Ireland; [3]The Beatson Institute for Cancer Research, Glasgow, UK; [4]Daniel Swarovski Research Laboratory, Innsbruck Medical University, Innsbruck, Austria; [5]Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands and [6]Institute of Medical Informatics, Statistics and Documentation, Medical University of Graz, Graz, Austria. Correspondence: Dr H Sill, Division of Hematology, Medical University of Graz, Auenbruggerplatz 38, Graz 8036, Austria.
E-mail: heinz.sill@medunigraz.at
[7]These authors contributed equally to this work.
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