NF1 Inactivation Revs Up Ras in Adult Acute Myelogenous Leukemia

Ann Mullally and Benjamin L. Ebert

Division of Hematology, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA.

Corresponding Author:
Benjamin L. Ebert
1 Blackfan Circle
Karp Building 5.210
Boston, MA 02115
bebert@partners.org

Phone: (617) 355-9060
Fax: (617) 355-9091

Summary

Mutations in the Ras pathway are common in myeloid malignancies. NF1, a tumor suppressor and negative regulator of Ras is inactivated in a subset of adult acute myelogenous leukemia (AML) cases. Loss of NF1 function sensitizes cells to inhibition of mTOR, a downstream effector of Ras activation, highlighting a potential therapeutic opportunity for some AML patients.
In this issue of *Clinical Cancer Research*, Parkin et al. report on the identification of a subset of adult acute myelogenous leukemia (AML) in which the tumor suppressor, NF1 is functionally inactive, resulting in increased Ras signaling and sensitivity to mTOR inhibition (1). The authors found that AML CD34+/C38- cells with absent NF1 expression are sensitive to treatment with rapamycin, thus implicating mTOR as a therapeutic target within the leukemia-initiating compartment of a small subgroup of adult AML patients.

AML is a highly malignant hematopoietic neoplasm characterized by a poor prognosis, especially in older patients. In addition to age, the major prognostic marker is cytogenetics and the mutational status of selected genes (e.g. *NPM1, FLT3*) strongly influence prognosis in the 50% of AML cases that are cytogenetically normal (2). The treatment of AML has changed little in the past several decades and the discovery of additional leukemia-initiating genes in this disease has the potential to identify novel therapeutic targets. The approach employed by Parkin et al. (1), using high-density SNP microarrays to identify chromosomal loci with recurrent somatic copy number abnormalities, has proved successful in this regard, and was used to identify TET2 and c-CBL as pathogenic genes in myeloid malignancies (3), (4).

Parkin et al. report on their detailed investigation of purified leukemic blasts and paired buccal DNA samples from 95 adult AML patients, the majority of whom had primary AML and were untreated (1). Using high-density SNP microarrays, the authors identified recurrent, somatically acquired microdeletions on chromosome 17q. They defined a minimally deleted region (MDR) spanning ~0.9Mb, distinct from the *p53* locus, containing the *NF1* gene. Copy number alterations in *NF1* were identified in 11/95 (12%) of the patients in the cohort (ten patients had heterozygous deletions and one had a copy gain). The authors sequenced all coding exons of *NF1* in each of these 11 samples and found acquired mutations in 2 cases. They measured *NF1* expression in all AML blast samples for which sufficient RNA was available and found reduced *NF1* expression in 7/10 samples with heterozygous deletions, absent *NF1* expression in 3/10 samples with heterozygous deletions and absent *NF1* expression in 3 samples that had normal *NF1* copy number. Increased Ras signaling was evident only when *NF1* expression was absent.

*NF1* encodes neurofibromin, a GTPase-activating protein and negative regulator of Ras. Ras signaling is an important biochemical pathway in myeloid malignancies. Activating somatic *NRAS* and *KRAS* mutations occur in approximately 20% of AML, 40% of chronic myelomonocytic leukemia (CMML) and 30% of juvenile myelomonocytic leukemia (JMML) cases (5). Children with Neurofibromatosis type 1, a dominant familial cancer syndrome have germline inactivating mutations in *NF1*, and are at markedly increased risk of developing JMML (6). Activating mutations in *PTPN11*, which encodes the protein tyrosine phosphatase SHP-2, potentiate Ras signaling and are present in approximately 35% of sporadic JMML. In AML, activating somatic mutations in the *FLT3* tyrosine kinase result in deregulated Ras signaling (5). Finally, *NF1* has recently been shown to be subject to copy number alteration in adult AML (7). In aggregate, these
genetic findings demonstrate that hyperactive Ras signaling is an important pathogenic pathway in myeloid cancers.

Direct therapeutic inhibition of Ras has proved difficult. As a result, therapeutic efforts have also focused on targeting downstream components of the Ras pathway e.g. Raf/MEK/ERK and PI3K/AKT/mTOR. The success of this strategy requires cancer cells to have a differential molecular dependency on downstream effectors of Ras for survival, as compared with normal cells. mTOR is a serine/threonine kinase that functions downstream of Ras and PI3K/AKT, and mTOR has been shown to be negatively regulated by NF1 (8). In a series of experiments described in this issue of *Clinical Cancer Research*, Parkin et al. move from bedside to bench by identifying a subset of primary AML blast cells with absent functional NF1, then demonstrating increased Ras signaling in these cells, and finally showing that AML blasts that do not express NF1 display differential sensitivity to apoptotic cell death in response to *in vitro* treatment with the mTOR inhibitor rapamycin, relative to blasts with intact NF1 function (Figure 1) (1). Given that there are few effective targeted therapies currently available in AML, these findings have important therapeutic implications and identify a subgroup of adult AML patients in whom clinical investigation of an mTOR inhibitor is warranted.

Validating mTOR dependence *in vivo* is an important next step, particularly in view of the limited clinical efficacy that single agent mTOR inhibitors have shown to date in clinical trials in relapsed or refractory AML (9). The rationale behind clinical trials of mTOR inhibitors in AML arose in part from the demonstration that constitutive phosphorylation of AKT is seen in the majority of primary AML samples (10), and that inhibiting downstream effectors of AKT would preferentially target AML cells; however mTOR inhibitor clinical trial results have not borne this out so far (9). Parkin et al. describe an analogous situation *in vitro* with respect to NF1 inactivation (1), so validating that AML cells that do not express NF1 remain mTOR dependent *in vivo* will be critical. On a practical level, the identification of AML patients with absent NF1 expression but normal NF1 copy number may prove challenging in routine clinical practice. To identify patients with mutated, deleted, or silenced NF1 might require the integrated use of fluorescent in situ hybridization (FISH), immunohistochemistry (IHC), DNA sequencing, and/or quantitative RT-PCR. Finally, AML patients with normal NF1 copy number but absent NF1 expression may be more likely to recover NF1 expression in response to mTOR inhibition than patients with an inactivating NF1 mutation.

In JMML, RAS, NF1 and PTPN11 mutations are usually mutually exclusive of each other (11) and given that activating mutations in NRAS and KRAS are more common than NF1 inactivation in AML, establishing if RAS mutated AML phenocopies NF1 inactivation in AML will be important. It has been shown that AML patients with RAS mutations derive the most benefit from high dose cytarabine (HIDAC) as post-remission therapy (12). Further studies to determine if the specific genetic mechanisms underlying Ras activation influence therapeutic susceptibilities in AML will be interesting, e.g. will AML...
patients with RAS mutations be sensitive to treatment with mTOR inhibitors and will patients with absent NF1 expression respond to HIDAC?

In summary, the study by Parkin et al. deepens our understanding of the pathogenic role of NF1 in adult AML (1), and in doing so identifies a molecular subgroup of AML with a rational therapeutic target. Progress in developing curative targeted therapies for AML will be made through delineating the full spectrum of genetic abnormalities that underpin this disease and determining their differential therapeutic susceptibilities.

References:

Figure Legend:

Illustration of a limited part of the Ras signaling pathway indicating loss of NF1 resulting in Ras activation and increased survival of AML cells. mTOR is a downstream effector of Ras and is inhibited by rapamycin (RAPA) resulting in apoptosis of AML cells. GF, growth factor; RTK, receptor tyrosine kinase.
Ras

GDP

GTP

PI3K

AKT

mTOR

Normal

AML

AML + Rapamycin

GF

RTK

Ras

GDP

GTP

NF1

NF1

π Survival

Apoptosis

Inactive

Active

Inhibition

Research.

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