

NF1 Inactivation Revs Up Ras in Adult Acute Myelogenous Leukemia

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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 mammalian target of rapamycin (mTOR), a downstream effector of Ras activation, highlighting a potential therapeutic opportunity for some AML patients. *Clin Cancer Res*; 16(16); 4074–6. ©2010 AACR.

In this issue of *Clinical Cancer Research*, Parkin and colleagues 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 mammalian target of rapamycin (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 and colleagues (1), using high-density single nucleotide polymorphism (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 and colleagues 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 spanning ~0.9 Mb, distinct from the *p53* locus, containing the *NF1* gene. Copy number alterations in *NF1* were identified in 11 out of 95 (12%) of the patients in the cohort (10 patients had heterozygous deletions and 1 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 out of 10 samples with heterozygous deletions, absent *NF1* expression in 3 out of 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, 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 show 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, for example, Raf/MEK/extracellular signal-regulated kinase (ERK) and phosphoinositide 3-kinase (PI3K)/AKT/mTOR. The success of this strategy requires cancer cells to have a

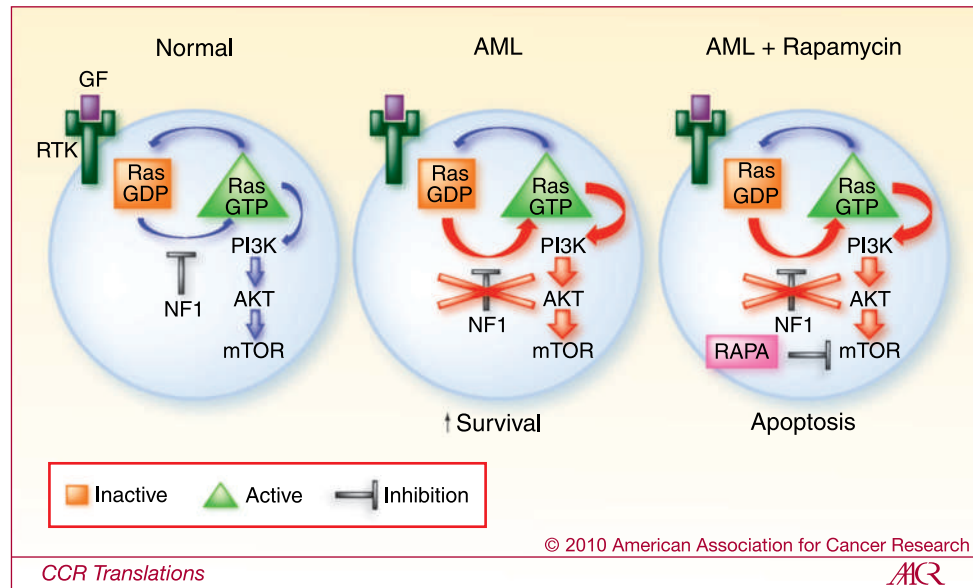
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Fig. 1. A limited part of the Ras signaling pathway indicating loss of NF1, resulting in Ras activation and increased survival of AML cells. mTOR is a downstream effector of Ras and is inhibited by rapamycin (RAPA), resulting in apoptosis of AML cells. GF, growth factor; RTK, receptor tyrosine kinase.



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 and colleagues move from bedside to bench by identifying a subset of primary AML blast cells with absent functional NF1, 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 (Fig. 1; ref. 1). Given that few effective targeted therapies are 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 showing 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 and colleagues describe an analogous situation *in vitro* with respect to NF1 inactivation (1); therefore, 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 with 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, immunohistochemistry, DNA sequencing, and/or quantitative reverse transcriptase-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 (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 postremission therapy (12). Further studies to determine if the specific genetic mechanisms underlying Ras activation influence therapeutic susceptibilities in AML will be interesting; for example, 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 and colleagues 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 by determining their differential therapeutic susceptibilities.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Parkin B, Ouilllette P, Wang Y, et al. NF1 inactivation in adult acute myelogenous leukemia. *Clin Cancer Res* 2010;16:4135–47.
2. Estey E, Dohner H. Acute myeloid leukaemia. *Lancet* 2006;368:1894–907.
3. Delhommeau F, Dupont S, Della Valle V, et al. Mutation in TET2 in myeloid cancers. *N Engl J Med* 2009;360:2289–301.
4. Dunbar AJ, Gondek LP, O'Keefe CL, et al. 250K single nucleotide polymorphism array karyotyping identifies acquired uniparental disomy and homozygous mutations, including novel missense substitutions of c-Cbl, in myeloid malignancies. *Cancer Res* 2008;68:10349–57.
5. Braun BS, Shannon K. Targeting Ras in myeloid leukemias. *Clin Cancer Res* 2008;14:2249–52.
6. Shannon KM, O'Connell P, Martin GA, et al. Loss of the normal NF1 allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. *N Engl J Med* 1994;330:597–601.
7. Walter MJ, Payton JE, Ries RE, et al. Acquired copy number alterations in adult acute myeloid leukemia genomes. *Proc Natl Acad Sci U S A* 2009;106:12950–5.
8. Johannessen CM, Reczek EE, James MF, Brems H, Legius E, Cichowski K. The NF1 tumor suppressor critically regulates TSC2 and mTOR. *Proc Natl Acad Sci U S A* 2005;102:8573–8.
9. Rizzieri DA, Feldman E, Dipersio JF, et al. A phase 2 clinical trial of deforolimus (AP23573, MK-8669), a novel mammalian target of rapamycin inhibitor, in patients with relapsed or refractory hematologic malignancies. *Clin Cancer Res* 2008;14:2756–62.
10. Xu Q, Simpson SE, Scialla TJ, Bagg A, Carroll M. Survival of acute myeloid leukemia cells requires PI3 kinase activation. *Blood* 2003;102:972–80.
11. Emanuel PD. Juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia. *Leukemia* 2008;22:1335–42.
12. Neubauer A, Maharry K, Mrozek K, et al. Patients with acute myeloid leukemia and RAS mutations benefit most from postremission high-dose cytarabine: a Cancer and Leukemia Group B study. *J Clin Oncol* 2008;26:4603–9.