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Novel recurrent mutations in the *RAS*-like GTP-binding gene *RIT1* in myeloid malignancies

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Availability of next-generation sequencing has provided many insights into the clonal architecture and dynamics of leukemogenic events,[1] but in a significant proportion (10–50%), causative molecular lesions have not yet been identified. Here we report the discovery of novel somatic mutations in the *RIT1* gene in patients with myeloproliferative or mixed myelodysplastic/myeloproliferative neoplasms (MDS/MPN), in particular, chronic myelomonocytic leukemia (CMML).

Somatic mutations of the Ras gene family are present in 20–30% of all human cancers,[2] including 15–20% of acute myeloid leukemia (AML), 10–50% of MDS and 11–27% of MDS/MPN cases.[3,4] In hematology, certain forms of congenital rasopathies are associated with juvenile myelomonocytic leukemia.[5,6] Although all Ras family members are characterized by guanosine-triphosphatase (GTPase) activity and maintain highly conserved domains (G1–G5), they possess distinct biochemical and biological activities and express both overlapping and unique functions under different conditions and in different cell types.[7] Ras-like-without-CAAX-1 (*RIT1*) gene is a new member of the family and has been found to be critical in neuron stress-mediated survival.[8] Moreover, *RIT1* has been reported to be overexpressed due to amplification and occasionally mutation in a proportion (25%) of patients with hepatocellular carcinoma (HCC).[9,10]

We applied whole-exome sequencing to a subgroup of patients with various forms of myeloid malignancies (seven MDS and AML with myelodysplasia-related changes (AML-MRC) and eight MDS/MPN) and found two closely positioned somatic *RIT1* mutations in two cases with CMML and AML-MRC (c.T244A and c.T245G) corresponding to the codon F82 (Figure 1). Sequencing of serial informative cases showed the presence of *RIT1* mutation early at the initial diagnosis, suggesting its ancestral origin (Supplementary Figure 1A). Screening for *RIT1* mutations was expanded to a total of 722 patients with various

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

myeloid neoplasms (Supplementary Table 1): all together, nine mutant cases were found: six in the amino acid F82, two in E81 and one in M90. All mutations were located on the Switch II effector domain of this protein, which is conserved among species (Figure 1). Of note, the implicated residues are very close to codon Q79; this codon is analogous to the amino acid Q61 in Ras family proteins such as NRAS or KRAS, where mutations frequently occur in cancer. Moreover, the experimental Q79L mutation in *RIT1* has been reported to confer constitutive activation of the protein.[11]

Parallel analyses using SNP-array-based karyotyping and sequencing demonstrated that mutations were heterozygous with the exception of one case, which showed 1q amplification involving the *RIT1* locus: in this case the mutant allele was duplicated (Supplementary Figure 2A). Complimentary DNA sequencing revealed that both mutant and wild-type alleles were equally expressed in all cases (Supplementary Figure 2B).

The E81Q somatic *RIT1* mutation and alterations of amino acids located toward the N-terminus of the protein have been described in solid cancers.[10] *RIT1* maps to the minimal common amplified region (1q21–22) in 1q gains frequently found in HCC (~25%); RIT1 amplification is associated with overexpression of the corresponding mRNA in these cases.[9] In our cohort we found 10 patients (1.8%) with 1q amplification involving the *RIT1* locus (Figure 1): RIT1 mutation was found only in one of these cases and, similar to HCC, amplifications were associated with mRNA overexpression (median relative ratio 1.29 in cases with 1q+ vs 0.18 in wild-type cases, P=0.041) (Figure 2A). The clinical phenotype of cases with 1q amplification resembled those with *RIT1* mutations, including CMML (n=3) and advanced MDS (n=3). The remaining four cases had myelofibrosis; three out of four were secondary to other forms of MPN (Supplementary Table 2). Similar to serial analyses of *RIT1* mutant cases, sequential cytogenetic evaluation showed the presence of 1q+ in the myelofibrotic stage, but absent at the initial polycythemia vera presentation (Supplementary Figure 1), consistent with the fact that chromosomal abnormalities are rare among chronic stages of MPN, but can be acquired at the time of fibrotic evolution.[12]

RIT1 has been shown to increase phosphorylation of AKT and thereby inhibit apoptosis and also activate proliferation through mitogen-activated protein kinase.[8,13,14] Consistent with these properties, we found that BAD expression was decreased while BCL2 increased in cells with RIT1 mutations or amplifications. Similarly, MYC mRNA levels were elevated in these cases.

Comparison of *RIT1* mutations with other somatic mutations in our whole-exome sequencing discovery cohort (Supplementary Table 9) demonstrated that mutations affecting Ras gene family are mutually exclusive (Figure 2d). Only in one *RIT1* mutant case with 1q amplification a concomitant *KRAS* mutation was found. *RIT1* lesions were less likely associated with *FLT3* mutations (2.4% vs 18.6%, P=0.003), but occurred more commonly with *DNMT3A* mutations (31% vs 16%, respectively, P=0.021). In myelodysplastic subcohort (MDS, MDS/MPN and AML-MRC), Ras and *RIT1* mutations were coincident with cohesin complex mutations (*STAG2*, *SMC1A*, *SMC3* and *RAD21*; 27% vs 5%, respectively, P=0.015). Similarly, when a larger cohort of patients was analyzed by Sanger sequencing, *TET2* mutant cases were coincident with *RIT1* abnormalities (30% of *RIT1*

mutant cases vs 9% in *RIT1* wild type, *P*=0.059) consistent with the presence of both mutations in MDS/MPN.

Because *RIT1* abnormalities occur in a diverse molecular context, a distinct phenotype was difficult to discern. However, *RIT1* abnormalities were significantly more frequent in CMML than other myeloid neoplasms (56% vs 9%, *P*=0.001) and also were associated with −7/del(7q) (33% vs 6%, *P*=0.017; Supplementary Table 3). *RIT1* mutant and amplified cases showed a trend toward having a shorter median overall survival (19 vs 14 months, *P*=0.053) in both the total cohort but more pronounced when studied in patients with MDS or MDS/MPN (24 vs 16 months, *P*=0.029) (Supplementary Table 4). Multivariate analysis showed that the impact of *RIT1* abnormalities was related to their occurrence with other high-risk features.

In conclusion, we demonstrate here that *RIT1* abnormalities, including activating mutations and locus amplifications, are novel lesions in a subgroup of patients with myeloid neoplasms, particularly frequent in CMML, where the percentage rose to 11% in our series, including 7% of mutations and 4% of locus duplications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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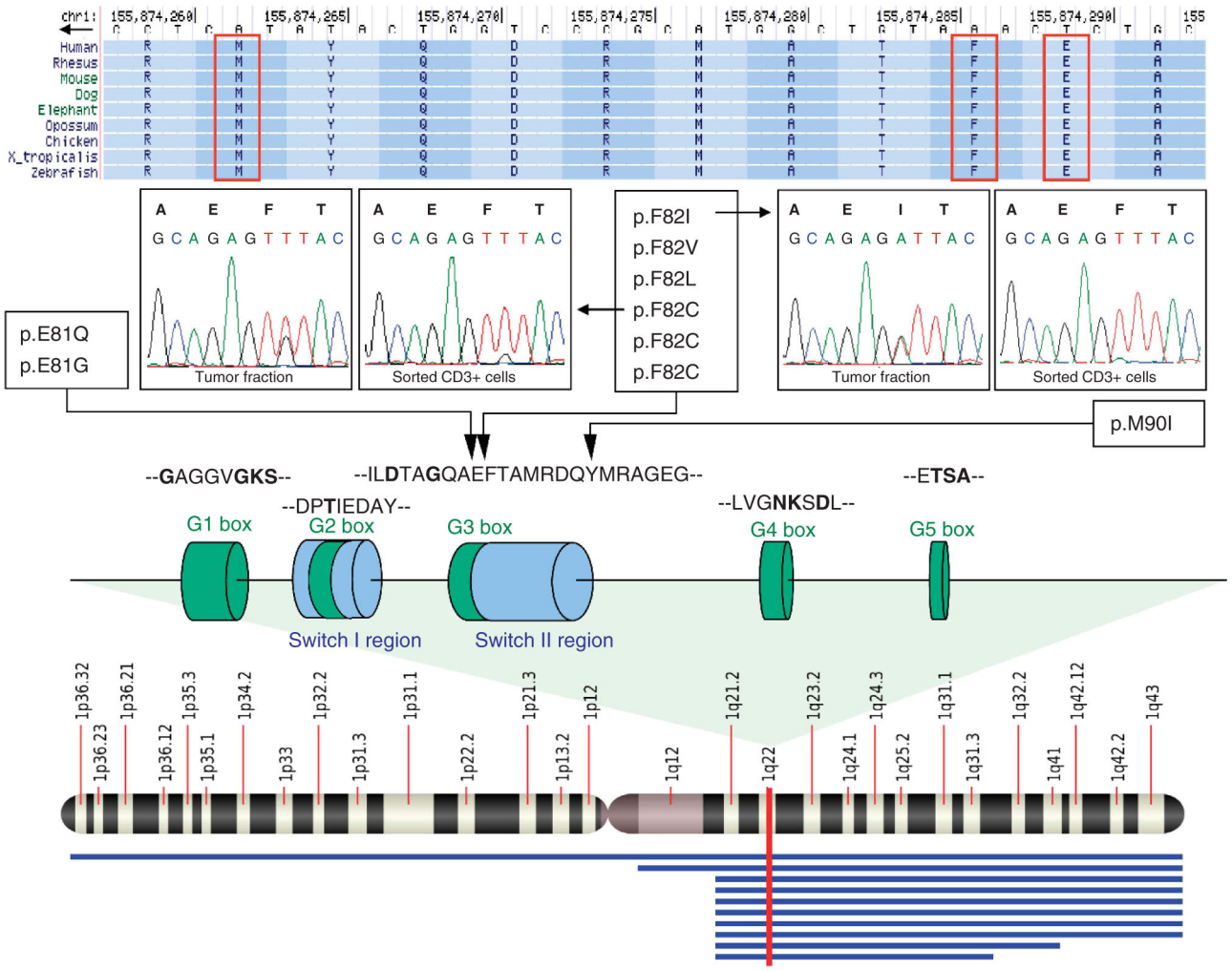

Figure 1.

Functional domains of RIT1, location of mutations and amplifications. Mutations are located in conserved sites among species. Amino acids in which a mutation was found are shown in a red rectangle. Sanger sequencing of tumor and CD3+ fraction of the two index cases with *RIT1* mutations. GTP-binding domains appear in green, whereas Switch effector domains appear in blue, both with codifying amino acids. *RIT1* is located at 1q22 (red bar). Blue bars show the amplified region in each patient.

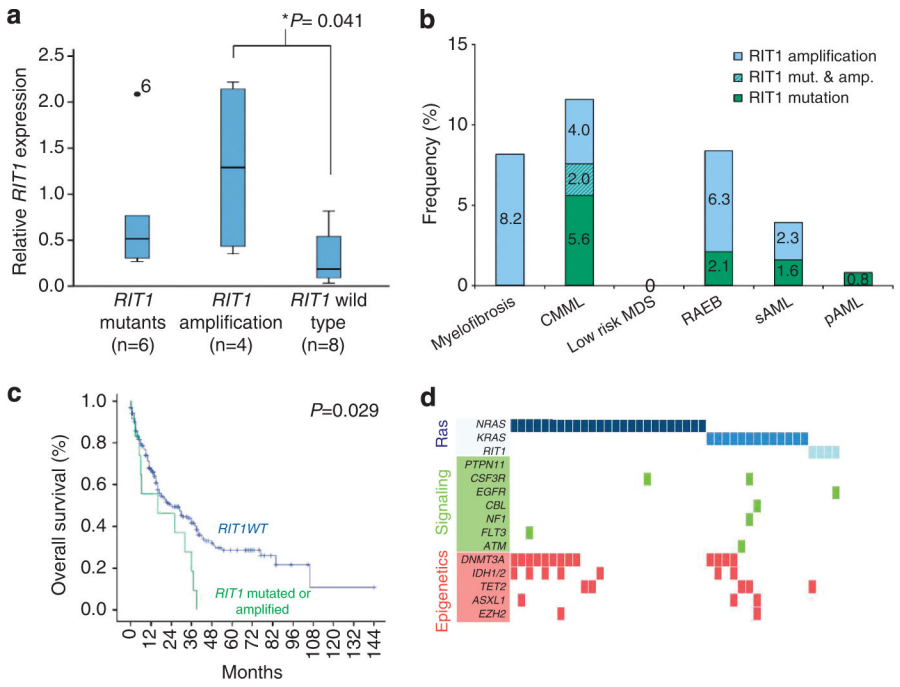

Figure 2.

(**a**) Relative *RIT1* expressions in patients with mutations, amplifications or wild-type gene. (**b**) Clinical distribution of *RIT1* anomalies in myeloid neoplasms. (**c**) Survival curve of patients with MDS or MDS/MPN diagnosis with and without *RIT1* abnormalities. (**d**) Molecular associations of Ras mutants in a cohort of patients studied by whole-exome sequencing, that appeared to be mutually exclusive with themselves and other mutations in genes of signaling pathways and to be related to mutations in genes involved in epigenetic regulation.