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Short communication

Acquired *PTPN11* mutations occur rarely in adult patients with myelodysplastic syndromes and chronic myelomonocytic leukemia

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Abstract

Myelodysplastic syndromes (MDS) are comprised of a heterogeneous group of stem cell disorders characterized by ineffective hematopoiesis and susceptibility to transform to acute myeloid leukemia. The molecular pathways underlying disease initiation and evolution are still largely unknown. We recently demonstrated that acquired mutations in *PTPN11* are a major event in JMML and occur with variable prevalence in children with other hematologic malignancies, including MDS. Here, we investigated contribution of *PTPN11* mutations to adult MDS and CMML pathogenesis. Our results indicate that *PTPN11* lesions might play a role in adult MDS/CMML pathogenesis but do not represent a major molecular event.

Keywords: SHP-2; *PTPN11*; RAS signaling; MDS; CMML

1. Introduction

Myelodysplastic syndromes (MDS) are comprised of a heterogeneous group of stem cell disorders characterized by ineffective hematopoiesis and susceptibility to transform to acute myeloid leukemia (AML). These conditions are characterized by peripheral blood cytopenias associated with hypercellular bone marrow with dysplastic features and show a considerable variability with respect to the degree of cell differentiation, apoptosis and blast proliferation. A heterogeneous clinical course is also observed, with some patients experiencing an extended indolent course while others evolve rapidly to AML. According to the French–American–British (FAB) classification [1], MDS are subdivided in five groups, including refractory anemia (RA), refractory anemia with ring sideroblasts (RARS), refractory anemia with excess of blasts (RAEB), refractory anemia with excess of blasts in transformation (RAEB-T), and chronic myelomonocytic leukemia (CMML). More recently, CMML has been included, along with juvenile myelomonocytic leukemia (JMML), in a hybrid myelodys-

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plastic/myeloproliferative group by the World Health Organization [2]. The molecular events and pathways underlying MDS disease initiation and evolution are still largely unknown. MDS is considered a preleukemic condition, with high risk of progression to AML as a result of additional somatic lesions conferring a proliferative advantage to the myeloid precursor cell. Specific chromosomal abnormalities [monosomy 7/del(7q) or monosomy 5/del(5q)] and oncogenic mutations in *NRAS* and *KRAS2*, which up-regulate the RAS/MAPK pathway, are frequently observed, and are associated with an increased risk of progression and shorter survival.

We recently demonstrated that acquired mutations in *PTPN11*, the gene encoding the cytoplasmic protein tyrosine phosphatase SHP-2, are a major molecular event in JMML, and occur with variable prevalence in children with other hematologic malignancies, including MDS [3–6]. SHP-2 is a signal transducer that relays signals from activated growth factor and cytokine receptors to RAS and other intracellular signaling molecules and is required for sustained activation of the MAPK cascade [7,8]. Accumulating evidence supports that *PTPN11*, *NRAS*, and *KRAS2* mutations are largely mutually exclusive in JMML and other hematologic malignancies [3–6]. These genetic findings suggest that mutated SHP-2 and RAS proteins elicit their effects through a common pathway, and that missense mutations in *PTPN11* represent a novel class of lesions that lead to hyperactive RAS.

Adult MDS and CMML share clinical features with pediatric MDS and JMML. Based on the occurrence of *PTPN11* mutations in JMML and childhood MDS with high-risk clinical features (RAEB and RAEB-T) as well as the high prevalence of somatic mutations of *NRAS* and *KRAS2* in adult MDS and CMML, we reasoned that *PTPN11* mutations might also contribute to adult MDS and CMML pathogenesis. By analyzing a large and clinically well-characterized MDS/CMML cohort, we provide evidence that *PTPN11* mutations might play a role in adult MDS and CMML pathogenesis but do not represent a major molecular event.

2. Materials and methods

Frozen material from 318 patients aged between 18 and 89 years with myelodysplastic disorders were included in the study. Ninety-nine patients (CMML, $N = 49$; MDS, $N = 50$) had cryopreserved bone marrow specimens available for molecular analysis from the MD Anderson Cancer Center (Houston, TX), while DNA specimens from 212 patients (CMML, $N = 28$; MDS, $N = 184$) were available at the Heinrich-Heine University (Düsseldorf, Germany). Seven additional CMML cases were available from the University of Freiburg Medical Center (Freiburg, Germany). Informed consent was obtained for each patient of the three cohorts, and each local institutional review board approved the study. Diagnosis was established according to the French–American–British group (FAB) criteria, and patients were classified as follows: RA ($N = 63$), RARS ($N = 57$), RAEB ($N = 71$), RAEB-T ($N = 43$), and CMML ($N = 84$). According to the FAB guidelines, 39 CMML cases were classified as myelodysplastic, while the remaining were myeloproliferative (WBC counts $> 13 \times 10^9\,l^{-1}$) [9]. Characterization of the MDS/CMML cohort is reported in Table 1. Mononuclear cells were separated from aspirated bone marrow samples using a Ficoll gradient and genomic DNAs were isolated from lysates of these cells using standard protocols. *PTPN11*, *NRAS*, and *KRAS2* mutational screening was carried out by DHPLC analysis and direct sequencing, as previously described [3–5]. Based on our previously generated data, exons 2, 3, 4, 7, 8, 12, and 13 of *PTPN11*, and exons 1 and 2 of *NRAS* and *KRAS2* were screened.

Table 1
Characterization of the adult MDS/CMML cohort included in the study

	RA	RARS	RAEB	RAEB-T	CMML
Number of cases	63	57	71	43	84
Gender					
Male	30	31	49	28	65
Female	33	26	22	15	19
Age					
ACC cohort					
Median (range)	72 (23–81)	76[a]	58 (22–81)	69 (42–84)	65 (31–78)
HHU cohort					
Median (range)	63 (18–87)	69 (25–89)	66 (41–88)	62 (31–88)	67[b] (48–83)
PTPN11 mutation	1	0	2	0	1[c]
NRAS/KRAS2 mutation	ND	ND	ND	ND	22[d]

[a] Single case.
[b] Seven CMML patients from the University of Freiburg Medical Center are also included; AAC indicates MD Anderson Cancer Center; HHU, Heinrich-Heine University.
[c] Patient with myeloproliferative CMML.
[d] Eighteen patients exhibited myeloproliferative CMML; ND: not determinated.