Human Pathology (2014) **45**, 2471–2479

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

The detection of *SRSF2* mutations in routinely processed bone marrow biopsies is useful in the diagnosis of chronic myelomonocytic leukemia ☆

Birgit Federmann MD[a,1], Manuel Abele[a,1], David Santiago Rosero Cuesta MD[a,b], Wichard Vogel MD[c], Leonardo Boiocchi MD[d], Lothar Kanz MD[b], Leticia Quintanilla-Martinez MD[a], Attilio Orazi MD[e], Irina Bonzheim PhD[a,1], Falko Fend MD[a,*,1]

[a]*Institute of Pathology and Neuropathology Comprehensive Cancer Center Tuebingen and University of Tuebingen, 72076 Tuebingen, Germany*
[b]*Department of Pathology, Hospital Universitario Miguel Servet, 50001 Zaragoza, Spain*
[c]*Department of Internal Medicine, Hematology and Oncology, Comprehensive Cancer Center Tuebingen and University of Tuebingen, 72076 Tuebingen, Germany*
[d]*Department of Molecular and Translational Medicine, Pathology Section, University of Brescia, 25100 Brescia, Italy*
[e]*Department of Pathology and Laboratory Medicine, Weill Cornell Medical College/New York Presbyterian Hospital, 10065 NY, USA*

Received 27 April 2014; revised 21 August 2014; accepted 24 August 2014

Keywords:
Chronic myelomonocytic leukemia;
Myeloproliferative neoplasms;
SRSF2 mutation;
JAK2 mutation;
Bone marrow biopsy

Summary Diagnosis of chronic myelomonocytic leukemia (CMML) is based on a combination of clinical, laboratory, and morphological parameters, including persistent peripheral blood monocytosis. Recently, mutations of serine/arginine-rich splicing factor 2 (*SRSF2*) have been identified in 40% to 50% of CMMLs and occasionally in other myeloid disorders. In this study, we established a robust assay for the detection of *SRSF2* mutations in decalcified, paraffin-embedded bone marrow (BM) biopsies and investigated its diagnostic utility. BM biopsies of 78 patients with myeloid neoplasms, including 36 CMMLs, 22 myelodysplastic syndromes (MDS), and 20 Ph− myeloproliferative neoplasms (MPN) were analyzed. The region around hot spot P95 in exon 1 of *SRSF2* was amplified and bidirectionally sequenced. In addition, a restriction fragment length polymorphism analysis was established. The *JAK2* V617F mutation was investigated by allele-specific polymerase chain reaction. *SRSF2* mutations were identified in 16 (44%) of 36 CMMLs, including 1 of 3 cases with associated systemic mastocytosis, 4 (20%) of 20 Ph− MPN, and 1 (4.5%) of 22 MDS. Restriction fragment length polymorphism analysis detected all mutations with the exception of a single P95A. Of note, 2 cases of *JAK2* V617F+ primary myelofibrosis with *SRSF2* mutation initially were diagnosed as CMML based on significant peripheral blood monocytosis. In CMML, no correlation with histopathology and/or clinical parameters was observed, but *SRSF2* mutations were associated with normal karyotype ($P < .001$). In summary, *SRSF2* mutations are frequent in CMML and a useful diagnostic feature demonstrable in BM biopsies, allowing a definitive diagnosis for cases with

☆ Disclosure: The authors report no conflict of interest or funding disclosures.

* Corresponding author. Institute of Pathology and Neuropathology, Liebermeisterstrasse 8, 72076 Tuebingen, Germany.

[1] Contributed equally.

E-mail address: falko.fend@med.uni-tuebingen.de (F. Fend).

http://dx.doi.org/10.1016/j.humpath.2014.08.014

minimal dysplasia and normal karyotype. The role of *SRSF2* mutations in cases with hybrid features between primary myelofibrosis and CMML needs further investigation.

1. Introduction

Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic disorder with both myelodysplastic and myeloproliferative features and was therefore assigned to the category of myelodysplastic/myeloproliferative neoplasms (MDS/MPN) in the 2008 WHO classification [1]. The diagnosis of CMML is based on a combination of clinical, laboratory, morphological, and molecular findings. The diagnostic features include persistent peripheral blood (PB) monocytosis of greater than 1×10^9/L, the absence of *BCR-ABL1* or *PDGFRA/B* rearrangements, less than 20% blasts in the PB and bone marrow (BM), and dysplasia in one or more myeloid lineages. Without dysplasia, the diagnosis can still be made if there is an acquired clonal cytogenetic or molecular genetic alteration, or if the monocytosis persists for at least 3 months and other causes of monocytosis are excluded [1].

However, because of overlapping clinical and morphological features, it often remains difficult to distinguish between CMML and common differential diagnoses such as other MDS/MPN, myelodysplastic syndromes (MDS), or occasionally myeloproliferative neoplasms (MPN) such as early stage primary myelofibrosis (PMF). In the absence of clonal cytogenetic aberrations, discrimination from reactive monocytosis may require prolonged observation. BM histopathology and immunohistochemistry are important adjuncts for confirming a diagnosis of CMML, but there is no single BM feature that is pathognomonic for the disease, and it remains impossible to make the diagnosis without clinical information and PB counts [2–4]. Histologically, hypercellularity, varying dysplasia in the hematopoietic cell lines, and an increased number of CD14-positive monocytes are common findings in CMML [3,5].

The prognosis of CMML varies widely, and many studies have systematically analyzed risk factors and devised prognostic scores. Because of the negative impact of blast counts in BM and PB on survival, CMML is divided into 2 subgroups: CMML-1 with less than 10% medullary, and less than 5% peripheral blasts including promonocytes and CMML-2 with 10% to 19% medullary, 5% to 19% peripheral blasts including promonocytes [1]. In contrast, the previous distinction between a myelodysplastic and a myeloproliferative form based on an arbitrary cutoff of 13×10^9/L PB leukocytes has not been integrated into the WHO classification [6]. Transformation into secondary acute myeloid leukemia (AML) occurs in about 15% to 30% of patients, but the risk varies depending on the subtype of the disease [2]. Cytogenetic alterations, none of them specific for CMML, are encountered in 15% to 30% of CMML cases and seem to be another prognostic factor [1].

Within the last years, a variety of recurrent gene mutations have been identified in MDS/MPN including CMML. These mutations target genes involved in epigenetic regulation (*ASLX1*, *TET2*, *EZH2*, *IDH1/2*, *DNMT3A*), transcription (*TP53*, *RUNX1*), and signaling (*N-/KRAS, CBL, JAK2*); but none of these mutations is specific for a single disease, and they occur in a broad range of myeloid neoplasms [7,8]. Recently however, identification of frequent mutations of RNA splicing machinery components such as *SRSF2, SF3B1, ZRSF2*, and *U2AF1* as novel mechanisms of oncogenesis in MDS and MDS/MPN has shed some light on the molecular pathogenesis of these disorders [9,10].

In particular, mutations in serine/arginine-rich splicing factor 2 (*SRSF2*) have been identified in a high frequency in CMML. In the largest study to date, *SRSF2* mutations were identified in 47% of CMML patients and were associated with higher age, elevated hemoglobin, and lack of chromosomal aberrations [11]. *SRSF2* mutations also occur in MDS and PMF, albeit at a lower frequency [12]. In MDS and PMF, mutations of *SRSF2* are associated with poor overall survival [12,13], whereas in CMML, the prognostic relevance is not entirely clear so far [10,11].

SRSF2 belongs to the SR-protein family, which is necessary for constitutive pre-mRNA splicing and regulates alternative splicing. SRSF2 interacts with other splicing factors and plays an important role in the formation of the early splicing complex and splice site selection [9]. The pathogenetic mechanism of splicing factor mutations in myeloid disorders is not clear yet, but it has been hypothesized that they provide the second step for development of full-blown malignancy [10].

Although PB or BM aspirates are the preferred material for mutational analysis in myeloid disorders, BM biopsies sometimes represent the only available material, especially for retrospective studies. The purpose of the current study was therefore to develop an assay for the detection of *SRSF2* mutations in formalin-fixed, EDTA-decalcified, and paraffin-embedded BM trephines of MDS/MPN and related disorders and to correlate the mutational status with clinical and histopathological features to further evaluate the role of *SRSF2* as a diagnostic tool in CMML, especially for cases with minimal dysplasia and normal karyotype.

2. Materials and methods

2.1. Patients and histopathological workup

BM trephine biopsies of patients with a confirmed or suspected diagnosis of CMML were selected from the files of the Institute of Pathology, Tuebingen University, between 2000 and 2013. In addition, 22 cases of MDS, 18 t(9;22)-negative