Case report

A novel del(8)(q23.2q24.11) contributing to disease progression in a case of *JAK2/TET2* double mutated chronic myelomonocytic leukemia

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ABSTRACT

We have identified a novel 7.7 Mb del(8)(q23.2q24.11) in a patient progressing to acute myeloid leukemia (AML) following a 12-year stable phase of chronic myelomonocytic leukemia (CMML). A surprisingly high *JAK2+* allelic burden of 92% at the time of AML led us to delineate the molecular aberrations relevant for leukemogenesis. While a frameshift mutation in the *TET2* gene was stably present throughout the course of disease the *JAK2* mutation was acquired after initial diagnosis of CMML. At progression aCGH revealed del(8q)(q23.2q24.11) encompassing various cancer relevant genes of which *RAD21* and *CSMD3* are of particular interest.

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1. Introduction

Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic stem cell neoplasm with overlapping myelodysplastic and myeloproliferative features. While progression to acute myeloid leukemia (AML) is common, the driving molecular aberrations involved in transformation remain obscure [1]. Clonal cytogenetic abnormalities predict adverse prognosis and are evident in up to 40% of cases [2]. Next generation sequencing has identified molecular aberrations in approximately 90% of CMML patients with *TET2* gene mutations (*TET2+*) being particularly frequent. On the contrary, the JAK2 V617F mutation (*JAK2+*) is uncommon [3]. Concomitant *TET2+* and *JAK2+* have been described in chronic myeloproliferative disorders, but none of these are sufficient to drive blastic transformation. Moreover, transformation of myeloproliferative disorders most often results in loss of *JAK2+* indicating the transformation to arise in a *JAK2*-negative subclone [4]. We here report a case of *TET2+* CMML who acquired *JAK2+* and progressed into overt AML 12 years after initial diagnosis. Array comparative genomic hybridization (aCGH)

revealed a novel del(8q)(q23.2q24.11) (del(8q)) comprising genes with recently reported relevance in cancer, including AML.

2. Case presentation

In 1998, a 64-year old male presented with pleural effusion, pericarditis, and leukocytosis of 38×10^9/L with 30% monocytes. A complete overview of the clinical course of disease is given in Table 1. A bone marrow (BM) biopsy revealed CMML-1. There was no splenomegaly or cytopenias. In 2004, pleural effusion reemerged and microscopy of pleural fluid revealed numerous CD68 positive monocytes. Lymph node enlargement emerged in 2007 and a biopsy showed infiltration of 50% fully differentiated monocytes. Despite these various CMML-related manifestations, no cytopenias were evident. However, in 2010 BM failure developed with decreasing hemoglobin and thrombocyte counts and increasing leukocyte count. Morphology revealed persistent CMML-1. Nine months later, in June 2011 at the age of 76 the patient was admitted with abdominal pain and severely deranged hematopoiesis and was diagnosed with AML. Flow cytometry of peripheral blood showing 40% myeloblasts staining positive for CD34, CD117, HLA-DR, and CD13. A CT scan showed splenomegaly, and signs of malignant infiltration in the renal parenchyma and the soft tissue along the lower thoracic vertebras, suspicious of myelosarcomas.

The patient received palliative care, but succumbed from progressive renal failure in July 2011.

Routine molecular analysis at AML diagnosis showed a *JAK2+* allelic burden of 92% of mononuclear cells (MNCs) from peripheral blood (PB). Since *JAK2+* is usually lost upon transformation this led us to retrospectively delineate the longitudinal evolution of molecular aberrations at critical time points, summarized in Table 1.

In short, DNA was extracted from diagnostic paraffin embedded BM biopsies (1998), from thawed BM MNCs from the time of disease progression (2010), and from PB MNCs at the time of overt AML (2011). *JAK2+* was analyzed for by standard quantitative polymerase chain reaction (qPCR). While the diagnostic CMML sample was negative for *JAK2+*, the allelic burden was above the 90% level both in the transformation- and AML phase of disease. The entire coding sequences and all splice sites of the *TET2* gene (exons 3–11) were scanned for mutations by PCR combined with denaturing gradient gel electrophoresis (DGGE) as described in Ref. [5]. In all samples, a stable frameshift mutation in exon 6 was found (c.3658_3658del A, Thr1220fs. RefSeq NM_001127208.2). DNA extracted from a benign skin biopsy from 1990 proved negative for *TET2* mutations, hence excluding germline mutation (Fig. 1A). By multiplex fluorescent-labeled PCR followed by capillary gel electrophoresis we retrospectively analyzed MNCs from the time of AML-diagnosis for recurrent AML related mutations, namely *FLT3*-ITD, *FLT3*-D835, *cKIT* D816V, *IDH1* R132, *CEBPA*, *NPM1* exon 12, and *WT1* exon 7 of which all came out negative. Standard G-band karyotyping was not performed at initial diagnosis in 1998, but the karyotype was 46,XY[20] two and a half years from diagnosis in stable phase of disease. However, in September 2010 the karyotype had changed to 46,XY,dup(9)(p21p21)[6]/46,XY[19].

Since progressive BM failure was not evident until September 2010, we considered this time point pivotal in AML transformation. Consequently, we focused on identifying candidate aberrations of potential impact by applying aCGH using human Cancer CytoChip at this time point. Regions of gain or loss contained within copy number variable regions (CNVs) were discarded. Reference genome was NCBI build 36.1 (hg18). High-resolution aCGH analysis revealed an interstitial deletion in chromosome 8 at bands q23.2-q24.11 of maximal size 7,7 Mb (position 110.679.916–118.334.826) (Fig. 1B and C). To confirm this finding and to longitudinally analyze for the del(8q), we performed fluorescence *in situ* hybridization (FISH) with the following directly labeled probes: RP11-11A18 (SpectrumOrange-labeled, BlueGnome, Cambridge, UK) located at 8q23.3 (position 113.491.837–113.659.918) and centromere SE 8 probe (FITC-labeled, Kreatech, Amsterdam, The Netherlands). Chromosomes and nuclei were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride. The FISH analysis confirmed the presence of del(8q) in the progression- and AML phase and absence of del(8q) in the stable phase of CMML in 2001 (Fig. 1D). Array CGH did not detect the dup(9)(p21p21) found with standard G-banding karyotyping in September 2010, since the fraction of dup(9) positive cells is below the sensitivity of aCGH.

The region 8q23.2-q24.11 encompasses genes as depicted in Fig. 1C. We performed an NCBI gene database and PubMed search on each of these genes for their reported relevance in carcinogenesis. Importantly, several of these are of notified relevance; *TRPS1* and *EIF3H* are reported as candidate oncogenes with increased expression in breast cancer [6,7]. In a study of non-small cell lung carcinomas the CUB and sushi multiple domains 3 gene (*CSMD3*) was identified as the second most frequently mutated gene and loss of function cause increased proliferation of airway epithelial cells *in vitro* [8]. While the CSMD gene family has recently been suggested tumor suppressor genes in colorectal cancer [9], the role of *CSMD3* in hematological cancers remains to be elucidated. Strikingly, *RAD21* has been associated with AML as it encodes a protein in the cohesin complex, which is involved in chromosome segregation during cell division, regulation of transcription, and DNA double strand break repair. Recently, genes belonging to the cohesin complex were described as novel recurrent and mutually exclusive mutations in myeloid neoplasms [10].

Table 1
Longitudinal registration of disease stage, laboratory values, cytogenetic and molecular analyses.

	December 1998	June 2001	September 2010	June 2011
Clinical features	Pericarditis and pleuritis	Routine follow-up	Progressive BM failure	BM failure, splenomegaly, and possible myelosarcoma
Lab results				
Hemoglobin (mmol/L)	7.9	8.5	5.1	5.3
Platelets (10^9/L)	206	241	218	25
Leukocytes (10^9/L)	23.7	33.9	55.5	84.2
Neutrophils (10^9/L)	12.6	8.8	46.6	61.5
Monocytes (10^9/L)	5.45	18.6	3.03	4.21
Morphology	CMML-1	CMML-1	CMML-1	Dry tap, inconclusive
Blasts incl. promonocytes in BM	< 5%	< 5%	< 5%	20%[a]
Flow cytometry	ND	ND	CMML (BM) 3% CD34+CD117+myeloblasts 84% CD64+myelomonocytoid cells	AML (PB) 40% CD34+CD117+myeloblasts 10% co-expression of CD64
G-banding	ND	46,XY[20]	46,XY,dup(9)(p21p21)[6]/46,XY[19]	45,X–Y[5]/46,XY[20]
TET2 **mutation**	Positive	ND	Positive	Positive
JAK2 **mutation**	Negative	ND	93% of BM MNCs	92% of PB MNCs
Array CGH	ND	ND	del(8)(q23.2q24.11)	ND
Interphase nuclei FISH	ND	BM Neg. for del(8q)	BM 88% cells with del(8q)	PB 15% cells with del(8q)

BM: bone marrow; ND: not done; PB: peripheral blood; MNCs: mononuclear cells.

[a] Blast count in PB, due to dry tap.

A
TET2 mutational status

| Skin biopsy | 1998 | 2010 | 2011 |

c.3658delA
p.C1225X
(Exon 6)

C T C A C A G G G G G G G C C A G T

B
Array CGH

C
Del(8q) affected genes

Chromosome 8

-2.0   0   2

SYBU
GOLSYN
AX748380
KCNV1
KIAA1894
miR2053
CSMD3
TRPS1
LINC00536
EIF3H
UTP23
RAD21
miR3610
RAD21-AS1
BC031280
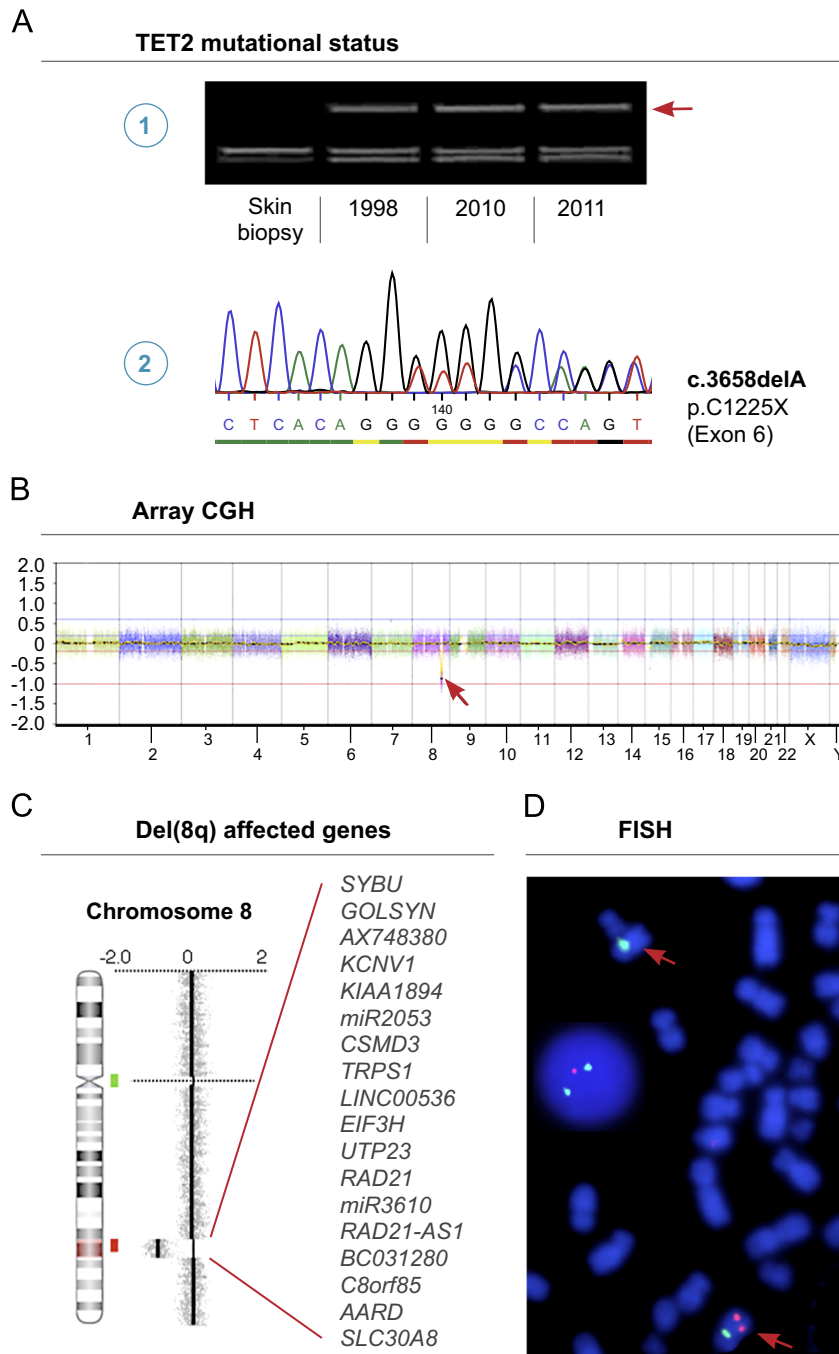*C8orf85*
AARD
SLC30A8

D
FISH

Fig. 1. Output data of applied analyses. (A) DGGE analysis showing mutant *TET2* being present in all samples as indicated by the heteroduplex band (arrow) (1). Sanger sequencing of the heteroduplex band showing presence of both mutant (del A) and normal alleles (reverse strand shown) (2). (B) High-resolution aCGH analysis at the time of progressive BM failure in 2010 revealing a submicroscopic deletion at chromosome 8 (arrow). The *X*-axis at the bottom denotes the chromosomal position. (C) Zoom view of genomic profile at chromosome 8 showing the deleted region 8q23.2–24.11 as indicated by red shade on chromosome 8 ideogram. The genes located in the deleted region are listed. Locations of BAC probes for subsequent FISH are indicated (red and green bars). (D) Confirmatory FISH using BAC probe RP11-11A18 at 8q23.3 in the *CSMD3* gene (red) and centromeric SE 8 probe (green) showing the deletion on one chromosome 8 (red arrows) in metaphases and interphase nuclei (1R2G signal pattern). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Conclusion

We have longitudinally described the molecular aberrations present at critical time points from initial diagnosis of CMML to overt AML applying a comprehensive panel of molecular analyses. Firstly, the consistent and non-germ line *TET2*+ in all stages of disease verify that the AML stage indeed was transformed from CMML and not a *de novo* event. Secondly, in this case, *TET2*+ and *JAK2*+ represent founder mutations. It cannot be ruled out that the massive *JAK2*+ allelic burden have contributed to genomic instability and, speculatively, also influenced the proliferative potential of the leukemic blasts. Thirdly, aCGH revealed a novel del(8q). Our results indicate that the leukemic transformation of an otherwise long-standing and stable CMML could have been driven by loss of gene function resulting from this exact deletion. Of the deleted genes, *RAD21* and *CSMD3* are of particular interest, since their loss of function have recently been reported to play a role in carcinogenesis. However, their role in hematological cancers remains to be studied. Finally, this case report emphasizes the relevance of aCGH in the search for candidate genes involved

in leukemogenesis, not least in heterogeneous neoplasms such as CMML.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication.

There has been no significant financial support for this work that could have influenced its outcome.

Contributions

All authors contributed to the writing, experimentation, and preparation of the manuscript.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship.

We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication.

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References

[1] Patnaik MM, Parikh SA, Hanson CA, Tefferi A. Chronic myelomonocytic leukaemia: a concise clinical and pathophysiological review. Br J Haematol 2014;165:273–86.

[2] Such E, Cervera J, Costa D, Sole F, Vallespi T, Luno E, et al. Cytogenetic risk stratification in chronic myelomonocytic leukemia. Haematologica 2011;96:375–83.

[3] Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M, et al. Prognostic score including gene mutations in chronic myelomonocytic leukemia. J Clin Oncol 2013;31:2428–36.

[4] Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. Leukemia 2010;24:1128–38.

[5] Asmar F, Punj V, Christensen J, Pedersen MT, Pedersen A, Nielsen AB, et al. Genome-wide profiling identifies a DNA methylation signature that associates with TET2 mutations in diffuse large B-cell lymphoma. Haematologica 2013;98:1912–20.

[6] Hu J, Su P, Jia M, Wu X, Zhang H, Li W, et al. TRPS1 expression promotes angiogenesis and affects VEGFA expression in breast cancer. Exp Biol Med 2014;239:423–9.

[7] Mahmood SF, Gruel N, Chapeaublanc E, Lescure A, Jones T, Reyal F, et al. A siRNA screen identifies RAD21, EIF3H, CHRAC1 and TANC2 as driver genes within the 8q23, 8q24.3 and 17q23 amplicons in breast cancer with effects on cell growth, survival and transformation. Carcinogenesis 2014;35:670–82.

[8] Liu P, Morrison C, Wang L, Xiong D, Vedell P, Cui P, et al. Identification of somatic mutations in non-small cell lung carcinomas using whole-exome sequencing. Carcinogenesis 2012;33:1270–6.

[9] Zhang R, Song C. Loss of CSMD1 or 2 may contribute to the poor prognosis of colorectal cancer patients. Tumour Biol 2014;35:4419–23.

[10] Kon A, Shih LY, Minamino M, Sanada M, Shiraishi Y, Nagata Y, et al. Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. Nat Genet 2013;45:1232–7.