phase of MPN suggesting a role of *IDH1* mutation in conversion from chronic MPN to acute leukemia.[8-9]

In order to test whether MPN also carries *IDH1* mutations, we investigated 160 BM biopsies of MPN patients, including CML (n=13), ET (n=73), PV (n=33), PMF (n=35) and unclassifiable MPN (n=6) using the $IDH1^{R132H}$ mutation specific antibody. We found 2 ET and one PMF case with positive hematopoietic cells (Table 1). Thus *IDH1* mutations occur not only in AML but can also be infrequently found in the chronic MPN.

$IDH1^{R132H}$ was detectable in the cytoplasm of granulocyte precursors, megakaryocytes and single erythroblasts. The number of $IDH1^{R132H}$ positive cells varied between almost 100% in case A and 1-3% in cases B and C (Table 1 and Figure 1). Sequencing the *IDH1* gene of the 3 immunohistochemically positive cases confirmed the presence of R132H mutation in case A but not in cases B and C (Figure 1). The fraction of *IDH1* mutant cells in cases B and C is below the sensitivity threshold of direct sequencing which requires the presence of approximately 20% of mutant allele. Thus our data indicate that immunohistochemistry with the mutation specific antibody is a more sensitive method for detection of bone marrow cells harboring $IDH1^{R132H}$ when compared to direct sequencing.[11]

For case A we were able to assess the chronology of *IDH1* and *JAK2* mutations based on the analyses of two consecutive bone marrow biopsies: the first taken at the initial diagnosis and the second two years later. The $IDH1^{R132H}$ mutation was detectable by immunohistochemistry and direct sequencing in both the initial and the recurrent lesion (Figure 1 A1, A2). In contrast, the *JAK2* V617F mutation was absent in the initial BM biopsy but detectable in the follow-up biopsy. Furthermore, in the later biopsy the majority of bone marrow cells harbor the $JAK2^{V617F}$ allele (Figure 1, A2, lower row). This indicates that *IDH1* R132H and *JAK2*V617F mutations are present in the same cells and not in two different cell clones. This also clearly demonstrates that $IDH1^{R132H}$ mutation, similar to *TET2* mutation,[12] can occur early in the course of MPN and precede the *JAK2* mutation. Additionally, this case shows that $IDH1^{R132H}$ was present for more than two years in virtually all hematopoietic cells but the patient did not progress to AML.

Notably, none of $IDH1^{R132H}$ harboring cases progressed to AML within the follow-up period of 26, 16 and 118 months for cases A, B and C, respectively. This finding indicates that $IDH1^{R132}$ mutation alone may not be sufficient for conversion of MPN to AML.

Taken together, our data demonstrate the presence of *IDH1* R132H mutation in MPN with a lower frequency than that reported in AML. Because other *IDH2* mutations are more frequent in AML, additional studies need to be carried out in order to find *IDH1* and *IDH2* mutations in chronic phase of MPN.

Furthermore, we demonstrate that standard immunohistochemistry with antibody H9 (Dianova, Hamburg, Germany) is a sensitive and reliable method to detect IDH1 R132H mutation in MPN.

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

Mindaugas Andrulis,[1] David Capper,[2,3] Jochen Meyer,[2] Roland Penzel,[1] Christian Hartmann,[2,3] Hanswalter Zentgraf,[4] and Andreas von Deimling[2,3]

[1]Department of General Pathology and [2]Department of Neuropathology, Institute of Pathology, Ruprecht-Karls-University Heidelberg, Heidelberg; [3]Clinical Cooperation Unit Neuropathology, and [4]Monoclonal Antibody Unit, German Cancer Research Center, Heidelberg, Germany

Correspondence: Andreas von Deimling, Department of Neuropathology, Institute of Pathology, Im Neuenheimer Feld 220/221, D-69120 Heidelberg, Germany. Phone: international +49.06221.562603/2604, Fax: international +49.06221.564566. E-mail: andreas.vondeimling@med.uni-heidelberg.de

Funding: this work was supported by the Bundesministerium für Bildung und Forschung grants BMBF01ES0730 and BMBF01GS0883.

Citation: Andrulis M, Capper D, Meyer J, Penzel R, Hartmann C, Zentgraf H, and von Deimling A. IDH1 R132H mutation is a rare event in MPN as determined by a mutation specific antibody. Haematologica 2010; 95(10):1797-1798. doi:10.3324/haematol.2010.024430

©Ferrata Storti Foundation

References

1. Campbell PJ, Green AR. The myeloproliferative disorders. N Engl J Med. 2006;355(23):2452-66.
2. Beer PA, Delhommeau F, Lecouedic JP, Dawson MA, Chen E, Bareford D, et al. Two routes to leukemic transformation following a JAK2 mutation-positive myeloproliferative neoplasm. Blood. 2010;115(14):2891-900.
3. Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol. 2009;118(4):469-74.
4. Chou WC, Hou HA, Chen CY, Tang JL, Yao M, Tsay W, et al. Distinct clinical and biological characteristics in adult acute myeloid leukemia bearing isocitrate dehydrogenase 1 (IDH1) mutation. Blood. 2010;115(14):2749-54.
5. Andrulis M, Capper D, Luft T, Hartmann C, Zentgraf H, von Deimling A. Detection of isocitrate dehydrogenase 1 mutation R132H in myelodysplastic syndrome by mutation-specific antibody and direct sequencing. Leuk Res. 2010;34(8):1091-3
6. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature. 2009;462(7274):739-44.
7. Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Coller HA, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. Cancer Cell. 2010;17(3):225-34.
8. Abdel-Wahab O, Manshouri T, Patel J, Harris K, Yao J, Hedvat C, et al. Genetic analysis of transforming events that convert chronic myeloproliferative neoplasms to leukemias. Cancer Res. 2010;70(2):447-52.
9. Green A, Beer P. Somatic mutations of IDH1 and IDH2 in the leukemic transformation of myeloproliferative neoplasms. N Engl J Med. 2010;362(4):369-70.
10. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood. 2005;106(6): 2162-8.
11. Capper D, Weissert S, Balss J, Habel A, Meyer J, Jager D, et al. Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. Brain Pathol. 2010;20(1):245-54.
12. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Masse A, et al. Mutation in TET2 in myeloid cancers. N Engl J Med. 2009;360(22):2289-301.

TET2 gene is not deleted in chronic myelomonocytic leukemia: a FISH retrospective study

We read with interest the paper *TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic*

Table 1. Cases with abnormal karyotype.

Sample	Diagnosis	Karyotype
1	CMML	47,XY,+8[8]/46,XY[3]
2	CMML	46,XY,del(22)t(11;22)(q13;q13)[20]
3	CMML	45,XX,del(5)(q13q33),-7,del(13)(q14)[6]/47,X,-X,add(3)(q29),+5,+7,del(13)(q14), -14,+15,+19,-20[4]
4	CMML	47,XY,+8[20]
5	CMML	45,XY,-7[15]
6	CMML	45,XY,-7[21]/46,XY[5]
7	CMML	46,XY,del(3)(q25),del(5)(q13q33),del(12)(p11), t(12;16)(q13;p13)[6]/46,XY,del(12)(p11),t(12;16)[14]
8	CMML	46,XY,del(7)(q22)[6]/46,XY[14]
9	CMML	45-46,XX,der(2),der(6)/polyploid cells with der(2),der(16)[2]/46,XX[24]
10	CMML	47,XX,+8,der(21)t(13;21)(q11;q22)[12]/46,XX[8]
11	CMML	48,XY,+8,+11[9]/47,XY,+8[1]/46,XY[10]
12	CMML	45,XX,-15[15]/46,XX[5]
13	CMML	46,XY,del(20)(q11q13)[3]/46,XY[17]
14	CMML	46,XY,del(20)(q11q13)[19]/46,XY[1]
15	CMML	45,X,-Y[3]/46,XY[25]
16	CMML	45,X,-Y[8]
17	CMML-1	46,X,idic(X)(q13)[18]/46,XX[2]
18	CMML-2	45,X,-Y[15]
19	CMML-2	47,XY,+8[9]/47,XY,+8,add(17)(p13)[5]/46,XY[6]
20	sAML	46,XY,del(5)(q13q33),del(12)(p11),t(12;16)[20]
21	sAML	47,XX,+8[19]/49,XX,+7,+8,+11[1]

CMML: chronic myelomonocytic leukemia; sAML: secondary acute myeloid leukemia.

© Ferrata Storti Foundation

leukemia, published in this Journal in December 2009. This study contributes significantly to the study of chronic myelomonocytic leukemia (CMML), providing important molecular and survival information. It reported the incidence and importance of TET2 mutations in this pathology.[1] Taking this into account, the aim of the present study was to determine the copy number status of the *TET2* gene by FISH in patients with CMML and acute myeloid leukemia (AML) secondary to CMML. As well as to evaluate the usefulness of fluorescence *in situ* hybridization (FISH) to analyze the status of *TET2* in this cohort of patients.

Regarding *TET2* (4q24), acquired somatic mutations in the coding sequence of this gene, as well as loss of heterozygosity or uniparental disomy of chromosome 4, were identified in hematopoietic cells from patients with myeloproliferative disorders (MPD) or myelodysplastic syndromes (MDS).[2] Kosmider *et al.*[1] detected TET2 mutations in 44 of 88 (50%) patients with CMML; *TET2* deletion was not observed by cytogenetics in any of the cases. Array comparative genomic hybridization (CGH-A) was also performed and they compared the leukemic cell profile to normal DNA in 28 of the 88, detecting *TET2* deletion in one of 10 studied patients with a mutated *TET2* copy. Thus, copy number alterations and deletion of the wild-type *TET2* copy in *TET2*-mutant CMML cases seem uncommon.

Besides, Delhommeau *et al.*,[3] using a combination of molecular, cytogenetic, CGH and single-nucleotide-polymorphism (SNP) arrays analyses, obtained the coding sequence of *TET2* gene in 320 patients with myeloid malignancies. They found 3 patients diagnosed of AML that presented 4q24 deletion, corroborating the results by FISH. Also, bone marrow cells from 3 patients with MDS and one with MPD had a similar deletion. However, they detected *TET2* defects in 15 of 81 patients with MDS (19%), in 24 of 198 patients with MPD (12%), in 5 of 21 patients with secondary AML (sAML) (24%), and in 2 of 9 patients with CMML (22%); suggesting that deletions or mutations in *TET2* are early events. Nevertheless, CGH/SNP arrays and sequencing of *TET2* are costly and time consuming.

The study cohort included 79 patients diagnosed with CMML and 4 with AML transformed from CMML. Patients were selected from 1990 until 2010. Conventional routine cytogenetic study was performed at the moment of diagnosis from bone marrow samples in a 24-hour culture without mitogens. FISH analysis was applied using BAC clones from a 32K library (http://bacpac.chori.org/) following the standard procedures in the bone marrow fixed cells.[4] Two labeled probes, RP11-542F11 (corresponding to the *TET2* gene) and RP11-1377H10 (control probe for chromosome 4) were used to identify possible alterations in the status of *TET2*. To establish the cut-offs, 10 peripheral blood normal samples (5 male and 5 female) were used as controls. The cut-off value to consider one sample as positive was defined as average plus three standard deviations, and was 3.88% for deletions. Both controls and samples were analyzed counting 200 nuclei by 2 independent observers. Additionally, a positive control was used to make sure that the probe detected TET2 deletions. The case used was a patient diagnosed with AML with a translocation involving a loss of 4q24 band [t(4;15(q24;q26)] resulting in a deletion of the *TET2* gene that was corroborated by FISH. The study was conducted with the approval of the ethical committee from our institution and in keeping with the guidelines of the Declaration of Helsinki.

Cytogenetic results revealed that 25.3% (21/83) presented an abnormal karyotype; trisomy 8 was the most

frequent alteration (6/21) followed by loss of Y chromosome (4 cases) and –7/7q- and del(5q) (3 cases) (Table 1). No chromosomal structural and numerical alterations were found in chromosome 4 for any of the cases included in the series. FISH results revealed that no cases with the diagnosis of CMML or AML transformed from CMML presented a deletion of *TET2*.

Other authors have used SNP arrays to study the status of *TET2* showing that the deletions are present in few cases. Langemeijer *et al.* examined 102 patients and found 2 cases with *TET2* deletion, both with a diagnosis of RAEB-2.[5] Also, Jankowska *et al.* used SNP arrays and sequencing in 396 patients. Eight of the patients studied had deletion of the gene and their diagnosis corresponded to MDS and sAML.[6] A similar study to ours was published by Viguié *et al.* in 2005, who, before the discovery of the involvement of the *TET2* gene in hematologic malignancies, studied 4 cases of AML with the 4q24 deletion suggesting the implication of a tumor suppressor gene.[7]

Taking into account our results and those previously reported, we can conclude that *TET2* is not deleted in CMML patients, although it is mutated in a high proportion of cases, as had been previously reported.[1,3,5-6] In addition, FISH is not a useful technique for analyzing the status of *TET2* in CMML.

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

Mar Mallo,[1,2] Gemma Osca,[2] Julia Solórzano,[3] Leonor Arenillas,[1] Lourdes Florensa,[1] and Francesc Solé[1,2]

[1]Laboratori de Citogenètica Molecular, Laboratori de Citologia Hematològica, Servei de Patologia, Parc de Salut Mar, GRETNHE, IMIM-Hospital del Mar, Barcelona; [2]Departament de Biologia Cellular, Fisiologia i Immunologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra; and [3]Facultat de Ciències de la Salut i de la Vida. Universitat Pompeu Fabra, Barcelona, Spain

Correspondence: Francesc Solé, Laboratori de Citogenètica Molecular, Servei de Patologia, Hospital del Mar, Passeig Marítim, 25-29 08003 Barcelona, Spain. Phone: international +34.93.2483521. Fax: international +34.93.2483131. E-mail: fsole@parcdesalutmar.cat

Key words: TET2, CMML, FISH, cytogenetics.

Acknowledgments: we would like to thank Blanca Espinet and Marta Salido for the cytogenetic analysis, and Carme Melero and María Rodríguez-Rivera for their expert technical assistance.

Funding: this work has been partially supported by grants from Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Spain (FI07/00107 and PI07/1009) and Ministerio de Ciencia e Innovación, Red Temática de Investigación Cooperativa en Cáncer (RTICC): RD07/0020/2004 FEDER.

Citation: Mallo M, Osca G, Solórzano J, Arenillas L, Florensa L, and Solé F. TET2 gene is not deleted in chronic myelomonocytic leukemia: a FISH retrospective study. Haematologica 2010; 95(10):1798-1800. doi:10.3324/haematol.2010.027920

References

1. Kosmider O, Gelsi-Boyer V, Racoeur C, Jooste V, Vey N, Quesnel B, et al. TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia. Haematologica. 2009;94(12): 1676-81.
2. Reiter A, Invernizzi R, Cross NC, Cazzola M. Molecular basis of myelodysplastic/myeloproliferative neoplasms. Haematologica. 2009;94(12):1634-38.
3. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Massé A, et al. Mutation in TET2 in myeloid cancers. N Engl J Med. 2009;360(22):2289-301.
4. Villa O, Del Campo M, Salido M, Gener B, Astier L, Del Valle J, et al. Small supernumerary marker chromosome causing partial trisomy 6p in a child with craniosynostosis. Am J Med Genet A. 2007;143A(10):1108-13.
5. Langemeijer SMC, Kuiper RP, Berends M, Knops R, Aslanyan MG, Massop M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Gen. 2009;41(7):838-42.
6. Jankowska AM, Szpurka H, Tiu RV, Makishima H, Afable M, Huh J, et al. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. Blood. 2009,113(25):6403-10.
7. Viguié F, Aboura A, Bouscary D, Ramond S, Delmer A, Tachdjian G, et al. Common 4q24 deletion in four cases of hematopoietic malignancy: early stem cell involvement? Leukemia. 2005,19(8): 1411-5.

©Ferrata Storti Foundation