SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 2: 379-381, 2011

MDR-1 gene polymorphisms G2677T and C3435T in a case of Hodgkin's variant of Richter's syndrome

GIUSEPPA PENNA[1], ALESSANDRO ALLEGRA[1], ANDREA ALONCI[1], MOHAMED AGUENNOUZ[2], ANTONINO CANNAVÒ[1], SABINA RUSSO[1], ANGELA GRANATA[1] and CATERINA MUSOLINO[1]

[1]Division of Hematology, and [2]Department of Neuroscience, Policlinico G. Martino, University of Messina, Messina, Italy

Received November 11, 2010; Accepted January 12, 2011

DOI: 10.3892/ol.2011.243

Abstract. Richter's syndrome is defined as the transformation of low-grade lymphoma to a more aggressive high-grade malignant form, usually diffuse large B-cell lymphoma. Hodgkin's lymphoma variant of Richter transformation is relatively rare, and only approximately 100 cases have been reported in the literature. This study examined a case of a 53-year-old woman who developed Hodgkin's lymphoma almost 5 years after the diagnosis of chronic lymphocytic leukemia (CLL). The major points of interest regarding CLL with Hodgkin's transformation were also considered, such as the potential role of MDR-1 gene polymorphisms. The patient was evaluated for two MDR-1 gene polymorphisms, G2677T polymorphism in exon 21 and silent C3435T polymorphism in exon 26, to ascertain whether polymorphisms affect the risk of Hodgkin's lymphoma variant of Richter transformation and whether genomic polymorphisms provide prognostic information on the clinical progression of the disease. According to the data obtained, the analysis of polymorphisms in the MDR1 gene exons 21 and 26 revealed that the T2677T and T3435T alleles are not a predisposing factor to Richter transformation, while the presence of the wild-type genotype may be associated with a more favorable response to therapy.

Introduction

Richter syndrome (RS) is defined as the transformation of low-grade lymphoma to a more aggressive high-grade malignant form, usually diffuse large B-cell lymphoma. Hodgkin's lymphoma (HL) variant of Richter transformation is relatively rare (0.4%) (1), and only approximately 100 cases have been reported in the literature (2-6).

The prognosis of patients with HL transformation of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) is poor in contrast with that of *de novo* HL, with a median overall survival of less than one year in small series (7).

In certain cases, results of studies using single-cell analysis have shown an identical immunoglobulin gene rearrangement. CLL and Reed-Sternberg cells were considered to belong to the same clonal population, although RS-like cells are found in B-CLL in the absence of clinical transformation to HL, whereas in other cases this transformation has not been validated (8,9). In the first case, a progression from underlying CLL cells is hypothesized; in the remaining cases, Hodgkin's disease may indicate a second malignancy (10). In addition, a role for the Epstein-Barr virus (EBV) in the pathogenesis of the transformation to HL has been suggested (11-13).

This study reported a case of a 53-year-old woman who developed HL almost 5 years after the initial diagnosis of CLL. Major points of interest in CLL with Hodgkin's transformation were considered, such as the possible role of MDR-1 gene polymorphisms (14).

The patient was evaluated for two MDR-1 gene polymorphisms, G2677T polymorphism in exon 21 and silent C3435T polymorphism in exon 26, to ascertain whether polymorphisms affect the risk of the HL variant of Richter transformation and whether genomic polymorphisms provide prognostic information on the clinical progression of the disease.

Case report

Patient and methods. A 47-year-old Caucasian female was diagnosed with CLL/SLL in 2003. A staging CT scan of the chest, abdomen and pelvis revealed axillary, pelvis, cervical lymphadenopathy and hepatosplenomegaly.

The patient's white blood cell count was 9,400/mm^3 with 48% lymphocytes. Hemoglobin was 6.9 g/dl and the platelet count was 97,000/mm^3. IgVH gene analysis and determination of Zap-70 and CD38 expression were performed (15). The results indicated IgVH mutation (>2%) and the patient was negative for ZAP-70 and CD38 expression (<30%).

A cervical lymph node biopsy was performed to confirm the diagnosis. The immunophenotype of the cervical lymph nodes and bone marrow (20% small lymphocytes) was consistent with the diagnosis of CLL (CD20$^+$, CD5$^+$, CD23$^+$, MUM1$^+$ and BCL6$^-$). The diagnosis was B-CLL Rai stage IV.

The patient received fludarabine (50 mg for 5 consecutive days every month from July to September 2003). Treatment

Correspondence to: Professor Caterina Musolino, Division of Hematology, Policlinico G. Martino, University of Messina, Via Colapesce 20, 98100 Messina, Italy
E-mail: cmusolino@unime.it

Key words: Richter syndrome, Hodgkin's lymphoma, chronic lymphoid leukemia, MDR-1 gene, gene polymorphism

was then interrupted due to the onset of pericarditis and acute renal failure.

In March 2004, chlorambucil, 15 mg/m^2 was administered daily for 5 consecutive days for 6 months. Following treatment, partial remission was achieved.

Four years later, the patient was admitted to hospital. Axillary and cervical lymph nodes were enlarged, but the liver and spleen were normal. A trephine bone marrow biopsy showed minimal infiltration with CLL (<10%). Following histopathological examination of the lymph node biopsy, a diagnosis of HL of classical type (mixed cellularity classical HL) was confirmed. *In situ* hybridization for EBER-1 RNA was negative for EBV.

Subsequently, the patient received chemotherapy with ABVD for eight cycles. After completing the chemotherapy, she was discharged and has since been in sustained CR for CLL and HL as documented by the results of the PET and CT scans.

Pathological findings. Prior to initiating chemotherapy, two MDR-1 gene polymorphisms, i.e., G2677T polymorphism in exon 21 and C3435T polymorphism in exon 26, were evaluated. For MDR-1 genotyping, the genomic DNA was extracted from the peripheral blood of the patient using standard procedures. Two MDR-1 gene polymorphisms were detected based on a polymerase chain reaction (PCR), using primers to amplify a short fragment of DNA containing the polymorphic sites. The PCR primers for G2677T polymorphism in exon 21 were: 5'-TGC AGG CTA TAG GTT CCA GG-3' and 5'-TTT AGT TTG ACT CAC CTT CCC G-3', producing a 224-bp fragment. Those for the C3435T polymorphism in exon 26 were: 5'-GCT GCT TGA TGG CAA AGA AA-3' and 5'-ATT AGG CAG TGA CTC GAT GAT GA-3', producing a 208-bp fragment. The PCR reaction was performed in a 40-μl reaction volume containing 100 ng of genomic DNA, 10 pmol of each primer, 0.2 mM of each deoxynucleotide triphosphate, 1X PCR buffer [50 mM KCl and 10 mM Tris-HCl (pH 8.3)], 1.5 mM $MgCl_2$ and 1 unit of *Taq* polymerase. The PCR program for exon 21 consisted of 35 cycles at 94˚C for 30 sec, 58˚C for 30 sec, 72˚C for 30 sec, and a final elongation step at 72˚C for 10 min. The PCR program for exon 26 consisted of 35 cycles at 94˚C for 30 sec, 54˚C for 30 sec, 72˚C for 30 sec, and a final elongation step at 72˚C for 10 min. The PCR products were evaluated on a 1.5% agarose gel, photographed using Polaroid film and subjected to RFLP analysis.

To distinguish the single nucleotide polymorphisms (SNPs), the restriction enzyme *Ban*I (New England BioLabs, Ipswich, MA, USA) was used for G2677T and *Dpn*II (New England BioLabs) was used for C3435T. Each 20 μl of PCR products was digested overnight with 5 units of *Ban*I (G2677T in exon 21) and *Dpn*II (C3435T in exon 26) at 37˚C. The digestion products were separated on a 4% agarose gel.

Our patient presented MDR1 G2677G and C3435C genotype, then she presented the wild-type genotype.

Discussion

Chemotherapy and radiotherapy are known risk factors for secondary cancers, while genetic factors affect risk factors only for selected secondary tumors following lymphoproliferative disorders (16).

The patient in this study was administered chlorambucil, an established human carcinogen, and fludarabine. It is known that treatment of CLL with fludarabine has been associated with secondary malignancies (17,18), although Cheson *et al* found no significantly increased risk of secondary malignancies (19).

HL has been reported as a second malignancy during continued chemotherapy (20), and an altered expression of the MDR1 gene may represent an additional risk factor for developing a secondary cancer. In previous studies, a higher frequency of the T allele in position 3435 of MDR1, as well as the T allele at position 2677, were detected in patients with colorectal cancers as compared to healthy subjects. These observations indicate that the MDR1 polymorphism is associated with an increased risk of cancer that has been attributed to lower protection against specific P-gp-dependent carcinogens (21-25).

Although Goreva *et al* pointed out that the T2677T and T3435T alleles are not a predisposing factor to lymphoproliferative diseases (27), Jamroziak *et al* showed that the gene polymorphism C3435T is associated with P-glycoprotein activity in B-cell CLL (26). In a previous study, we noted a significant difference in the genotype distribution between B-CLL patients and the control group as well as significant differences in the genotype distribution between patients with high and low risk of progression, with a correlation with IgVH gene analysis and CD38 positivity (28). Notably, since it is well known that the probability of transformation in RS is strongly associated with the CD38 polymorphism, the G allele is an independent risk factor for RS development (29).

The clinical definition of RS is heterogeneous and encompasses at least two biologically different conditions. CLL transformation may be due to a clonally related lymphoma, which accounts for the majority of cases or the development of an aggressive high-grade malignant form unrelated to the CLL clone. In clonally related RS, the pathogenetic link between CLL and RS has been substantiated by the acquisition of novel molecular lesions at the time of clinicopathologic transformation (30).

No data are currently available in the literature regarding a correlation between the MDR1 polymorphism and RS. Genetic polymorphisms, which determine differences in the activity of enzymes involved in transport and metabolism of mutagens, are a promising area in the search for factors providing an enhanced risk of developing hematologic neoplasms. The alteration of the cellular defense mechanism mediated by P-gp has been speculated to be closely associated with the development of various types of cancer. These observations suggest that MDR1 plays a significant role in the elimination of carcinogens, and that the malfunction of MDR1 may cause various human malignancies, including HL or RS.

In the present study, we examined the association of the development of the HL variant of Richter transformation and gene polymorphisms. According to the data obtained, the study of polymorphisms in the MDR1 gene exons 21 and 26 revealed that the T2677T and T3435T alleles are not a predisposing factor to Richter transformation, while the presence of the wild-type genotype may be associated with a more favorable response to therapy.

It is not possible to verify the prognostic role of common SNPs based on the genotyping results of a single patient, given the rarity of the HL variant of RS. However, it is quite difficult to perform a case-control study in which the MDR-1 genotype of CLL cases transformed to the HL variant is compared to that of a cohort of CLL not transformed to RS. Further studies involving a large series of patients may indicate new paths of investigation leading to an improved understanding of RS.

References

1. Tsimberidou AM, O'Brien S, Kantarjian HM, Koller C, Hagemeister FB, Fayad L, Lerner S, Bueso-Ramos CE and Keating MJ: Hodgkin transformation of chronic lymphocytic leukemia: the MD Anderson Cancer Center experience. Cancer 107: 1294-1302, 2006.
2. Almhanna K, Wongchawart N and Sweetenham J: Intracerebral Hodgkin's lymphoma in a patient with chronic lymphocytic leukemia/small lymphocytic lymphoma: a case report and literature review. Cancer Invest 27: 215-220, 2009.
3. Prochorec-Sobieszek M, Majewski M, Sikorska A, Kos K, Huszcza S and Maryniak RK: Localized gastric diffuse large B-cell lymphoma and Hodgkin's lymphoma as secondary neoplasms in two patients with chronic lymphocytic leukemia. Leuk Lymph 47: 2244-2246, 2006.
4. Roddie C, Cwynarski K, Craig C, Diss T and McNamara C: Hodgkin transformation of newly diagnosed small lymphocytic lymphoma in the gastrointestinal tract. Leuk Lymph 48: 1644-1646, 2007.
5. Adiga GU, Abebe L and Wiernik PH: Partially successful treatment of a patient with chronic lymphocytic leukemia and Hodgkin's disease: case report and literature review. Am J Hematol 72: 267-273, 2003.
6. Robertson LE, Pugh W, O'Brien S, Kantarjian H, Hirsch-Ginsberg C, Cork A, McLaughlin P, Cabanillas F and Keating MJ: Richter's syndrome: a report on 39 patients. J Clin Oncol 11: 1985-1989, 1993.
7. Brecher M and Banks PM: Hodgkin's disease variant of Richter's syndrome. Report of eight cases. Am J Clin Pathol 93: 333-339, 1990.
8. Kanzler H, Kuppers R, Helmes S, Wacker HH, Chott A, Hansmann ML and Rajewsky K: Hodgkin and Reed-Sternberg-like cells in B-CLL chronic lymphocytic leukemia represent the outgrowth of single germinal-center B-cell-derived clones: potential precursors of Hodgkin and Reed-Sternberg cells in Hodgkin's disease. Blood 95: 1023-1031, 2000.
9. Fong D, Kaiser A, Spizzo G, Gastl G and Tzankov A: A Hodgkin's disease variant of Richter's syndrome in chronic lymphocytic leukemia patients previously treated with fludarabine. Br J Haematol 129: 199-205, 2005.
10. Dujardin F, Lefrancq T, Bléchet C, Boni-Boka M, Sénecal D, Desmoulins I and Guyetant S: Syndrome de Richter de type pseudohodgkinien. A propos de deux cas et revue de la literature. Annales de Pathologie 28: 311-316, 2008.
11. Lazzarino M, Orlandi E, Baldanti F, Furione M, Pagnucco G, Astori C, Arcaini L, Viglio A, Paulli M, Gerna G and Bernasconi C: The immunosuppression and potential for EBV reactivation of fludarabine combined with cyclophosphamide and dexamethasone in patients with lymphoproliferative disorders. Br J Haematol 107: 877-882, 1999.
12. Niscola P, Principe MI, Palombi M, *et al*: Hodgkin lymphoma in a mutated ZAP-70-negative B-cell chronic lymphocytic leukemia patient. Leuk Res 32: 362-365, 2008.
13. Cerny J, Wang S, Miron P, Hutchinson L and Brettler D: EBV-negative Hodgkin lymphoma in ZAP-70-positive B-cell lymphocytic leukemia patient. Ann Hematol 88: 495-497, 2009.
14. Pescaramona E, Pignoloni P, Mauro FR, Cerretti R, Anselmo AP, Mandelli F and Baroni CD: Hodgkin/Reed-Sternberg cells and Hodgkin's disease in patients with B-cell chronic lymphocytic leukaemia: an immunohistological, molecular and clinical study of four cases suggesting a heterogeneous pathogenic background. Virchows Arch 437: 129-132, 2000.
15. Hamblin TJ, Davis Z, Gardiner A, Oscier DG and Stevenson FK: Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 94: 1848-1854, 1999.
16. Landgreen O, Pfeiffer RM, Stewart L, Gridley G, Mellemkjaer L, Hemminki K, Goldin LR and Travis LB: Risk of second malignant neoplasms among lymphoma patients with a family history of cancer. Int J Cancer 120: 1099-1102, 2007.
17. World Health Organization: Monographs on the evaluation of carcinogenic risks to humans – overall evaluation of carcinogenety. IARC, Lyon, 1987.
18. Maddocks-Christianson K, Slager SL, Zent CS, Reinalda M, Call TG, Habermann TM, Bowen DA, Hoyer JD, Schwager S, Jelinek DF, Kay NE and Shanafelt TD: Risk factors for development of a second lymphoid malignancy in patients with chronic lymphocytic leukaemia. Br J Haematol 139: 398-404, 2007.
19. Cheson BD, Vena DA, Barrett J and Freidlin B: Second malignancies as a consequence of nucleoside analog therapy for chronic lymphoid leukemias. J Clin Oncol 17: 2454-2460, 1999.
20. Alebouyeh M, Moussavi F, Haddad-Deylami H and Vossough P: Hodgkin lymphoma as second malignancy during continuing chemotherapy for childhood acute lymphoblastic leukemia. Klin Padiatr 220: 388-390, 2008.
21. Potocnik U, Ravnik-Glavac M and Glavac D: Functional MDR1 polymorphisms (G2677T and C3435T) and TCF4 mutations in colorectal tumors with high microsatellite instability. Cell Mol Biol Lett 7: 92-95, 2002.
22. Golapud S and Gupta S: Anti-P-glycoprotein antibody-induced apoptosis of activated peripheral blood lymphocytes: a possible role of P-glycoprotein in lymphocyte survival. J Clin Immunol 21: 420-430, 2001.
23. Wulf G, Kluding H, Ho AD, Doerner M, Doehner H, Manegold C and Hunstein W: Multidrug resistance phenotype in patients with chronic lymphocytic leukemia as detected by immunofluorescence (FACS) and northen blot analysis. Leuk Res 18: 475-484, 1994.
24. Marzolini C, Paulus E, Buclin T and Kim RB: Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. Clin Pharmacol Ther 75: 13-33, 2004.
25. Kim DH, Park JY, Sohn SK, Lee NY, Baek JH, Jeon SB, Kim JG, Suh JS, Do YR and Lee KB: Multidrug resistance-1 gene polymorphisms associated with treatment outcomes in the novo acute myeloid leukemia. Int J Cancer 118: 2195-2201, 2005.
26. Jamroziak K, Balcerczak E, Smolewski P, Robey RW, Cebula B, Panczyk M, Kowalczyk M, Szmigielska-Kapłon A, Mirowski M, Bates SE and Robak T: MDR1 (ABCB1) gene polymorphism C3435T is associated with P-glycoprotein activity in B-cell chronic lymphocytic leukemia. Pharmacol Rep 58: 720-728, 2006.
27. Goreva OB, Grishanova AY, Mukhin OV, Domnikova NP and Lyakhovich VV: Possible prediction of the efficiency of chemotherapy in patients with lymphoproliferative diseases based on MDR1 gene G2677T and C3435T polymorphisms. Bull Exp Biol Med 136: 183-185, 2003.
28. Penna G, Allegra A, Alonci A, *et al*: MDR-1 polymorphisms (G2677T and C3435T) in B-chronic lymphocytic leukemia: an impact on susceptibility and prognosis. Med Oncol: May 22, 2010 (E-pub ahead of print).
29. Aydin S, Rossi D, Bergui L, D'Arena G, Ferrero E, Bonello L, Omedé P, Novero D, Morabito F, Carbone A, Gaidano G, Malavasi F and Deaglio S: CD38 gene polymorphism and chronic lymphocytic leukemia: a role in transformation to Richter syndrome? Blood 111: 5646-5653, 2008.
30. Rossi D and Gaidano G: Richter syndrome: molecular insights and clinical perspectives. Hematol Oncol 27: 1-10, 2009.