unnecessary samples. However, it should be pointed out that three out of our 16 hematologic cases had the HFE mutations related to HH.

One important question remains to be answered. Is transferrin saturation as good a screening test in Southern Europe as it is in Northern Europe?[5,6] In this regard, a number of studies have demonstrated a lower prevalence of the C282Y HFE mutation in these areas (from 0.5 to 3%).[3,4,9] Moreover, a number of potentially confounding pathologies are more frequent in our countries (hepatitis C virus infection,[10] thalassemia, alcoholism, etc.).

In conclusion, in tertiary level medicine, additional criteria (e.g., hepatitis C virus infection, alcoholism, hematologic disorders, transfusions) together with a positive iron overload screening test are necessary to isolate HH from other pathologies.

Angel F. Remacha, Marina Carrasco,* M. Pilar Sardà,* M. Jesús Barceló,# Irene Blesa,° Montserrat Baiget#*

**Hematology, °Gastroenterology and #Genetics Departments, Hospital de Sant Pau, Barcelona, Spain.*

Key words

Hereditary hemocromatosis, HFE, iron.

Correspondence

Angel F. Remacha, M.D., Hematology Department, Hospital de Sant Pau, Avda Padre Claret 167, Barcelona 08025, Spain. Phone: international +34-93-2919290 – Fax: international +34-93-2919192 – E-mail: 2107@hsp.santpau.es

References

1. Pietrangelo A, Camaschella C. Molecular genetics and control of iron metabolism in hemochromatosis. Haematologica 1998; 83:456-61.
2. Piperno A. Classification and diagnosis of iron overload. Haematologica 1998; 83:447-55.
3. Baiget M, Barceló MJ, Gimferrer E. Frequency of the HFE C282Y and H63D mutations in distinct ethnic groups living in Spain. J Med Genet 1998; 35:701.
4. Sánchez M, Bruguera M, Bosch J, Rodés J, Ballesta F, Oliva R. Prevalence of the Cys282Tyr and His63Asp HFE gene mutations in Spanish patients with hereditary hemochromatosis and in controls. J Hepatol 1998; 29:725-8.
5. Looker AC, Johnson CL. Prevalence of elevated serum transferrin saturation in adults in the United States. Ann Intern Med 1998; 129:940-5.
6. Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. A population-based study of the clinical expression of the hemochromatosis gene. N Engl J Med 1999; 341:718-24.
7. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class-I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996; 13:399-408.
8. Adams PC, Chakrabarti S. Genotypic/phenotypic correlations in genetic hemochromatosis: evolution of diagnostic criteria. Gastroenterology 1998; 114:319-23.
9. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative haemochromatosis mutations. J Med Genet 1997; 34:275-8.
10. Remacha AF, Carrasco M, Sardá MP, Barceló MJ, Baiget M. HFE mutation analysis in patients with hepatitis C virus with positive screening for iron overload. Haematologica 1999; 84:284-5.

Cytoplasmic overexpression of p53 and p21^{ras} in megaloblastic anemia

In this study, we describe, for the first time, cytoplasmic overexpression of p53 and p21^{ras} proteins in megaloblastic anemia (MA). Nuclear p53 expression and mutations of p53 and N, H, and K-ras genes were absent. These results suggest that wild-type p53 protein might induce apoptosis in MA and help maintain genetic stability in this disease.

Sir,

We found overexpression of p53 and p21^{ras} proteins in a patient with megaloblastic anemia (MA). We studied the expression of p53 and p21^{ras} in bone marrow cytospins from this patient with MA and in 6 normal control samples from bone marrow donors. The levels of these proteins were assessed by the alkaline phosphatase-antialkaline phosphatase procedure (APAAP) with monoclonal antibodies PAb 1801 (p53) and Y13-259 (p21^{ras}) (Oncogene Science – Calbiochem). The patient was a 41-year old man with anemia. Laboratory data showed red blood cells 2.54 $\times 10^{12}$/L, hemoglobin 7.8 g/dL, hematocrit 21.6%, mean corpuscular volume 85 fL, reticulocyte count 1.3%, white blood cells 11.5×10^9/L, platelets 414×10^9/L, serum folate 3.73 ng/mL (normal 3-17 ng/mL), serum B_{12} <100 pg/mL (normal 300-1,000 pg/mL), and serum lactate dehydrogenase 5,954 U/L (normal 150-450 U/L). Bone marrow aspirate revealed typical megaloblastic morphology. A diagnosis of heterozygous α^+ thalassemia was also confirmed by Southern blotting. We found that p53 protein was highly expressed (+++ intensity) in the cytoplasm of all bone marrow cells in the cytospin (Figure 1), while p21^{ras} was moderately overexpressed (++ intensity). No expression was observed in the nucleus. All the control samples exhibited very low levels of p53 and p21^{ras} expression. Six months after successful treatment with folate and vitamin B_{12}, the patient's p53 and p21^{ras} expression was similar to that of controls. Additionally, polymerase chain reaction (PCR)-single-strand conformation polymorphism (SSCP) analysis of the patient's bone marrow sample was negative for mutations in codons 12, 13 and 61 of N-, K-, and H-ras genes as well as in exons 2-11 of the p53 gene.

The patient showed an unusually high expression of wild-type p53 protein (wt-p53) in the cytoplasm of all bone marrow cells at diagnosis as demonstrated by the absence of p53 mutation, while normal bone marrow controls exhibited weak cytoplasmic p53 expression. Nuclear p53 expression was absent. As in our controls, small amounts of p53 were found by Katsumoto *et al.*[1] in the cytoplasm of normal peripheral blood lymphocytes using the same monoclonal antibody. It has already been shown that exogenous p53 can rapidly and reversibly shuttle between cytoplasm and nucleus,[2] a phenomenon that endogenous p53 can also undergo in response to DNA damaging agents.[3] Thus, cytoplasmic localization does not imply inactivation of wt-p53 functions[4] such as cell cycle arrest or apoptosis. The overexpression of p53 found in this patient may have been triggered by DNA

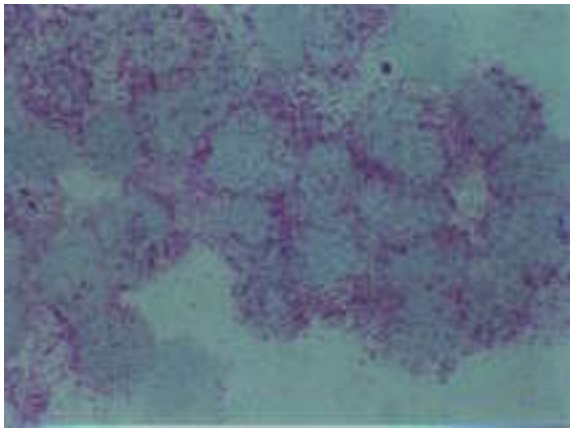

Figure 1. High expression of cytoplasmic wild-type p53 in megaloblastic marrow at diagnosis (APAAP, x1,000).

damage caused by uracil misincorporation into DNA, in agreement with a report that high levels of wt-p53 are associated with an increased rate of apoptosis in late-stage erythroblasts in folate deficiency states.[5] We suggest, therefore, that p53 is one of the main mediators which induces apoptosis in MA and helps to maintain genetic stability in this disease. On the other hand, the overexpression of $p21^{ras}$ may reflect the activation of this signal transducer in part by elevated levels of erythropoietin determined by the anemia or other hematopoietic growth factors known to stimulate this signaling pathway.

Elizabeth Schulz, Valder R. Arruda, Fernando F. Costa, Sara T.O. Saad

Hemocentro de Campinas, Departamento de Clinica Médica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Brasil

Key words

p53, $p21^{ras}$, megaloblastic anemia.

Funding

We would like to thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), and CAPES (Fundação de Coordenação de Aperfeicoamento de Pessoal de Nível Superior) for financial support for this study.

Correspondence

Sara T.O. Saad, M.D., Hemocentro de Campinas UNICAMP Barão Geraldo - Campinas - SP – Brazil P.O. Box 6198 Zip Code 13081-970. Phone: international +55-19-7888733 or 55-19-2891089 – Fax 55-19-2891089 – E-mail: sara@obelix.unicamp.br

References

1. Katsumoto T, Higaki K, Ohno K, Onodera K. Cell-cycle dependent biosynthesis and localization of p53 protein in untransformed human cells. Biol Cell 1995; 84:167–73.
2. Middeler G, Zerf K, Jenovai S, et al. The tumor suppressor p53 is subject to both nuclear import and export, and both are fast, energy-dependent and lectin-inhibited. Oncogene 1997; 14:1407-17.
3. Clarke AR, Purdie CA, Harrison DJ, et al. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature 1993; 362:849–52.
4. Goldman SC, Chen CY, Lansing TJ, Gilmer TM, Kastan MB. The p53 signal transduction pathway is intact in human neuroblastoma despite cytoplasmic localization. Am J Pathol 1996; 148:1381–5.
5. Koury MJ, Horne DW, Brown ZA, et al. Apoptosis of late-stage erythroblasts in megaloblastic anemia: association with DNA damage and macrocyte production. Blood 1997; 89:4617–23.

Acute monocytic leukemia in the adult presenting with associated extramedullary gastric infiltration and ascites

Involvement of the stomach in acute myelogenous leukemia has been rarely described. We report a case of an adult patient with acute monocytic leukemia who presented with an abdominal mass and ascites due to massive intramural involvement. Leukemic gastric infiltration in the adult leading to a tumoral presentation and melena has, to our knowledge, not been previously reported.

Sir,

Acute monocytic leukemia (AMoL), referred to as M5 in the FAB classification, is the morphologic subtype of acute myelogenous leukemia (AML) that most frequently presents with extramedullary involvement, including liver, spleen, lymph nodes, gingiva, skin, eyes, larynx, lung, bladder, meninges and the central nervous system. Involvement of the gastrointestinal tract is rare, the mouth, rectum and anal canal being the most affected sites.[1] By contrast, leukemic infiltration of the stomach has been rarely described, and when it has, mainly in children.[2,3] We report a case of an adult patient with AMoL who presented with an abdominal mass, ascites and melena due to massive intramural gastric infiltration.

A 32-year old woman was referred to our department with a 1-month history of constitutional symptoms, abdominal pain and melena, with a normal blood smear. There was no relevant past medical history. Examination upon admission revealed pallor, ascites and an epigastric mass, without palpable lymphadenopathy, hepatomegaly or splenomegaly. Examination of ascitic fluid revealed an exudate containing abundant monocytic blasts. Laboratory tests showed a hemoglobin of 99 g/L, MCV 88 fL, WBC 15.7×10^9/L (1% eosinophils, 28% neutrophils, 35% lymphocytes, 13% monocytes, 23% blast cells) and a platelet count of 84×10^9/L. Prothrombin and partial thromboplastin times were normal. Biochemistry data were unremarkable except for LDH 16 mkat/L (normal 3.9-6.7), albumin 36 g/L (normal 39-50), total proteins 59 g/L (normal 64-80) and β_2-microglobulin 3.7 mg/L (normal 0.8-2.4).

Bone marrow examination revealed a blast population making up more than 50% of the cellular marrow components. The blast cells had an immature monocytic appearance and a diagnosis of acute monocytic leukemia (FAB M5a) was made. Blast cells were positive for non-specific esterase and negative for peroxi-