calculated using the Genepix Pro3.0 software (Axon, USA). In quantitative analysis, the wild-type-to-mutant signal ratios (W/M) is the spot of wild type probe (denoted as W) to mutant probe (denoted as M), can be employed to detect the genotype of point mutations. We employed an intensity ratio of 5:1 as the threshold for categorizing the positive and negative signals. Used to detect point mutations, the W/M produced unequivocal assignment of samples genotype, with W/M > 5 for normal subjects, ~1 for heterozygotes, and < 0.2 for homozygous mutations. The Q-probe group was used to detect three deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$). Q1 probe was complementary to a 378-base-long fragment of normal $\alpha 2$ gene produced by 5'-Cy5- ctctcagggcagaggatcac. Q2 probe was complementary to a 384-base-long fragment of the mutation ($-\alpha^{3.7}$) produced by 5'-Cy5-ctctcagggcagaggatcac. Q3 probe was complementary to a 253-base-long fragment produced by linear amplification of PCR product ($--^{SEA}$) by 5'-Cy5-gcgatctgggctctgtgttct. Q4 probe was complementary to a 155-base-long fragment produced by linear amplification of PCR product ($-\alpha^{4.2}$) by 5'-Cy5-gcagaggttgcagtgagcta. When the signal of a deletion ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$) allele was present, the Q1 probe was used to provide a reliable identification of heterozygous, compound heterozygous and homozygous α thalassemia genotypes. Again, we employed an intensity ratio of 5:1 as the threshold to categorize the positive and negative signals in the Q probe group. The hybridization result with a sample (β(41-42) heterozygote, $\alpha\alpha/--^{SEA}$) is shown in Figure 1. It is apparent from these results that the discrimination of single-base mismatches is excellent. In addition, the results demonstrate the microarray's ability to detect and discriminate both homozygous and heterozygous mutations in human genomic DNA.

To demonstrate the accuracy of the method in genotyping thalassemia, we investigated 1,880 unrelated individuals whose thalassemia alleles had been typed previously. There was 100% agreement in all 1,880 samples. Based on these results we conclude that the method described here is a valuable technique, suitable for population screening.

Ye Bang-Ce, Liu Hongqiong,
Zhang Zhuanfong, Lei Zhengsong, Gao Jianling

**State Key Laboratory of Bioreactor Engineering, East China University of Science & Technology, Shanghai, 200237, China; °Jiangnan Biotechnology Inc. Huzhou, Zhejiang, 313000, China*

Funding: This work was supported in part by 863 Program of China (2002AA2Z2043).

Acknowledgments: we are grateful to Dr. Li Dehao (the Family Planning Bureau of Hainan) and Mr. Lin Weixiong (the First Affiliated Hospital of Guangxi Medical University), who kindly provided us samples for this study.

Key words: thalassemia, microarray, genotyping, population screening

Correspondence: Ye Bang-Ce, State Key Laboratory of Bioreactor Engineering, East China University of Science & Technology, Shanghai, 200237, China. Phone: international +86.21.64252250. Fax: international + 86.21.64253904. E-mail: bcye@ecust.edu.cn

References

1. Chan K, Wong MS, Chan TK, Chan V. A thalassaemia array for Southeast Asia. Br J Haematol 2004;124:232-9.
2. Chong SS, Boehm CD, Higgs DR, Cutting GR. Single-tube multiplex-PCR screen for common deletional determinants of α-thalassemia. Blood 2000;95:360-2.
3. Guo Z, Gullfoyle FA, Thiel AJ, Wang R, Smith LM. Direct fluorescence analysis of genetic polymorphisms by hybridization with oligonucleotide arrays on glass supports. Nucleic Acids Res 1994; 22:5456-65.

Stem Cell Disorders

Lack of mutations in the human telomerase RNA component (*hTERC*) gene in Fanconi's anemia

As some patients with Fanconi's anemia (FA) present excessive telomere shortening correlating with poor outcome, we investigated whether human telomerase RNA component (*hTERC)* mutations also play a role in telomere shortening in 115 FA patients. Only one patient was heterozygous for the G58A polymorphism. No other mutation or deletion was found. We conclude that *hTERC* gene mutations do not contribute to telomere shortening in FA.

haematologica 2004; 89:1012-1013
(*http://www.haematologica.org/2004/8/1012)*

Fanconi's anemia (FA) is an autosomal recessive disorder clinically characterized by progressive pancytopenia due to bone marrow (BM) failure frequently evolving to acute leukemia, a variety of physical abnormalities, and increased predisposition to cancer.[1] The onset of BM failure may be variable, presenting at older ages in some patients due, at least in part, to a myriad of genetic abnormalities. Eleven complementation groups (A to J) of FA cells have been described.[2] Increased telomere shortening also correlates with BM failure in FA.[3] Telomeres are responsible for the integrity and stability of chromosome ends, and telomere length is maintained by telomerase-mediated addition of telomeric repeats.[4] Excessive telomere shortening is observed in other types of BM failure syndromes, such as dyskeratosis congenita (DC) and acquired aplastic anemia. Mutations in genes encoding telomerase complex components cause the X-linked type of DC (*DKC1 gene*) and the autosomal dominant type of DC (*hTERC gene*).[1,5] Mutations in the *hTERC* gene are also found in a small subgroup of patients with acquired aplastic anemia.[6-10]

In this study, we investigated whether *hTERC* mutations may also contribute to telomere shortening in FA. Peripheral blood samples were collected from 115 patients with FA before bone marrow transplantation and screened for *hTERC* mutations by direct sequencing, as previously described.[6,7] Seventy-one were Brazilian patients treated at the Federal University of Paraná, Curitiba, PR, Brazil (37 males and 34 females; 42 were White, 25 Mulatto, seven Black and one Asian); the diagnosis was confirmed by diepoxybutane-induced chromosome breakage analysis. Complementation group analysis was available for 39 Brazilian patients (30 belonged to the FANC A group, four to the FANC C group, and five to the FANC G group). The other 44 cases studied were European patients treated at Hôpital Saint-Louis, Paris, France, and had a Caucasian ethnic background. Fifty-seven percent of these patients were male and 43% were females. Their diagnoses were confirmed by cytogenetics and nitrogen mustard-induced chromosome breakage analysis. Complementation group analysis was available for 17 patients (16 were FANC A and one was FANC C). Telomere length was determined in peripheral blood leukocytes by Southern blot in all European patients, as previously reported.[3] Nineteen patients had a telomere shortening below 200 bp/year, whereas in 25 patients telomere shortening was greater than 200 bp/year. Blood samples for genetic analysis were collected after informed consent had been obtained for this study which was approved by the respective local Ethics Committees.

One patient (a 12-year old, African-Brazilian male with FANC A complementation group, homozygous for the 3788-

3790 FANC A mutation) was heterozygous for the G58A transition. No other mutation or deletion was found among these patients. It is important to note, however, that other large deletions or promoter region mutations could not be detected if beyond the limits of our primers. The G58A substitution was previously described in one patient with constitutional aplastic anemia and in two with acquired aplastic anemia by Vulliamy *et al.*[6] Yamaguchi *et al.*[8] also found two Black patients with myelodysplastic syndrome and one patient with acquired aplastic anemia who were heterozygous for this substitution. More recently, Wilson *et al.*[9] described a 2-year old Black myelodysplastic patient heterozygous for the G58A transition. Four of eight relatives of this patient carry this gene variant, but none presents any hematologic abnormality. The prevalence of the G58A substitution varies from 4 to 20% in African-Americans and is not a disease-causing mutation, but a common polymorphism, especially among normal Black subjects.[8,9] In the present study, the only patient carrying this substitution was an African-Brazilian. BM failure was diagnosed at the age of 3 years, and the patient had a short stature, low weight, narrow eyelid gap, tenar hypotrophy, and *café-au-lait* spots. No HLA-matched donor was available, but the BM showed relative response to treatment with oxymetholone and the boy became transfusion independent.

Nucleotide G58, located immediately 3' of the template, is not phylogenetically conserved in vertebrates, does not influence the telomerase RNA tertiary structure, and does not influence telomerase activity *in vitro*.[11] Taken together, these data strongly suggest that this polymorphism is unlikely to play a role in the pathogenesis of BM failure in FA or in other BM failure syndromes.

In conclusion, although excessive telomere length shortening is observed in some patients with FA and seems to predict evolution to aplastic anemia, mutations in the *hTERC* gene do not contribute to this phenomenon in the setting of FA. However, mutations in other genes encoding other components of the telomerase complex might play a role. Alternatively, increased hematopoietic proliferative demands and/or the oxidative stress in response to BM failure may ultimately lead to increased telomere shortening in FA.

Rodrigo T. Calado,[+] Maria-Carolina Pintão,*[+] Vanderson Rocha,[@] Roberto P. Falcão,* Marco A. Bitencourt,[#] Wilson A. Silva Jr.,° Eliane Gluckman,[@] Ricardo Pasquini,[#] Marco A. Zago**

**Center for Cell-Based Therapy, Department of Clinical Medicine; °Department of Genetics, University of São Paulo at Ribeirão Preto Medical School, Ribeirão Preto, SP; [#]Bone Marrow Transplant Unit, Federal University of Paraná, Curitiba, PR, Brazil; [@]Hematopoietic Stem Cell Transplant Unit, Hôpital Saint Louis, Paris, France*

[+]These authors contributed equally to this study.

Funding: This work was supported in part by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant 98/14247-6.

Key words: Fanconi's anemia, bone marrow failure, telomerase, telomere.

Correspondence: Dr. Rodrigo T. Calado, Department of Clinical Medicine, University of São Paulo at Ribeirão Preto Medical School, Av. Bandeirantes, 3900 – Ribeirão Preto, SP 14049-900 Brazil. Phone: international +55.16.21019361. Fax: international + 55.16.21019309. E-mail: rtcalado@uol.com.br

References

1. Dokal I. Inherited aplastic anaemia. Hematology J 2003;4:3-9.
2. Levitus M, Rooimans MA, Steltenpool J, Cool NF, Oostra AB, Mathew CG, et al. Heterogeneity in Fanconi anemia: evidence for two new genetic subtypes. Blood 2004;103:2498-503.
3. Li X, Leteurtre F, Rocha V, Guardiola P, Berger R, Daniel MT, et al. Abnormal telomere metabolism in Fanconi´s anaemia correlates with genomic instability and the probability of developing severe aplastic anaemia. Br J Haematol 2003;120:836-45.
4. Collins K, Mitchell JR. Telomerase in the human organism. Oncogene 2002;21:564-79.
5. Vulliamy T, Marrone A, Goldman F, Dearlove A, Bessler M, Mason P, et al. The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. Nature 2001;413:432-5.
6. Vulliamy T, Marrone A, Dokal I, Mason PJ. Association between aplastic anaemia and mutations in telomerase RNA. Lancet 2002;359:2168-70.
7. Calado RT, Pintao MC, Silva WA, Falcao RP, Zago MA. Aplastic anaemia and telomerase RNA mutations. Lancet 2002; 360:1608.
8. Yamaguchi H, Baerlocher GM, Lansdorp PL, Chanock SJ, Nunez O, Sloand EM, et al. Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. Blood 2003;102:916-8.
9. Wilson DB, Ivanovich J, Whelan A, Goodfellow PJ, Bessler M. Human telomerase RNA mutations and bone marrow failure. Lancet 2003;361:1993-4.
10. Fogarty PF, Yamaguchi H, Wiestner A, Baerlocher GM, Sloand E, Zeng WS, et al. Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. Lancet 2003;362:1628-30.
11. Ly H, Blackburn EH, Parslow TG. Comprehensive structure-function analysis of the core domain of human telomerase RNA. Mol Cell Biol 2004;23:6849-56.

Acute Lymphoblastic Leukemia

Molecular diagnosis of leukemic cerebrospinal fluid cells in children with newly diagnosed acute lymphoblastic leukemia

Cytomorphology and IgH/T-cell receptor γ clonal gene rearrangements detected by polymerase chain reaction (PCR) homo/heteroduplex analysis and direct sequencing were evaluated in cerebrospinal fluid (CSF) free of red-blood cells at diagnosis of 37 children with acute lymphobalstic leukemia. Molecular CSF involvement was greater as detected by molecular analysis than observed by morphologic criteria (45.9% vs 5.4%). The 4-year event-free survival was lower in the group with molecularly detected CSF involvement (p = 0.01).

haematologica 2004; 89:1013-1015
(*http://www.haematologica.org/2004/8/1013)*

In the majority of treatment centers the diagnosis of central nervous system (CNS) involvement is defined by the presence of pleocytosis of more than 5 cells per cubic millimeters in the cerebrospinal fluid (CSF), with blast cells or the presence of cranial nerve palsies. The detection of CNS involvement has been associated with a high risk of relapse.[1,2] Some studies have also demonstrated that patients with any identifiable blast cells in CSF, as well CSF contamination by blastic cells during traumatic lumbar puncture, have an increased risk of CNS relapse.[3-5] Others, however, did not find this association.[6,7] Conventional cytological analysis has proven useful, but the analysis of cells in CSF, especially when the cell count is low, is more difficult than is widely admitted and nor is it always conclusive.

We analyzed 37 of 66 children with acute lymphoblastic leukemia (ALL) admitted to our institution (18 were classified as having standard risk and 19 as high risk). Twenty-nine patients were excluded, 13 because of the presence of red blood cells in the CSF, suggesting puncture accident, 10 because of the absence of stored CSF or bone marrow at

©Ferrata Storti Foundation