A 19-year follow-up of a patient with type 3 ectrodactyly–ectodermal dysplasia–clefting syndrome who developed non-Hodgkin lymphoma

Sevim Balci, MD,[a] Ozlem Engiz, MD,[a] Gulsen Okten, MD,[b] Murat Sipahier, DMD,[c] Guler Gursu, MD,[d] and Bedri Kandemir, MD,[e] Ankara and Samsun, Turkey
HACETTEPE UNIVERSITY AND ONDOKUZ MAYIS UNIVERSITY

The ectrodactyly–ectodermal dysplasia–clefting (EEC) syndrome is characterized by ectrodactyly, ectodermal dysplasia, and clefting. The development of a malignancy with EEC syndrome is very rare. Here we present follow-up on a Turkish boy with EEC syndrome type 3 who developed malignant lymphoma with high expression of *p63*. He had chronic renal failure due to recurrent urinary infections caused by ureterovesical reflux. Cervical, diffuse, large, B-cell non-Hodgkin lymphoma with high expression of *p63* was diagnosed, and the patient died at 19 years of age. The transcription factor *p63* is a key regulator of ectodermal, orofacial, and limb development. Mutations in the *p63* gene can cause syndromes of ectodermal dysplasia, ectrodactyly, and orofacial clefting. Malignant lymphoma is a very rare complication of EEC syndrome. We suggest that *p63* gene mutation analysis should be performed in every EEC syndrome patient with the possibility of developing malignant tumors. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:e91-e95)**

The ectrodactyly–ectodermal dysplasia–clefting (EEC) syndrome (MIM *129900) is characterized by split hand/foot malformation, anomalies of hair, teeth, nails, nipples, nasolacrimal ducts, and sweat glands, and cleft lip with or without cleft palate.[1,2] There are 3 types of EEC syndrome, and their gene loci have been identified: EEC syndrome type 1 (MIM 129900) was assigned to 7q11.2-q21.3,[3,4] type 2 (MIM 602077) to chromosome 19,[5] and type 3 (MIM 604292) to 3q27.[6] Recent studies have identified heterozygous mutations in the DNA-binding domain of the *p63* gene at 3q27 as the molecular basis for EEC type 3 (EEC3).[6-9]

The association of a malignancy with EEC syndrome is very rare.[10,11] Here we report on a follow-up of an 18-year-old Turkish boy with EEC type 3. This patient was first reported by Balci et al. in 1983.[12] Later on, this patient was reported by Ogütcen-Toller et al. as a letter to the editor.[13] The patient suffered from recurrent urinary infections and subsequently chronic renal insufficiency developed. Later on, non-Hodgkin large B-cell diffuse lymphoma developed, and the patient died at 19 years old. In this paper, we reevaluate this patient's clinical, radiologic, and histopathologic findings in detail and present the result of *p63* gene expression which was not performed previously. The *p63* gene may function as a tumor-suppressor gene.[10] The demonstration of p63 in cases with EEC3 may be considered to be an important indicator of malignancy.

CASE REPORT

The patient was a male newborn who was referred to our hospital with chief complaints of bilateral cleft lip and palate and ectrodactyly of left hand and foot. Syndactyly of the second and third fingers of the right hand was also observed. The delivery was uneventful at 36 weeks. The patient was the first child of a phenotypically normal 19-year-old mother and a 25-year-old father. The parents were third-degree relatives. Three younger sisters of the patient were phenotypically normal (Fig. 1). The family history revealed no similar malformation.

The patient had several reconstructive plastic surgery operations for his cleft lip and palate and hands at 2 years of age. Intravenous pyelogram revealed bilateral hydronephrosis and hydroureter (Fig. 2). When he was 3 years old, bilateral ureteroneocystostomy was performed. Clinical signs of ectodermal dysplasia, such as blonde hair and sparse eyebrows and eyelashes, were noted at the age of 4 years (Fig. 3). Complete cleft palate closure was achieved at this age. He was also operated on his right hand for the separation of syndactyly. At the age of 8 years, enamel hypoplasia and partial adontia became evident (Fig. 4).

[a]Department of Clinical Genetics, Hacettepe University Medical Faculty, Ihsan Doğramaci Children's Hospital.
[b]Department of Medical Genetics, Ondokuz Mayıs University Medical Faculty.
[c]Faculty of Dentistry, Ondokuz Mayıs University.
[d]Department of Plastic and Reconstructive Surgery, Hacettepe University Medical Faculty.
[e]Department of Pathology, Ondokuz Mayıs University Medical Faculty.
Received for publication Aug 15, 2007; returned for revision Apr 16, 2009; accepted for publication Apr 29, 2009.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2009.04.043

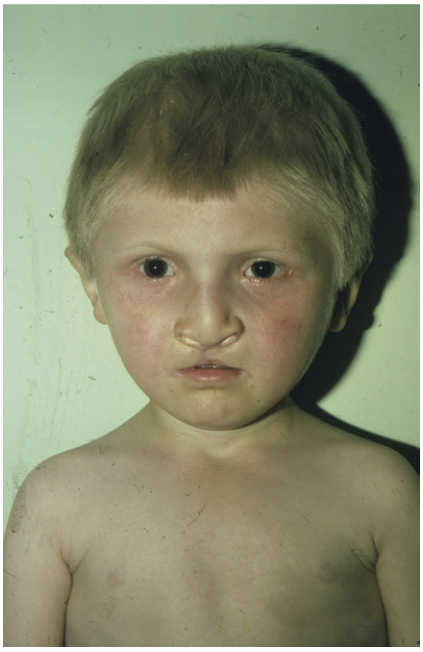

Fig. 1. Bilateral cleft lip-palate, ectrodactyly of left and foot of the patient is seen.

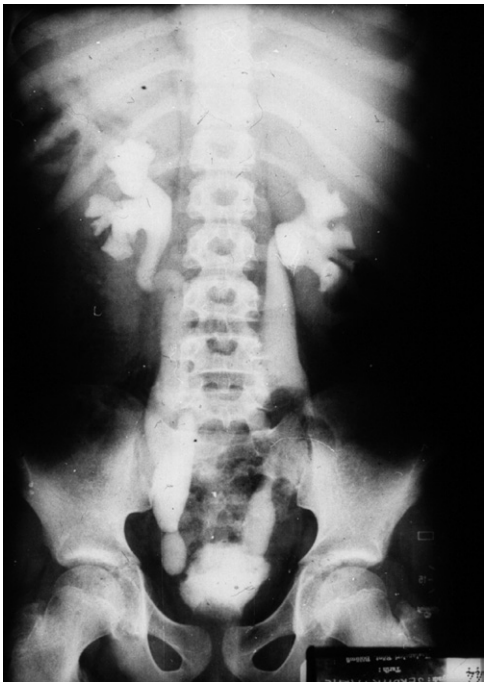

Fig. 2. Intravenous pyelogram demonstrating bilateral hydronephrosis and hydroureter.

At the age of 18 years, he had symptoms of weight loss and fever, and 3 separate masses were discovered during his physical examination. A $10 \times 7 \times 5.5$ cm cervical mass was removed surgically. The other masses were located in the postauricular and peritonsillar regions.

The surgical specimen was fixed in 10% neutral-buffered formaldehyte and parafin embedded. Sections of 7 μm thickness were stained with hematoxylin and eosin. Light microscopic examination revealed non-Hodgkin lymphoma composed of large atypical lymphoid cells. The cells had moderate amount of cytoplasm and round vesiculated nuclei with prominent nucleoli. Numerous mitoses and necrotic foci were encountered over all of the tumor tissue.

Fig. 3. Clinical signs of ectodermal dysplasia, such as blonde hair, sparse hair and eyebrows, are noted.

A tumor block without areas of necrosis or hemorrrhage was selected for immunohistochemistry, and sections of 4 μm thickness were cut. The immunohistochemistry panel consisted of LCA (CD45 Ab-2 Clone PD7/26/16+2B11; Labvision, Fremont, CA, USA), T-cell (CD3 Ab-2; Labvision), B-cell (CD20, Ab-1, Clone 2b; Labvision), Ki-67 (Clone SP6; Labvision), p53 (DO-7 mouse monoclonal antibody; Novocastro, Newcastle, U.K.), and p63 (Ab-1, clone4A 4; Labvision) monoclonal antibodies. Immunohistochemical staining was performed using the streptavidin-biotin-peroxidase technique. Endogenous peroxidase activity of the deparaffinized sections was removed by incubation in 3% hydrogen peroxide solution for 10 minutes. Subsequently, sections to be stained for Ki-67, T-cell, p53, and p63 were boiled in citrate buffer for 35 minutes for antigen retrieval and left to cool for 20 minutes. Antigen retrieval was not done for LCA and B-cell. Blocking solution was applied for 5 minutes. Then primary antibodies for B-cell, T-cell, p53, p63, and Ki-67 were allowed to react at room temperature for 60 minutes, and 30 minutes for LCA. After washing with phosphate-buffered saline, secondary antibody was applied for 10 minutes, followed by peroxidase-marked streptavidin for 10 minutes. Peroxidase was visualized by diaminobenzidine tetrahydrochloridine (DAKO, Carpinteria, CA, USA). Nuclei were stained with Mayer hematoxylin for 60 seconds. Appropriate controls were included in the immunohistochemical study. Tumor cells stained positive for B-cell (CD20) marker and stained negative for T-cell (CD3). Tumor cells were 3 to 4 times as large as a mature lymphocyte. Most of the tumor cells were

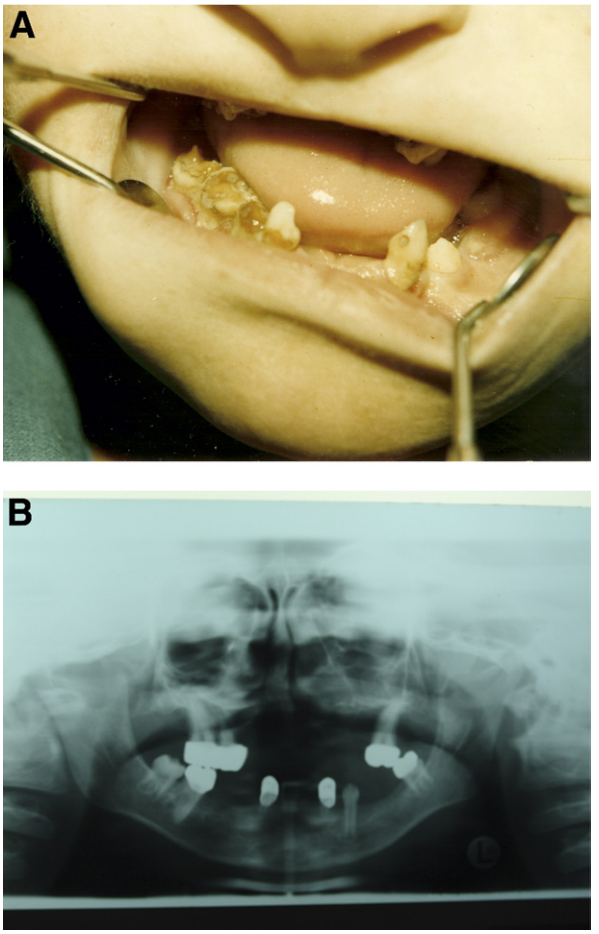

Fig. 4. **A,** Dental abnormalities, anodontia, and enamel hypoplasia are noted. **B,** Panaromic roentgen film showing anodontia.

large atypical lymphocytes which were much larger than a red blood cell (Fig. 5, *A*).

Normal lymphoid tissue cells used as control showed 80% positive staining for Ki-67 proliferation index in germinal centers. Tumor cells displayed 2% positive staining for Ki-67 proliferation index. p53 and p63 overexpression were assessed by comparing tumor tissue with normal lymphoid tissue. No positive staining was observed in normal lymphoid tissue, whereas 30% of the tumor cells showed overexpression for p53 (Fig. 5, *B*) and 60% for p63 (Fig. 5, *C*).

The pathologic diagnosis was diffuse, large, B-cell non-Hodgkin lymphoma. He was scheduled for chemotherapy. Unfortunately the patient died at 19 years of age.

DISCUSSION

The EEC syndrome is characterized by ectrodactyly, ectodermal dysplasia, and facial clefts. In ectodermal dysplasia, skin, hair, teeth, nails, and several exocrine glands, such as sweat and sebaceous glands, are abnormally developed. Ectrodactyly, which means clefts in

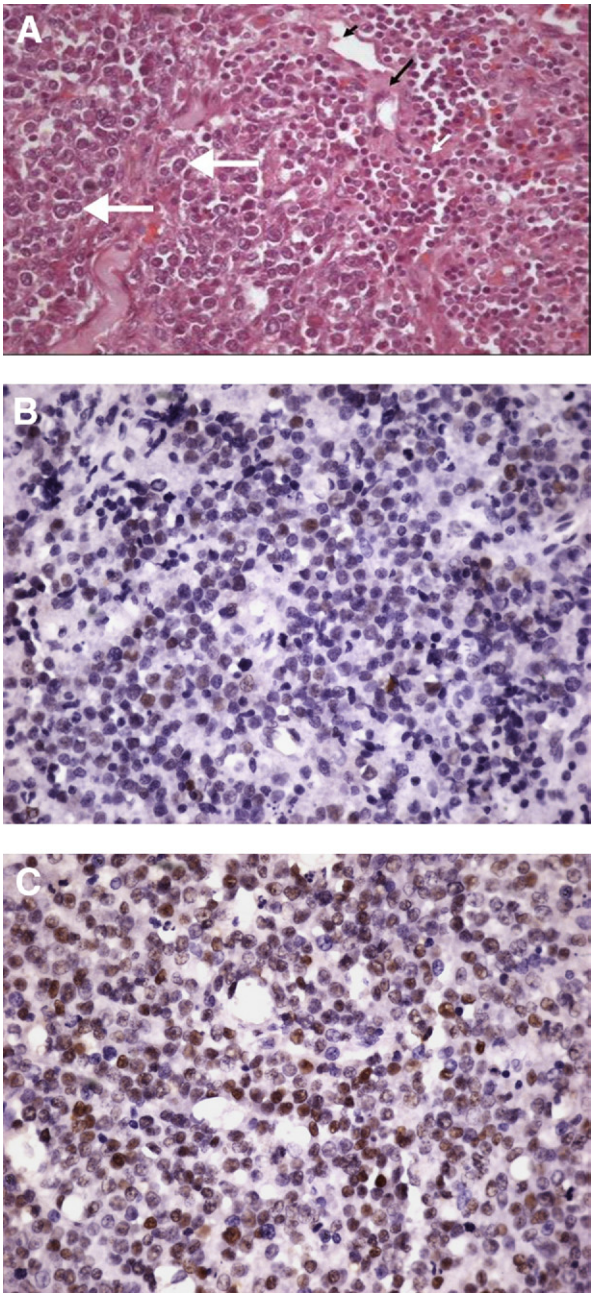

Fig. 5. **A,** Diffuse large B-cell lymphoma cells with large round nuclei and multiple nucleoli. *Short black arrow* shows red blood cell; *long black arrow* shows endothelial cell; *small white arrow* shows a mature lymphocyte; and 2 *white block arrows* show tumor cells. Hematoxylin and eosin, ×200. **B,** Neoplastic tumor cells with high nuclear expression of p53. (diaminobenzidine tetrahydrochloridine chromogen, ×200). **C,** Neoplastic tumor cells with high nuclear expression of p63. (diaminobenzidine tetrahydrochloridine chromogen, ×200).

hands and feet in conjunction with a lack of one or more central digits, is the second characteristic feature of the syndrome. Syndactyly can be seen in conjunction

with ectrodactyly, as seen in this patient. The third component of the syndrome is orofacial clefting. It is seen in the form of cleft lip and/or cleft palate.[14] Genitourinary malformations are commonly present in the form of kidney or collecting system duplication, dysplastic kidney, and hydroureter. Urinary infections are frequently seen in EEC syndrome.[15,16] The present patient demonstrated all 3 components of the syndrome as well as urinary tract malformation.

To date, there have been 3 reports documenting association of EEC syndrome with malignant lymphoma. Gerschoni-Baruch et al.[11] reported 2 brothers with EEC syndrome; one of them had mild primary hypothyroidism, developed Hodgkin disease, and died at the age of 16 years. Ogütcen-Toller et al.[13] reported the present case with EEC3 syndrome and diffuse large-cell non-Hodgkin lymphoma. Although these cases present a relationship between EEC syndrome and malignancy, *p63* expression analysis was not performed. Akahoshi et al.[10] reported a Japanese girl with EEC syndrome who developed diffuse large B-cell non-Hodgkin lymphoma. Molecular p63 gene mutation analysis was performed for this patient. An A-to-G substitution at nucleotide position 1,079 in exon 8 of *P63* was detected, the change predicting a heterozygous missense mutation, Asp312Gly (D312G). Because this mutant allele was not present among 100 normal control individuals, and another mutation (D312H) at the same nucleotide position has been reported in a patient with EEC syndrome,[17] the authors concluded that the D312G mutation detected in their patient contributed to her EEC syndrome. The present patient is possibly type 3 EEC syndrome characterized by ectrodactyly, ectodermal dysplasia, cleft palate, and specific genitourinary defects similar to the patient reported by Akahoshi et al. Thus this patient is the second documented patient with EEC3 and malignant lymphoma.

Ki-67 is a monoclonal antibody that is present in proliferating cells. Ki-67 expression in the diffuse large B-cell lymphoma is generally >40%. Agressive clinical course is generally associated with >80% expression. Ki-67 expression of 2% shows a favourable outcome. p53 overexpression correlates with *p53* mutation and indicates poor prognosis in diffuse large B-cell lymphoma.[18] p63 overexpression was found to be associated with the development of lymphoma, but its prognostic significance is still unclear.[19]

Recently the evidence of a relationship between mutations in the *p63* gene and EEC syndrome has been published.[6,9,17,20] The *p63* gene expression is essential for limb formation and epidermal morphogenesis, including the formation of adnexa (teeth, hair, and mammary and prostate glands).[21] Numerous studies have investigated the role of *p63* in neoplastic transformation and tumor progression.[22-25] There is growing evidence that *p63* is involved in oncogenesis through several mechanisms. Transactivating isoforms, such as TAp63/p73, show tumor suppressor gene properties similar to *p53*, and isoforms lacking N-terminal transactivating domain, such as DeltaNp63, induce a functional block against *p53* as well as TAp63/p73.[26] Also, the interaction of tumor-derived *p53* mutants with *p63* impairs the transcriptional activity of *p63* and this could provide survival advantage to cancer cells.[27] The identification of p53-like and p53-inhibitory version of p63 and the close interplay activities within the p53 family members give *p63* gene both tumor suppressor and oncogenic roles. Future reports implicating a relationship between p63 and malignancy are required to determine which of these roles are important in tumorigenesis.

In conclusion, *p63* gene may act as a tumor suppressor gene in patients with EEC3 syndrome and may cause malignant disorders, such as lymphoma. The identification of *p63* mutations may suggest malignant diseases in EEC3 syndrome. Therefore *p63* mutation analysis should be performed in every patient with EEC3 syndrome to predict the risk of malignant lymphoma.

REFERENCES

1. Rüdiger RA, Haase W, Passarge E. Association of ectrodactyly, ectodermal dysplasia and cleft lip-palate. Am J Dis Child 1970; 120:160-3.
2. Rodini ES, Richieri-Costa A. EEC syndrome: report on 20 new patients, clinical and genetic considerations. Am J Med Genet 1990;37:42-53.
3. Qumsiyeh MB. EEC syndrome (ectrodactyly, ectodermal dysplasia and cleft lip/palate) is on 7p11.2-q21.3. Clin Genet 1992;42:101.
4. Fukushima Y, Ohashi H, Hasegawa T. The breakpoints of the EEC syndrome (ectrodactyly, ectodermal dysplasia, and cleft lip/palate) confirmed to 7q11.21 and 9p12 by fluorescence in situ hybridization. Clin Genet 1993;44:50.
5. O'Quinn JR, Hennekam RCM, Jorde LB, Bamshad M. Syndromic ectrodactyly with severe limb, ectodermal, urogenital, and palatal defects maps to chromosome 19. Am J Hum Genet 1997;61(Suppl):A289.
6. Celli J, Duijf P, Hamel BC, Bamshad M, Kramer B, Smits AP, et al. Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. Cell 1999;99:143-53.
7. Ianakiev P, Kilpatrick MW, Toudjarska I, Basel D, Beighton P, Tsipouras P. Split-hand/split-foot malformation is caused by mutations in the p63 gene on 3q27. Am J Hum Genet 2000;67: 59-66.
8. Wessagowit V, Mellerio JE, Pembroke AC, McGrath JA. Heterozygous germline missense mutation in the p63 gene underlying EEC syndrome. Clin Exp Dermatol 2000;25:441-3.
9. Kosaki R, Ohashi H, Yoshihashi H, Suzuki T, Kosaki K. A de novo mutation (R279C) in the p63 gene in a patient with EEC syndrome. Clin Genet 2001;60:314-5.
10. Akahoshi K, Sakazume S, Kosaki K, Ohashi H, Fukushima Y. EEC syndrome type 3 with a heterozygous germline mutation in the p63 and B cell lymphoma. Am J Med Genet 2003;120A: 370-3.

11. Gershoni-Baruch R, Goldscher D, Hochberg Z. Ectrodactyly-ectodermal dysplasia-clefting syndrome and hypothalamo-pituitary insufficiency. Am J Med Genet 1997;68:168-72.
12. Balcı S, Tunçbilek E, Hazarlı GG, Yildiz P, Shyr Y, Edgerton ME. Ectrodactyly-ectodermal dysplasia-cleft syndrome (EEC). Özdemir Kardeşler Matbaacılık, XXI Türk Pediatri Kongresi, İstanbul. Türk Pediatri Kurumu Yayınları 1983;24:523.
13. Ogütcen-Toller M, Gulen O, Okten G, Elbistan M. Non-Hodgkin's lymphoma in a patient with ectrodactyly–ectodermal dysplasia–clefting syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;90:124-5.
14. Penchaszadeh VB, de Negrotti TC. Ectrodactyly–ectodermal dysplasia–clefting (EEC) syndrome: Dominant inheritance and variable expression. J Med Genet 1976:13:281-4.
15. Maas SM, de Jong TP, Buss P, Hennekam RC. EEC syndrome and genitourinary anomalies: an update. Am J Med Genet 1996; 63:472-8.
16. Nardi AC, Ferreira U, Netto Júnior NR, Magna LA, Rodini ES, Richieri-Costa A. Urinary tract involvement in EEC syndrome. A clinical study in 25 Brazilian patients. Am J Med Genet 1992;44:803-6.
17. van Bokhoven H, Hamel BC, Bamshad M, Sangiorgi E, Gurrieri F, Duijf PH, et al. p63 gene mutations in EEC syndrome, limb-mammary syndrome, and isolated split hand–split foot malformation suggest a genotype-phenotype correlation. Am J Hum Genet 2001;69;481-92.
18. Taylor CR, Cote RJ, editors. Immunomicroscopy: a diagnostic tool for the surgical pathologist. 3rd ed. Philadelphia: Saunders Elsevier; 2006. p. 117.
19. Hallack Neto AE, Siqueira SA, Dulley FL, Ruiz MA, Chamone DA, Pereira J. p63 protein expression in high risk diffuse large B-cell lymphoma. J Clin Pathol 2009;62:77-9.
20. Brunner HG, Hamel BC, Van Bokhoven H. The p63 gene in EEC and other syndromes. J Med Genet 2002;39:377-81.
21. Yang A, Schweitzer R, Sun D, et al. p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 1999;398:714-8.
22. Yamaguchi K, Wu L, Caballero OL, et al. Frequent gain of the p40/p51/p63 gene locus in primary head and neck squamous cell carcinoma. Int J Cancer 2000;86:684-9.
23. Massion PP, Taflan PM, Jamshedur Rahman SM, Charytonowicz E, Verbel D, Crum CP, et al. Significance of p63 amplification and overexpression in lung cancer development and prognosis. Cancer Res 2003;63:7113-21.
24. Urist MJ, Di Como CJ, Lu M, et al. Loss of p63 expression is associated with tumor progression in bladder cancer. Am J Pathol 2002;161:1199-206.
25. Hedvat CV, Teruya-Feldstein J, Puig P, Capodieci P, Dudas M, Pica N, et al. Expression of p63 in diffuse large B-cell lymphoma. Appl Immunohistochem Mol Morphol 2005;13:237-42.
26. Stiewe T, Theseling CC, Pützer BM. Transactivation-deficient Delta TAp73 inhibits p53 by direct competition for DNA binding: implications for tumorigenesis. J Biol Chem 2002;277: 14177-85.
27. Flores ER, Tsai KY, Crowley D, Sengupta S, Yang A, McKeon F, Jacks T. P63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature 2002;416:560-4.

Reprint requests:

Prof. Dr. Sevim Balci
Kıbrıs Sokak 17/8
Kavaklıdere 06690
Ankara
Turkey
sbalci@hacettepe.edu.tr