J Clin Immunol (2010) 30:132–137
DOI 10.1007/s10875-009-9340-6

BAK, *BAX*, and *NBK/BIK* Proapoptotic Gene Alterations in Iranian Patients with Ataxia Telangiectasia

Anna Isaian · Natalia V. Bogdanova · Masoud Houshmand · Masoud Movahadi · Asghar Agamohammadi · Nima Rezaei · Lida Atarod · Mahnaz Sadeghi-Shabestari · Seyed Hasan Tonekaboni · Zahra Chavoshzadeh · Seyed Mohammad Seyed Hassani · Reza Mirfakhrai · Taher Cheraghi · Najmoddin Kalantari · Mitra Ataei · Thilo Dork-Bousset · Mohammad Hossein Sanati

Received: 21 July 2009 / Accepted: 29 September 2009 / Published online: 7 November 2009
© Springer Science + Business Media, LLC 2009

Abstract
Introduction Ataxia telangiectasia (AT) is an autosomal recessive multisystem disorder characterized by variable immunodeficiency, progressive neurodegeneration, occulocutaneous telangiectasia, and an increased susceptibility to malignancies. This study was designed to study the role of proapoptotic *BAK*, *BAX*, and *NBK/BIK* genes in a group of patients with AT to elucidate the possible role of these genes in progression of malignancies in this disease.
Materials and Methods Fifty Iranian patients with AT were investigated in this study. The entire coding regions of the *BAK* gene (exons 2–6), *NBK/BIK* gene (exons 2–5), and *BAX* gene (exons 1–7) were amplified using polymerase chain reaction (PCR). The PCR products were separated by 2% agarose gel electrophoresis, and all positive samples were verified by direct sequencing of PCR products using the same primers used for PCR amplification, BigDye chemistry, and Avent 3100 Genetic Analyzer following the manufacturer's instructions (Applied Biosystems).
Results Eight of fifty Iranian AT patients (16%) exhibited a C>T transition in exon 2 (c342C>T) of the *BAK* gene, while none of the healthy controls had such alteration ($P=0.0001$). Higher frequency of another nucleotide substitution in the noncoding region of exon 7 in *BAX* gene (6855G >A) was also identified in 68% of the patient group versus 24% in the controls ($P<0.0001$). Sequence alteration in intronic region of the *NBK/BIK* gene IVS4-12delTC was observed in 52% of AT patients, which was significantly higher than 20% in the control group ($P=0.0023$). Another variant IVS1146C>T in the intronic region of the *BAX* gene was found in 78% of patients, which was significantly

A. Isaian · M. Houshmand · S. M. S. Hassani · R. Mirfakhrai · M. Ataei · M. H. Sanati (✉)
National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
e-mail: m-sanati@nigeb.ac.ir

A. Isaian
e-mail: isaiian@sina.tums.ac.ir

A. Isaian · M. Movahadi · A. Agamohammadi · N. Rezaei · L. Atarod · T. Cheraghi · N. Kalantari
Department of Pediatrics, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

N. Rezaei
e-mail: nima_rezaei@farabi.tums.ac.ir

A. Isaian · N. V. Bogdanova · T. Dork-Bousset
Gynaecology Research Unit, Medical School of Hannover, Hannover, Germany

T. Dork-Bousset
e-mail: Doerk.Thilo@mh-hannover.de

A. Agamohammadi · N. Rezaei
Growth and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran

M. Sadeghi-Shabestari
Division of Pediatric Immunology and Allergy, Children's Hospital, Tabriz University (Medical Sciences), Tabriz, Iran

S. H. Tonekaboni · Z. Chavoshzadeh
Mofid Children Hospital, Shaheed Beheshti University, Tehran, Iran

higher than 10% in the controls ($P<0.0001$). Frequency of alteration in intronic region of exon 3 of the *BAX* gene (IVS3+14A>G) was also significantly higher in the AT patients ($P<0.0001$).
Discussion Several alterations in the proapoptotic genes *BAK*, *NBK*/*BIK*, and *BAX* were found in our study, which could elucidate involvement of the mitochondrial pathway mediated apoptosis in accelerating and developing of cancers and in immunopathogenesis of AT. Such altered apoptosis in AT could play some roles in developing cancers in this group of patients.

Keywords Ataxia telangiectasia · *BAK* · *BAX* · *NBK/BIK* · cancer · polymorphisms

Introduction

Ataxia telangiectasia (AT, OMIM*208900) is an autosomal recessive multisystem disorder that affects~1:40,000–1:300,000 children in various ethnic groups and characterized by variable immunodeficiency, progressive neurodegeneration, occulocutaneous telangiectasia, and an increased susceptibility to malignancies [1–3]. Progressive cerebellar ataxia of early childhood with degeneration of Purkinje cells is the main neurological manifestations of patients with AT. Variable immunodeficiency in patients with AT can predispose them to a variety of infections. Chromosomal breakage and radiation hypersensitivity are main features of AT, which could lead to an increased susceptibility to cancers [4]. It is estimated that about one third of the patients with AT develop malignancies; T cell leukemia and B cell lymphoma are the most common cancers in this group of patients [5].

The ataxia telangiectasia gene mutated (*ATM*, OMIM* 607585), which is the responsible gene for AT, is localized on the chromosome 11q22.3. The ATM protein is a serine/threonine protein kinase, which responds to DNA damage by phosphorylating key substrates involved in oxidative stress, cell cycle, regulation, DNA repair, and apoptosis [2].

Apoptosis is a key process for maintenance of tissue homeostasis and in the pathogenesis of cancers. Evading apoptosis is an essential feature in the malignant transformation of normal B cells to lymphoma cells [6]. BCL2 homologous antagonist killer 1 (*BAK1*, OMIM* 600516) gene spans 7.6 kb, contains six exons, which is located on the chromosome 6p21.3-p21.2. The first exon is noncoding, and most of the largest final exon is untranslated. The *BAK* gene could be directly involved in activating the cell death machinery, which interacts with *BAX* to initiate membrane process leading to mitochondrial dysfunction and release of cytochome C [7, 8]. Mutations in the coding region of the *BAK* gene were described in a number of cancers, including gastric, colorectal, and uterine cervical cancers [9–11].

The proapoptotic *BAX* gene (OMIM*600040) is located on 19q13.3-q13.4. The *BAX* gene contains seven exons and encodes the protein which forms a heterodimer with Bcl2 and functions as an apoptotic activator [12]. Mutations in *BAX* have been described in acute lymphoblastic leukemia, gastric carcinoma, and colorectal cancers [13].

Genomic sequence analysis determined that the *NBK*/*BIK* gene (OMIM*603392) contains five exons and spans approximately 19 kb. The *NBK*/*BIK* gene is located on chromosome 22q13.3 [14]. Sequence analysis revealed that *NBK/BIK* lacked the BH(BCl-2 homology)1 and BH2 domains, but did share a 9-amino acid domain (designated BH3) with *BAX* and *BAK*, which suggests a critical determinant for the death-promoting activity of these proteins. The Bcl-2 family is classified into the following three subfamilies depending on the homology and functions of each protein: (i) a subfamily including Bcl-2, Bcl-xl, and Bcl-w; all of them exert anticell death activity and share sequence homology particularly within four regions, BH1 through BH4; (ii) a subfamily represented by *Bax* and *Bak*, which share sequence homology at BH1, BH2, and BH3, but not at BH4; all these proteins exert proapoptotic activity; and (iii) a subfamily including Bik and Bid; all of them are proapoptotic and share sequence homology only within BH3. BCL2-related proteins either promote cell survival or accelerate cell death. Mutation of the *NBK*/*BIK* gene is a frequent feature of B cell lymphomas [15].

Although apoptosis of neuronal cells is altered in AT [16], the data on this condition are limited. We studied the role of proapoptotic *BAK*, *BAX*, and *NBK*/*BIK* genes in a group of patients with AT for the first time to elucidate the possible role of these genes in progression of malignancies in this disease.

Materials and Methods

Subjects

Fifty patients with AT, who were previously diagnosed in Children's Medical Center in Tehran, enrolled in this study [17]. The suspicious diagnosis of AT was made according to standard criteria on the basis of typical clinical features of progressive ataxia and telangiectasia and laboratory evidence of elevated α-fetoprotein levels (>40 ng/dl) and altered serum immunoglobulin profiles. Only patients who had confirmed diagnosis of AT-based on cytogenetic analysis of chromosome breakage rates, radio-resistant DNA synthesis, or ATM mutation(s) in molecular studies were enrolled in this study. One hundred age- and sex-

matched healthy individuals were also selected as controls. This study was approved by the Ethical Committee of the hospital. Written informed consent was obtained from all subjects before sampling.

Mutation Analysis

Genomic DNA was isolated from peripheral white blood cells by routine phenol/chloroform extraction. The entire coding regions of the *BAK*, BAX and NBK/BIK genes were amplified by polymerase chain reaction (PCR). In order to detect alterations of the *BAK* gene in the subjects, PCR was performed using four primer pairs for exons 2–6 (Table I), under standard conditions in a 20 µl reaction mixture, containing 1 µl of template DNA, 1.2 µl MgCl2 (5 mM), dNTP (2 mM), and Taq polymerase(5 U/µl) according to manufacturer's protocol (Qiagen). Sequencing analysis was also performed for the entire coding region of *NBK*/*BIK* gene (exons 2–5) and *BAX* gene (exons 1–7; Table II).

For this purpose, genomic DNA was amplified with forward and reverse primers and incubated for 35 cycles with denaturation of 95°C annealing temperature between 60°C–63°C, extension of 72°C.

The PCR products were separated by 2% agarose gel electrophoresis. Positive and negative controls were included in each assay. All positive samples were verified by direct sequencing of PCR products using the same primers used for PCR amplification, BigDye chemistry, and Avent 3100 Genetic Analyzer (Applied Biosystems).

Statistical Analysis

The frequencies of each allele polymorphisms in exonic and intronic regions of the *BAK*, *BAX*, and *NBK*/*BIK* genes

Table I Primers Used for Amplification of *BAK*, *BAX*, and *NBK/BIK* Coding Regions

Primers	Sequence	Product size (bp)
BAK		
BAK/2F	5′–AAAGTCCCAGAGGACTAAGG–3′	350 bp
BAK/2R	5′–CACAGTGGGTGAACCGAGG–3′	
BAK/3F	5′–CTTTCCATGGGTGAGGACAG–3′	323 bp
BAK/3R	5′–AGCTCCCAGGACCTGCACAG–3′	
BAK/4F	5′–CTCATGTCTTAGAACACTGTC–3′	414 bp
BAK/4R	5′–AGCAGACATTGGACACTGAC–3′	
BAK/5F	5′–CTGCGGCCTTAATTCACAGC–3′	607 bp
BAK/6R	5–′CAGACAAGGCAAAGACTTCG–3′	
BAX		
BAX/1F	5′–CATTAGAGCTGCGATTGG–3′	406 bp
BAX/1R	5′–CTCAGTGCTTGGAGATCG–3′	
BAX/2,3F	5′–CCGTCACTTTATCTGCTAGG–3′	529 bp
BAX/2,3R	5′–GGCCCAGACTCCTAGTTCTTAG–3′	
BAX/4F	5′–TTT CATTTC AGCCTGGCTTG–3′	444 bp
BAX/4R	5′–CTGGCACATAGCAGGTCCAGT–3′	
BAX/5F	5′–CACTGTGCCTTCGGGTCTTC–3′	401 bp
BAX/5R	5′–TTGGGAGTCTGAGGCAGGAG–3′	
BAX/6F	5′–GGCAAAGAATTGACAAAGG–3′	407 bp
BAX/6R	5′–GTCAGCAGGGTAGATGAATC–3′	
BAX/7F	5′–AATGCCCGTTCATCTCAG–3′	
BAX/7R	5′–CCTCAAGACCACTCTTCC–3′	371 bp
NBK/BIK		
NBK/BIK/2F	5′–GCCAGCATGTAGCAGGATTC–3′	425 bp
NBK/BIK/2R	5′–CCCGCTCATAGAATGTCTGC–3′	
NBK/BIK/3F	5′–CTCTTATCCTCTGGGCCACTC–3′	246 bp
NBK/BIK/3R	5′–CCAGGTGTAGAGGCATAGGG–3′	
NBK/BIK/4F	5′–GCTGTGATGGTGTCATCTAAG–3′	325 bp
NBK/BIK/4R	5′–TGACACAGATGATGATACGG–3′	
NBK/BIK/5F	5′–CTTGGCACTCCGCTGTCAC–3′	411 bp
NBK/BIK/5R	5′–GTTAACCGGTGACAATTGCAG–3′	

Table II Summary of *BAK*, *BAX*, and *NBK/BIK* Sequence Alterations in AT Patients and Controls

Gene	Location	Alteration	Frequency	
			AT cases (%)	Controls (%)
BAK	exon 2, c.342	C>T	16%	0%
BAX	exon1 (IVS146)	C>T	78%	10%
BAX	exon 3 (IVS3+14)	A>G	73%	25%
BAX	exon 7 (6855)	G>A	68%	24%
NBK/BIK	exon 4 (IVS4-12delTC)	Del TC	52%	20%

were assessed in the patient group and compared with the control group. Chi-square test was performed. Odds ratio and *P* value were calculated from two by two tables, and statistical significance was assessed with Fisher's exact test. *P* value of less than 0.05 was considered statistically significant (Epi Info 6 program, version 6.2, World Health Organization, Geneva, Switzerland).

Results

Characteristics of Patients

Fifty patients with AT (25 males and 25 females) with mean age of 12.2±3.9 years were investigated in this study. The parents of 46 patients were consanguineous. There was a history of the same disease in the family of the 12 enrolled patients. There was a family history of malignancy in ten cases, including lymphoma, gastrointestinal cancer, lung cancer, and breast cancer.

Exonic Alterations

Fragments showed abnormal mobility in the exon 2 of the *BAK* gene, which are indicative of sequence alteration. Eight of fifty Iranian AT patients (16%) exhibited C>T transition in exon 2 (c342C>T; Table II), with no alteration in amino acid sequence (Fig. 1). All of these alterations were in the heterozygous state in AT patients. One hundred healthy controls were also sequenced to exclude polymorphism; none of them had such alteration (*P* value=0.0001). Characteristics of the AT patients with c342C>T in the *BAK* gene are presented in the Table III. Only two patients, among 50 AT patients, developed lymphoma; both were located in this group with such transition in the *BAK* gene. Indeed, half of these patients had history of malignancies in their family members.

Higher frequency of another nucleotide substitution in the noncoding region of *BAX* exon 7 (6855G>A) was also identified in 68% of the patient group (60% homozygotes and 8% heterozygotes) versus 24% in the controls, *P* value<0.0001, OR 6.73; 95%CI 2.99–15.36). There was not any exonic alteration in the *NBK/BIK* gene.

Intronic Alterations

Sequence alteration in intronic region of *NBK/BIK* gene IVS4-12delTC (Table II) was observed in 23 of 44 cases (52.3%) of AT patients (13 heterozygotes and ten homozygotes), which was significantly higher than 20% in the control group (*P* value=0.0023; OR 4.38; 95%CI 1.61–12.13).

Abnormal mobility was also detected in the intronic regions of *BAX* gene (Table II). Frequency of (IVS1146C>T) alteration was 78% (66% homozygotes and 12% heterozygotes) in the patients, which was significantly higher than 10% in the controls (*P* value<0.0001; OR 31.91; 95%CI 11.49–92.17). Frequency of alteration in intronic region of exon 3 of the *BAX* gene (IVS3+14A>G) was also

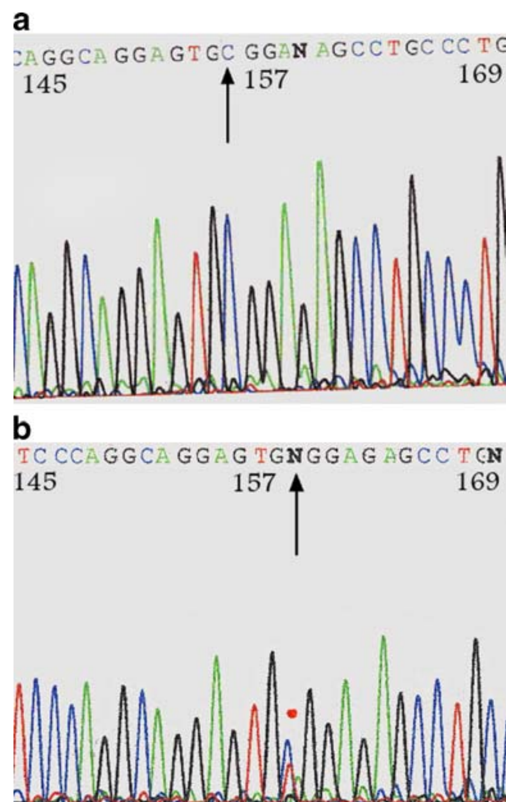

Fig. 1 Direct sequencing of exon 2 of *BAK* gene from PCR product. **a** Wild type. **b** Heterozygote

Table III Characteristics of AT Patients with *BAK* Exon 2 Alteration

Code	Age	Sex	Consanguinity	Status	Malignancy	Cancer in the family	AT in family
IR2	18 y	M	+	Alive	−	Lymphoma	No
IR11	15 y	F	+	Alive	−	−	No
IR14	5 y	F	+	Alive	−	Lung cancer	Brother
IR16	20 y	M	+	Died	Lymphoma	−	No
IR22	10 y	M	+	Alive	−	−	Brother
IR30	12 y	F	+	Alive	Lymphoma	Colon and gastric cancers (2 affected)	Brother
IR39	11 y	M	+	Alive	−	−	No
IR50	16 y	F	+	Died	−	Intestinal cancer	No

significantly higher in the AT patients (72.9% in the patients versus 25% in the controls; *P*-value <0.0001; OR 8.08; 95% CI 3.47–19.12).

Discussion

Bcl-2 family proteins are major regulators of mitochondria-dependent apoptosis, and *BAK* is one of proapoptotic member of the Bcl-2 family, which shows high homology to *BAX* in size sequence and biological activity [18].

Dysregulation of apoptosis occurs commonly in cancers. Mutations in the *BAK* gene may prevent apoptosis [9, 10]. The *BAK* gene mutations in exon 2 were observed in gastric cancers [19]. Another study in Korea indicated such mutation in about 3% of advanced gastric adenocarcinoma. All of these mutations were exclusively detected in exon 2 [10].

Our study revealed several sequence alterations of *BAK*, *NBK*/*BIK*, and *BAX* genes in AT. We identified c342C>T silent mutation in exon 2 of *BAK* gene with frequency of 16% (8/50) in AT patients; all of these patients were heterozygotes (C/T genotype). Meanwhile, there was not any case with such alteration in Iranian healthy controls. This genetic variant is also rare in other population reports (rs2227925 in the NCBI SNP database) [19]. Thus, it seems that Iranian AT patients have a significantly higher frequency of c.342C>T silent mutation in exon 2 of *BAK* gene than the normal population. Two of eight AT patients had lymphoma, while half of their first family also experienced malignancies.

Cancer is the second cause of death in the patients with AT. The risk of malignancy in individuals is 38%. Leukemia and lymphoma account for about 85% of malignancies [3]. It seems that in addition to the *ATM* gene, mutations in the proapoptotic genes such as *BAK*, *NBK*/*BIK*, and *BAX* could be relevant to progression of cancers in this group of patients, which can shorten the overall survival [20–22].

We have demonstrated an exonic alteration (6855 G>A) in exon 7 in noncoding region of *BAX* gene. The frequency of such alteration in the AT patients was 68%, which was significantly higher than control group (24%) and also other population (3.9%), based on NCBI SNP database (rs704243) [19].

Intronic alteration in the *NBK*/*BIK* gene (IVS4-12delTC) was also more common in the AT patients. Previous study indicated that mutation of *NBK*/*BIK* gene frequent feature of B cell lymphoma [15], while recent studies described only intronic mutations without functional significance in colorectal tumors [23]. Moreover, expression of *NBK*/*BIK* gene seems to be highly predictive of stage 1 lung cancer patients [24].

Intronic alteration in the *BAX* gene (IVS146C>T) was another finding in our AT patients, which had higher frequency of 78% in comparison with 10% in the control groups. Comparison of data with NCBI SNP Database (rs4645881) [19], with average C>T genotype frequency of 33.7%, could also indicate that this SNP has higher frequency in AT patients. We also observed intronic alteration in the *BAX* gene (IVS3+14A>G) with frequency of 73% in AT patients, which is significantly higher than control group (25%), as well as other Asian populations (53%; rs1805419) [19].

As pro- and antiapoptotic Bcl-2 proteins are in balance to control the mitochondrial pathway, overexpression of proapoptotic Bcl-2 proteins could be considered as an alternative strategy for the targeting of antiapoptotic factors [25]. Apoptosis deficiency was previously supposed as a critical factor for therapy resistance in some malignancies like metastasized melanoma. *NBK*/*BIK* and *BAX* proapoptotic genes may be employed efficient induction of apoptosis in melanoma cells and improved gene therapeutic strategies [26].

In conclusion, we observed different alterations of proapoptotic genes *BAK*, *NBK*/*BIK*, and *BAX*. A caveat of the present study is that the biological consequences of these noncoding sequence variants are unknown, and it is possible

that they are neutral or represent markers of other, yet, unknown loci. We can also not fully exclude the possibility that the associations observed in this pilot study are due to chance or may be related to some population stratification or sampling bias. For example, our AT patients may have a higher likelihood of being homozygotes by descent than our controls. Nevertheless, the results from our study would be in line with the proposed involvement of the mitochondrial pathway mediated apoptosis in accelerating and developing of cancers and in immunopathogenesis of AT, which would offer basics to establish mitochondrion-targeted therapeutic interventions in AT. It is known that the apoptotic capacity of AT cells is highly variable between individuals and cell lines, and it has been proposed that this may be subject to modulation by additional genetic variants [27]. Such altered apoptosis in AT could be via the mitochondrial pathway, and further research is required to investigate whether mitochondrial apoptosis, in addition to *ATM* mutations, plays some roles in developing cancers, which could also improve our understanding on the pathogenesis of nervous system damage.

Acknowledgment The authors are very grateful for all colleagues in Medical School of Hannover (Hannover, Germany) for their kind help and advices in the laboratory, and all the patients and their families for their kind collaboration in this study. We would like to thank Dr. Ali Rabbani for his help and support.

References

1. Yeganeh M, Gambineri E, Abolmaali K, Tamizifar B, Espanol T. Other well-defined immunodeficiencies. In: Rezaei N, Aghamohammadi A, Notarangelo LD, editors. Primary immunodeficiency diseases: definition, diagnosis and management, vol 1. Berlin: Springer; 2008. p. 251–90.
2. Sandoval N, Platzer M. Characterization of *ATM* gene mutations in 66 ataxia telangiectasia families. Hum Mol Genet. 1999;8:69–79.
3. Gatti RA. Ataxia-Telangiectasia. http://www.ncbi.nih.gov.book. Gene Reviews 2005.
4. Perlman S, Becker-Catania S, Gatti RA. Ataxia-telangectasia: diagnosis and treatment. Semin Pediatr Neurol. 2003;10:173–82.
5. Babaei M, Mitui M, Olson ER, Gatti RA. *ATM* haplotypes and associated mutations in Iranian patients with ataxia-telangiectasia: recurring homozygosity without a founder haplotype. Hum Genet. 2005;117:101–6.
6. Martini M, D'Alò F, Hohaus S, Leone G, Larocca LM. Absence of structural mutations of the *Bak* gene in B cell lymphomas. Haematologica. 2002;87:661–2.
7. Cartron PF, Juin P, Oliver L, Martin S, Meflah K, Vallette FM. Nonredundant role of Bax and Bak in Bid-mediated apoptosis. Mol Cell Biol. 2003;23:4701–12.
8. Wang X. The expanding role of mitochondria in apoptosis. Genes Dev. 2001;15:2922–33.
9. Kondo S, Shinomura Y, Miyazaki Y, Kiyohara T, Tsutsui S, Kitamura S, et al. Mutation of the *Bak* gene in human gastric and colorectal cancers. Cancer Res. 2000;60:4328–30.
10. Kim SP, Hwang MS, Cho YR, Kwon SY, Kang YN, Kim IH, et al. Mutation of *Bak* gene are infrequent in advanced gastric adenocarcinomas in Koreans. Cancer Lett. 2003;195:87–91.
11. Wani KM, Huilgol NG. Genetic alterations in coding region of the *Bak* gene in uterine cervical carcinoma. Br J Cancer. 2003;88:1584–6.
12. Peng H, Aiello A, Packham G, Isaacson PG, Pan L. Infrequent *Bax* gene mutations in B cell lymphomas. J Pathol. 1998;186:378–82.
13. Gil J, Yamamoto H, Zapata JM, Reed JC, Perucho M. Impairment of the pro apoptotic activity of Bax by missense mutations found in gastrointestinal cancers. Cancer Res. 1999;59:2034–7.
14. Suleka V, Budarf ML. Structural analysis of human pro-apoptotic gene *BIK*. Gene. 2000;254:157–62.
15. Arena V, Martini M, Luongo M, Capelli A, Larocca LM. Mutations of *Bik* gene in human peripheral B cell lymphomas. Genes Chrom Cancer. 2003;38:91–6.
16. Takagi M, Delia D. Defective control of apoptosis, radiosensivity, and spindle checkpoint in ataxia telangiectasia. Cancer Res. 1998;58:4923–9.
17. Moin M, Aghamohammadi A, Kouhi A, Tavassoli S, Rezaei N, Ghaffari SR, et al. Ataxia-telangiectasia in Iran: clinical and laboratory features of 104 patients. Pediatr Neurol. 2007;37:21–8.
18. Reed JC. Bcl-2 family proteins and hematologic malignancies: history and future prospects. Blood. 2008;111:3322–30.
19. http://www.ncbi.nlm.nih.gov/SNP/index.html.
20. Cartron PF, Juin P, Oliver L, Meflah K, Vallette FM. Impact of proapoptotic proteins Bax and Bak in tumor progression and rerponse to treatment. Expert Rev Anticancer Ther. 2003;3:563–70.
21. Meijerink JP, Mensink EJ, Wang K, Sedlak TW, Slöetjes AW, de Witte T, et al. Hematopoietic malignancies demonstrate loss-of-function mutations of BAX. Blood. 1998;91:2991–7.
22. Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, et al. Somatic frameshift mutations in the *Bax* gene in colon cancers of the microsatellite mutator phenotype. Science. 1997;275:967–9.
23. Castells A, Ino Y, Louis DN, Ramesh V, Gusella JF, Rustgi AK. Mapping of a target region of allelic loss to a 0.5 cM interval on chromosome 22q13 in human colorectal cancer. Gastroenterology. 1999;117:831–7.
24. García N, Salamanca F, Astudillo-de la Vega H, Curiel-Quesada E, Alvarado I, Peñaloza R, et al. A molecular analysis by gene expression profiling reveals Bik/NBK overexpression in sporadic breast tumor samples of Mexican females. BMC Cancer. 2005;5:93.
25. Eberle J, Hossini AM. Expression and function of bcl-2 proteins in melanoma. Curr Genomics. 2008;9:409–19.
26. Eberle J, Fecker LF, Hossini AM, Kurbanov BM, Fechner H. Apoptosis pathways and oncolytic adenoviral vectors: promising targets and tools to overcome therapy resistance of malignant melanoma. Exp Dermatol. 2008;17:1–11.
27. Fernet M, Angele S, Dörk T, Hall J. Variation in radiation-induced apoptosis in ataxia-telangiectasia lymphoblastoid cell lines. Int J Radiat Biol. 2003;79:193–202.