Association of *Fas*-670 gene polymorphism with risk of cervical cancer in North Indian population

D.M. Kordi Tamandani, R.C. Sobti, M. Shekari

Department of Biotechnology, Panjab University, Chandigrah (India)

Summary

Objectives: Cervical cancer is the second most common cancer among women in the world, with approximately 470,000 new cases and 231,000 deaths occurring each year. Incidence is greater in developing countries such as India, where this is the most common female malignancy with almost 100,000 new cases each year. Apoptosis must be considered as a safe mechanism that controls the integrity of the cell erasing abnormal clones and it is likely that failure of apoptosis constitutes a key factor responsible for tumor formation, progression and resistance to drugs. The *Fas* gene plays a key role in regulation of apoptotic cell death and corruption of this signaling pathway has been shown to participate in immune escape and tumorgenesis. *Study design:* A single-nucleotide polymorphism at -670 of *Fas* gene promoter (A/G) was examined in a total of 400 blood samples from normal healthy women and cervical cancer patients, using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) technique. *Results:* Significant association was observed for AG (OR = 3.0, 95% CI = (1.68-5.09, $p < 0.001$) and combined AG+GG (OR = 2.54, 95% CI = 1.47-4.40, $p < 0.001$) genotype with risk of cervical cancer. Heterozygous genotype (AG) in SCC showed a highly significant association with risk of cervical cancer (OR = 2.57, 95% CI = 1.47-4.50 $p < 0.001$). Similarly, combined AG+GG genotype had a 2.25-fold risk for SCC patients (OR = 2.25, 95% Cl = 1.30-3.90, $p < 0.001$). There was high increase risk of cervical cancer in passive smokers with AG and combined (AG+GG) genotypes (OR = 4.6, 95% CI = 2.07-10.32, $p < 0.001$ - OR = 4.9, 95% CI = 2.20-10.32, $p < 0.001$), respectively. *Conclusion:* This is the first study to provide evidence for the association of a *Fas* -670 (A/G) gene polymorphism with the risk of cervical cancer in a North Indian population.

Key words: Fas gene; Polymorphism; Cervical cancer.

Introduction

Cervical cancer is the second most common cancer in women worldwide, and is a preventable and a curable disease - especially if identified at an early stage. It is widely accepted that specific human papillomavirus (HPV) types are central etiologic agents of cervical carcinogenesis. Other environmental and host factors also play decisive roles in the persistence of HPV infection and further malignant conversion of cervical epithelium [1]. Although many previous reports have focused on HPV and environmental factors, the role of host susceptibility to cervical carcinogenesis is largely unknown. Smoking exposes the body to many carcinogens that affect more parts of the body than the lungs. Smoking contributes to a weakening of the immune system and tobacco by products have been found in cervical mucosa in women who smoke [2]. One recent study has found that women who smoke and have oncogenic HPV with abnormal Pap tests were more likely to be diagnosed later with precancerous or severe cervical dysplasia (CIN III) or cancer compared to nonsmokers [3]. Researchers believe that these substances damage the DNA of cervical cells and may contribute to the development of cervical cancer. Smokers are about twice as likely as nonsmokers to get cervical cancer; however, the exact biologic relationship of smoking to oncogenic HPV is less clear [4]. Exposure to passive cigarette smoking is potentially modifiable, and hence this may have implications for strategies to prevent cervical cancer. The results of several case-control and cross-sectional studies have indicated that women married to smokers experience a higher risk of cervical neoplasia than those married to nonsmokers [5]. Apoptosis is a physiological process that regulates normal homeostasis, and alterations of apoptosis-related genes are likely to contribute to the pathogenesis of autoimmune disease [6] and malignant tumors [7]. Among various cell surface death receptors, Fas (CD95/APO-1) has a key role in known apoptosis pathways and is a member of the tumour necrosis factor receptor superfamily. The binding of Fas-L to the cell surface death receptor Fas activates intracellular cascades that ultimately cause apoptotic death of the cell [8, 9]. Downregulation of Fas with resultant resistance to death signals has been reported in many cancers [10]. The transcriptional expression of *Fas* gene is regulated by a number of genetic elements located in the 5' upstream region of the gene. The promoter region of the *Fas* gene consists of basal promoter, enhancer, and silencer regions [11]. Single nucleotide polymorphism at –670 in the enhancer region (A/G) situated at a binding element of gamma interferon activation signal (GAS), G allele results in an abolishment of the GAS element and a significant decrease in *Fas* gene expression in response to interferon (IFN-gamma) stimuli. The A allele has been

Revised manuscript accepted for publication January 31, 2008

associated with autoimmune diseases [12, 13] and cervical carcinogenesis [14]. The Fas –670 A allele has also shown a higher binding affinity for the signal transducer and activator of transcription (STAT) 1 protein [15]. This would then lead to a reduction of CTL response which is beneficial for HPV in establishing persistent infections.

The aim of the study was to further investigate the Fas receptor SNP as a susceptibility factor for cervical carcinoma in a North Indian population.

Materials and Methods

Study subjects

This case-control study involved collection of peripheral blood samples (2-5 ml) from 400 North Indian subjects. The 200 cases were newly diagnosed, previously untreated and histologically confirmed as cervical cancer patients. The samples were collected from the Post graduate Institute of Medical Education and Research (PGIMER), Chandigarh and the Government Medical College (GMC), Chandigarh. The control peripheral blood samples (n = 200) were collected from the same institute with no history of cancer or precancer.

Informed consent was obtained from all the cases and controls. Detailed data regarding age, menarche, and menopausal status, number of children, age at marriage and birth of first child, cigarette smoking history and spouse's smoking history were also obtained.

Methods

Genomic DNA was extracted from EDTA anti-coagulated peripheral blood samples according to a standard proteinase K digestion and phenol chloroform extraction method [16]. The *Fas-G670A* polymorphism was typed as described previously by Huang *et al.* [17] with the following minor modifications in polymerase chain reaction: 5 min at 95˚C, 30 cycles of 30 sec at 95˚C, 30 sec at 62˚C, and 1 min at 72˚C, followed by a final extension for 7 min at 72˚C. Primer sequences were 5'-CTA CCT AAG AGC TAT CTA CCG TTC-3' and 5'-GGC TGT CCA TGT TGT GGC TGC-3'. The 332 bp PCR product was digested with MvaI for 5 hrs at 37˚C . Allele G yielded three fragments of 99 bp, 189 bp, and 44 bp, whereas allele A yielded two fragments of 99 bp and 233 bp. Digested fragments were separated on 3% agarose gels and visualised after ethidium bromide staining.

Age, age at marriage and at birth of first child, age at menarche and menopouse, smoking status (passive smokers, active smokers, active+passive smokers) and genotypes of the *Fas-G670A* gene were tabulated for cases and controls. Cases were further categorised into histological subtypes to check for significant correlations.

Under the hypothesis that *Fas-G670A* genotype might be associated with risk of cervical cancer, we tested a combination of *Fas* genotype with squamous cell carcinoma (SCC) and adenocarcinoma (AC), and the interactions of this gene with smoking habits were checked .

Statistical analysis

The association between a polymorphism in the *Fas-G670A* gene with the risk of cervical cancer was estimated by computing the odds ratio (OR) and 95% confidence intervals (95% CI), using a multivariate logistic regression analysis that included several potential confounding variables. Statistical analysis was performed using SPSS version 10.0 (SPSS, Chicago, IL) and Epical version 3.2.

Results

The genotypes of the *Fas* gene at the -670 in cervical cases and healthy controls derived from a North Indian population was analysed.

Demographic variables for such cases and controls have been summarised in Table 1. The variables have also been categorised for squamous cell carcinoma (SCC) and adenocarcinoma (AC) cervical cancer; 175 were identified as SCC and 25 as AC.

The average age in years was calculated to be 48.55 ± 9.43 and 48.81 ± 9.64 for cases and controls, respectively. Compared with the controls, the study group was younger at the time of the marriage (16.36 ± 3.03) and birth of the first child (18.39 ± 3.39) and had a greater mean number of children (4.11). Ages at menarche and menopause were found to be comparable between cases and controls.

There were no significant differences in the *Fas-670* genotype distribution and allelic frequencies between the healthy Indian controls and the other four healthy ethnic control groups (Table 2).

As shown in Table 3, the incidence of *Fas-670* wild genotype (AA) in cases were lower (13.0%) than controls (27.5%). The distribution of heterozygous genotype (AG) was higher among cases (83.5%) than controls (60.5%). The association between the *Fas-670* gene and cervix cancer has been summarised in Table 3. A significant association was observed for AG genotype (OR = 3, 95% CI = 1.68-5.09, p < 0.001) and risk of cervical cancer. Also a statistically significant risk (OR = 2.54, 95% CI =

Table 1. — *Demographic characteristics of cervix cancer cases and controls.*

Variable	Cases (200)	Controls (200)	SCC (175)	AC (25)
Age ±	48.55 ±9.43	48.81± 9.64	48.39 ± 9.42	49.68 ± 9.64
Age at menarche ±	14.87 ± 1.14	14.02 ± 1.09	14.90 ± 1.16	14.68 ± 1.00
Age at marriage ±	16.36 ± 3.03	20.31 ± 3.46	16.68 ± 2.97	14.74 ± 2.56
Age at first birth ± child	18.39 ± 3.39	22.31 ± 4.30	18.61 ± 3.16	16.84 ± 4.45
Children no	4.11	2.50	4.09 ± 1.51	4.21 ± 1.84
Age at menopause ±	48.31±3.56	48.26 ± 2.39	48.29 ± 3.62	48.44 ± 3.40
smoking status				
– non smoker	110 (55.0)	138 (69.0)	99 (56.6)	11 (44.0)
– active smoker	0	0	0	0
– active + passive smoker	2 (1.0)	6 (3.0)	2 (1.1)	0
– passive smoker	88 (44.0)	56 (28.0)	74 (42.3)	14 (56.0)
OR	2		1.0	1.3
P	0.002			

AC, adenocarcinoma; CI, confidence interval; OR, odds ratio; SCC, squamous cell carcinoma. Significance set at < 0.05.

Table 2. — *Fas-670 genotype and allele frequencies in several healthy ethnic controls.*

	AA	AG	GG	A	G
Chinese (n = 124)	41 (33)	64 (52)	19 (15)	59	41
Dutch (n = 206)	46 (23)	118 (57)	42 (20)	51	49
Australian (n = 183)	46 (25)	97 (53)	40 (22)	52	48
Korean (n = 84	25 (30)	46 (55)	13 (15)	57	43
Indian (n = 200)	55 (27.5)	121 (60.5)	24 (12.0)	58	42

Table 3. — *Fas-670 genotypes in cervical cancer and healthy controls.*

FAS genotypes	Case (%) 200	Control (%) 200	OR (95% CI)	p
AA	26 (13.0)	55 (27.5)	1.0 (ref)	–
AG	167 (83.5)	121 (60.5)	3.0 (1.68-5.09)	p < 0.001
GG	7 (3.5)	24 (12.0)	0.62 (0.21-1.76)	
AG+GG	174 (87)	145 (72.5)	2.54 (1.47-4.40)	p < 0.001

Abbreviation: OR, odds ratio. Significance set at p ≤ 0.05. 1.0 (Reference). OR adjusted with age and smoking.

Table 4. — *Determinants of interaction between Fas-670 genotypes and type of cervical cancer.*

FAS-670	Type of cancer	n[c]	OR (95% CI)	p
AA	intact	26/55	1.0 (ref)	–
AG	SCC	147/121	2.57 (1.47-4.50)	p < 0.001
	AC	20/121	0.35 (0.17-0.71)	p = 0.003
GG	SCC	7/24	0.62 (0.21-1.76)	–
AG+GG	SCC	154/145	2.25 (1.30-3.90)	p < 0.001
	AC	20/145	0.29 (0.14-0.56)	

OR, odds ratio. Significance set at p ≤ 0.05. 1.0 (Reference). OR adjusted with age and smoking.

Table 5. — *Assessments of interaction between Fas-670 genotypes and smoking in cervical cancer cases and controls.*

FAS genotypes	Statue of smoking	Case %	Control %	OR (95% CI)	p
AA	Never smoked	14 (12.7)	34 (24.8)	1.0 (ref)	–
AA	Passive smoking	12 (46.2)	18 (32.7)	1.62 (0.56-4.72)	
AG	Passive	70 (79.5)	37 (66.1)	4.6 (2.07-10.32)	p < 0.001
	active + passive	2 (100.0)	3 (50.0)	1.4 (0.43-4.38)	
GG	Passive	6 (6.8)	1 (1.8)	3.0 (1.72-5.02)	p = 0.007
AG+GG	Passive	76 (86.4)	38 (67.85)	4.9 (2.20-10.32)	p < 0.001

OR, odds ratio. Significance set at p ≤ 0.05. 1.0 (Reference). OR adjusted with age.

1.47-4.40, p < 0.001) was observed for combined AG+GG genotype.

As shown in Table 4 interaction between *Fas-670* genotypes and type of cancer, heterozygous genotype (AG) in SCC showed a highly significant association with risk of cervical cancer (OR = 2.57, 95% CI = 1.47-4.50, p < 0.001). Similarly combined AG+GG genotypes had a 2.25-fold risk for SCC patients (OR = 2.25, 95% Cl = 1.30-3.90, p < 0.001).

The effects of smoking on the relationship between *Fas-670* genotypes and cervical cancer are shown in Table 5. When OR was assessed with the stratification of smoking status, a statistically significant association was found for heterozygous (AG) and combined (AG+GG) genotypes of *Fas* with an increased risk of cervical cancer in passive smokers (OR = 4.6, 95% CI = 2.07-10.32, p < 0.001 - OR = 4.9, 95% CI = 2.20-10.32, p < 0.001), respectively. There was significance increase in OR (OR = 3.0, 95% CI = 1.72-5.02, p = 0.007) in passive smokers with mutant (GG) genotype.

Discussion

In the present study, we genotyped *Fas-670* polymorphism in the North Indian population with cervical cancer and healthy controls, and found that the polymorphism is associated with cervical cancer. This study suggests that *Fas-670* polymorphism might play a role in susceptibility of cervical cancer in the North Indian population. There is an expanding body of literature suggesting that host factors, including genetic polymorphisms, may explain some of the individual differences in cancer occurrence. Polymorphisms in the promoter region or 5' flanking region of genes can lead to different levels of gene expression and have also been implicated in a number of diseases. SNP at -670 of the *Fas* gene promoter (A/G) has been found with potentially different transcriptional efficiency [18]. Since this polymorphism of the *Fas* gene is located in the promoter region, it may affect the level of transcription of the Fas protein. Bauvois *et al.* [19] suggested that the substitution of G to A in the position –670 (TTCCAG G/AAA) would change the gamma interferon activation site (GAS) (TTC-nnnGAA). This site was involved in interferon gamma and interferon alpha signaling [20]. GAS elements are known to bind to homodimers of a phosphorylated form of the 91-kDa transcription factor, STAT1. Interferon gamma could cause tyrosine phosphorylation of STAT1 by the interferon gamma receptor-associated Janus kinases 1 and 2. Subsequently, phosphorylated STAT1 formed homodimers and translocated into the nucleus where it induced transcription of GAS containing genes [21]. Fas has been significantly upregulated by interferon gamma according to several reports [22]. Several studies have addressed the association of this SNP with autoimmune disease and Fas promoter -670 polymorphism analysis on cervical cancer showed that the frequency of A allele and AA genotype increased in accordance with the multiple step carcinogenesis [23]. Ueda *et al.* [24] suggested that *Fas* gene promoter -670 polymorphism (A/G) may be closely associated with cervical carcinogenesis in a Japanese population. Also, they reported that there was an increased OR for AG+GG genotype in HSIL cases compared to controls among the patients with high-risk HPV. The same trend was observed in our study in that AG+GG genotype increased the risk of developing cervical cancer (OR 2.54, 95% Cl = 1.47-4.40, p < 0.001) in North Indian women. Engelmark *et al.* [25] and Dybikowska *et al.* [26] have demonstrated that AA genotype in the *Fas* gene promoter at -670 position may not be engaged in the development of cervical neoplasia in Swedish and Polish populations. These discrepancies may be due to the ethnic variation of HPV prevalence and genotype frequency of the *Fas* gene promoter in different geographical regions. Zhang *et al.* [27] demonstrated that gene-environment interaction of the *FAS* polymorphism and smoking was associated with increased risk of lung cancer. Similarly, results raised in this study in passive smoking cancer patients with AG and GG genotypes of Fas –670, (OR - 4.6, CI, 95% = 2.07-10.32. p < 0.001, OR = 3.0, CI, 95% = 1.72-5.02, p = 0.007), respectively. The -670 *Fas* polymorphism has been reported to be associated with Alzheimer's disease and to interact with the apolipoprotein – E variant [28], indicating that it has potential biological significance. However Fas polymor-

phism does not appear to have an impact on non-melanoma skin cancer [29]. Also Xia *et al.* [30] could not find any significant association between *Fas*-670 polymorphism and inflammatory bowel disease in Chinese patients. It is imperative to test if the polymorphism could be used as a disease marker for the natural history of cervical lesions in the setting of a longitudinal cohort study. Functional analysis of *Fas*-670 polymorphism in infiltrating lymphocytes and stromal cells from patients with precancerous lesions will be important in order to understand molecular mechanisms precipitating to cervical carcinogenesis. The limitation of the present study is that it was hospital-based and took place in that environment, therefore it can not be free from any selection bias. In conclusion, to the best of our knowledge, this is the first study to date that provides evidence for an association between *Fas* gene polymorphism and risk of cervical caner in a North Indian population.

Acknowledgments

The authors are grateful to all staff and the patients who took part in this study. We are also thankful to PGIMER and GMCH Chandigrah for providing the clinical samples.

References

[1] Zur Hausen H.: "Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis". *J. Natl. Cancer Inst.,* 2000, 3, 92, 690.

[2] Palefsky J., Handley J.: "What your doctor may not tell you about HPV and abnormal Pap smears". New York, NY, Warner Books Inc., 2002.

[3] McIntyre-Seltman K., Castle P.E., Guido R., Schiffman M., Wheeler C.M., ALTS Group: "Smoking is a risk factor for cervical intraenpithelial neoplasia grade 3 among oncogneic human papillomavirus DNA-positive women with equivocal or mildly abnormal cytology". *Cancer Epidemiol., Biomark. Prevent.,* 2005, 1165.

[4] Shepherd J., Peersman G., Weston R., Napuli I.: "Cervical cancer and sexual lifestyle: a systematic review of health education interventions targeted at women". *Health Edu. Res.,* 2000, 681.

[5] Buckley J.D., Harris R.W., Doll R., Vessey M.P., Williams P.T.: "Case-control study of the husbands of women with dysplasia or carcinoma of the cervix uteri". *Lancet,* 1981, *2,* 1010.

[6] Lorenz H.M., Herrmann M., Winkler T., Gaipl U., Kalden J.R.: "Role of apoptosis in autoimmunity". *Apoptosis,* 2000, *5,* 443.

[7] Zornig M., Hueber A., Baum W., Evan G.: "Apoptosis regulators and their role in tumorigenesis". *Biochim. Biophys. Acta,* 2001, 1551, F1.

[8] Nagata S.: "Fas and Fas ligand: a death factor and its receptor". *Adv. Immunol*., 1994, *57,* 129.

[9] Nagata S., Golstein P.: "The Fas death factor". *Science,* 1995, *267* 1449.

[10] Shimonishi T., Isse K., Shibata F., Aburatani I., Tsuneyama K., Sabit H. *et al.*: "Up-regulation of fas ligand at early stages and down-regulation of Fas at progressed stages of intrahepatic cholangiocarcinoma reflect evasion from immune surveillance". *Hepatology,* 2000, *32*, 761.

[11] Rudert F., Visser E., Forbes L., Lindridge E., Wang Y., Watson J.: "Identification of a silencer, enhancer, and basal promoter region in the human CD95 (Fas/APO-1) gene". *DNA Cell. Biol.,* 1995, *14*, 931.

[12] Lee Y.H., Kim Y.R., Ji J.D., Sohn J., Song G.G.: "Fas promoter -670 polymorphism is associated with development of anti-RNP antibodies in systemic lupus erythematosus". *J. Rheumatol.,* 2001, *28,* 2008.

[13] Kanemitsu S., Ihara K., Saifddin A., Otsuka T., Takeuchi T., Nagayama J. *et al*.: "A functional polymorphism in fas (CD95/APO-1) gene promoter associated with systemic lupus erythematosus". *J. Rheumatol.,* 2002, *29,* 1183.

[14] Lai H.C., Sytwu H.K., Sun C.A., Yu M.H., Yu C.P., Liu H.S. *et al.*: "Single nucleotide polymorphism at Fas promoter is associated with cervical carcinogenesis". *Int. J. Cancer,* 2003, *103*, 221.

[15] Engelmark M.T., Renkema K.Y., Gyllensten U.B.: "No evidence of the involvement of the *Fas*-670 promoter polymorphism in cervical cancer in situ". *Int. J. Cancer,* 2004, *112,* 1084.

[16] Roe B.A., Crabtree J.S., Khan A.S.: "Methods for DNA isolation. Part III". In: Protocols for recombinant DNA isolation, cloning, and sequencing [Internet edition]. Norman, OK: University of Oklahoma; 1995. [Available from: http://www.genome.ou.edu/protocol_book/protocol_index.html; also available in paper copy as: DNA Isolation and Sequencing; New York: Wiley; 1996].

[17] Huang Q.R., Morris D., Manolios N.: "Identification and characterization of polymorphisms in the promoter region of the human Apo-1/Fas (CD95) gene". *Mol. Immunol.*, 1997, *34,* 577.

[18] Shirasawa H., Tomita Y., Fuse A., Yamamoto T., Tanzawa H., Sekiya S. *et al.*: "Structure and expression of an integrated human papillomavirus type 16 genome amplified in a cervical carcinoma cell line". *J. Gen. Virol.,* 1989, *70,* 1913.

[19] Bauvois B., Djavaheri-Mergny M., Rouillard D., Dumont J., Wietzerbin J.: "Regulation of CD26/DPPIV gene expression by interferons and retinoic acid in tumor B cells". *Oncogene,* 2000, *19,* 265.

[20] Shuai K.: "Interferon-activated signal transduction to the nucleus". *Curr. Opin. Cell. Biol*., 1994, *6,* 253.

[21] Gao J., Morrison D.C., Parmely T.J., Russell S.W., Murphy W.J.: "An interferon-gamma-activated site (GAS) is necessary for full expression of the mouse iNOS gene in response to interferon-gamma and lipopolysaccharide". *J. Biol. Chem.,* 1997, *272,* 1226.

[22] Schwartzberg L.S., Petak I., Stewart C., Turner P.K., Ashley J., Tillman D.M. *et al.*: "Modulation of the Fas signaling pathway by IFN-gamma in therapy of colon cancer: phase I trial and correlative studies of IFN-gamma, 5-fluorouracil, and leucovorin". *Clin. Cancer Res.,* 2002, *8,* 2488.

[23] Ueda M., Terai Y., Kanda K., Kanemura M., Takehara M., Yamaguchi H. *et al.*: "Fas gene promoter –670 polymorphism in gynecological cancer". *Int. J. Gynecol. Cancer,* 2006, *16* (suppl. 1), 179.

[24] Ueda M., Hung Y.C., Terai Y., Yamaguchi H., Saito J., Nunobiki O. *et al.*: "Fas gene promoter – 670 polymorphism (A/G) is associated with cervical carcinogenesis". *Gynecol. Oncol.,* 2005, *98,* 129.

[25] Engelmark M.T., Renkema K.Y., Gyllensten U.B.: "No evidence of the involvement of the *Fas*-670 promoter polymorphism in cervical cancer in situ". *Int. J. Cancer,* 2004, *20,* 112, 1084.

[26] Dybikowska A., Sliwinski W., Emerich J., Podhajska A.J.: "Evaluation of Fas gene promoter polymorphism in cervical cancer patients". *Int. J. Mol. Med.*, 2004, *14*, 475.

[27] Zhang X., Miao X., Sun T., Tan W., Qu S., Xiong P *et al.*: "Functional polymorphisms in cell death pathway genes FAS and FASL contribute to risk of lung cancer". *J. Med. Genet.*, 2005, *42,* 479.

[28] Feuk L., Prince J.A., Breen G., Emahazion T., Carothers A., St Clair D., Brookes A.J.: "Apolipoprotein-E dependent role for the FAS receptor in early onset Alzheimer's disease: finding of a positive association for a polymorphism in the TNFRSF6 gene". *Hum. Genet.,* 2000, *107,* 391.

[29] Nelson H.H., Kelsey K.T., Bronson M.H., Mott L.A., Karagas M.R.: "Fas/APO-1 promoter polymorphism is not associated with non-melanoma skin cancer". *Cancer Epidemiol. Biomarkers Prev.,* 2001, *10*, 809.

[30] Xia B., Yu Y., Guo Q., Li X., Jiang L., Li J.: "Association of Fas-670 gene polymorphism with inflammatory bowel disease in Chinese patients". *World J. Gastroenterol.,* 2005, *11*, 415.

Address reprint requests to:
D.M.K. TAMANDANI, M.D.
Department of Biotechnology
Panjab University
Chandigarh (India)
e-mail: dor_kordi@yahoo.com