Immunology Letters 172 (2016) 84–88

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Significant association between FasL gene -844T/C polymorphism and risk to hepatocellular carcinoma in Egyptian patients

Rania H. Khalifa[a,*], Dina M. Rasheed Bahgat[a], Hatem Abdel Hamid Darwish[b], Rasha Mohamad Hosny Shahin[a]

[a] Department of Clinical & Chemical Pathology, Kasr Al-Ainy, School of Medicine, Cairo University, Egypt
[b] Department of Internal Medicine, Kasr Al-Ainy, School of Medicine, Cairo University, Egypt

ARTICLE INFO

Article history:
Received 20 November 2015
Received in revised form 12 February 2016
Accepted 12 February 2016
Available online 15 February 2016

Keywords:
Fas/Fas ligand
Polymorphism
Hepatocellular carcinoma
PCR-RFLP
Egypt

ABSTRACT

Fas/Fas ligand (FasL) system is the most critical apoptotic signaling entity in the extrinsic apoptotic pathway; hence mutations affecting this pathway may prevent the immune system from the removal of newly-formed tumor cells, and thus lead to tumor formation. The present study investigated the association between the FasL -844T/C polymorphism and the risk of hepatocellular carcinoma (HCC) in a cohort of Egyptian patients and explored the relationship of various clinical and pathological parameters with this single nucleotide polymorphism (SNP).

Blood samples were withdrawn from hundred HCC patients and 100 age-, sex- and ethnically matched controls. The FasL -844 T/C (rs763110) gene polymorphism was typed from genomic DNA using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. Genotype distributions and allelic frequencies between patients and control subjects showed that the TT homozygous patients were two times more likely to develop HCC ($p = 0.011$). Also, the T allele was found to be a significant risk factor for the disease (OR 1.970, 95% CI 1.250–3.105, $p = 0.003$). No association was detected between different parameters of the disease and the SNP. For the first time, our results suggest that the -844T/C polymorphism in the FasL gene confers risk to HCC. The alarming increase in the incidence of HCC in Egypt encourages further studies to document our results in a larger sample, and recommends more genetic studies hoping to define a genomic risk prediction specific to this cancer in our population.

1. Introduction

Annually, more than 560,000 people are diagnosed with hepatocellular carcinoma (HCC) and approximately the same number dies with it, demonstrating the gloomy outcome of this malignancy [1]. Worldwide, HCC is one of the most common cancers associated with poor prognosis [2].

Egypt had witnessed a remarkable increase in the incidence of HCC over the past decade, and studies show that there has been almost a doubling in the proportion of HCC among chronic liver disease (CLD) patients from 4.0% to 7.2% over a decade [3] to rank as the second and 6th most common cancer in Egyptian men and women respectively [4].

The magnitude of the problem had made studies targeting every aspect of the disease a national call; hence a bulk of researches is running within this field. Single nucleotide polymorphisms (SNPs) have been shown to play an important role in the genetic susceptibility to cancer [5]. Of these SNPs, are the functional mutations in the Fas and FasL genes, which have been proposed to be associated with an increased risk of many types of malignancies [6,7].

Fas/Fas ligand (FasL) system is one of the key apoptotic signaling pathways, Fas (TNFRSF6/CD95/APO-1) and Fas ligand (TNFSF6/CD178) belong to the tumor necrosis factor superfamily [8]. Fas is a cell-surface receptor involved in apoptotic signal transmission in many cell types [9] and interacts with its natural ligand, Fas ligand (FasL) which was first described as a cytotoxic protein that is only expressed in activated T cells. However, it is now known to be present in many other cell types in various organs, such as the eyes, brain, placenta and testicles [10].

The death signal cascade initiated by Fas/Fas ligand interaction is important for T-cell homeostasis, cytotoxic T-cell activity, and maintaining immune-privileged sites in the body [11].

Cancers exhibit two opposite effects of the *FAS/FASL* system. Expression of FAS on tumor cells, may assist FAS-triggered killing of tumors by the infiltrating lymphocytes [12], on the other hand, the

* Corresponding author at: Department of Clinical Pathology, School of Medicine, Cairo University, 29 Abdel-Aziz Alsood Street, El Manial, Egypt.
E-mail address: drrhk@hotmail.com (R.H. Khalifa).

http://dx.doi.org/10.1016/j.imlet.2016.02.007
0165-2478/

Table 1
Genotypic and allelic frequencies of the FasL-844 polymorphism between cases and controls and their associations with the risk of HCC.

Genotypes	Cases (n = 100)	Controls (n = 100)	OR	95%CI	*P*
T/T	60(60%)	42(42%)	2.071	1.178–3.639	*0.011
T/C	40(40%)	50(50%)	–	–	–
CC	0	8(8%)	–	–	–
T	160(80%)	134(67%)	1.970	1.250–3.105	*0.003
C	40(20%)	66(33%)	–	–	–

* $p < 0.05$ is statistically significant.

expression of FASL on tumor cells may resist the antitumor immune response, rendering the tumor an immuno-privileged site [13]. In view of these findings, we hypothesized that the polymorphisms of these crucial genes of the apoptosis pathway may influence the susceptibility to cancer.

The FasL gene maps on chromosome 1q23. The FasL -844T/C polymorphism locates in the gene promoter. It has been shown that the FasL -844C allele and its flanking sequence constitute CAAT box that is the binding site for CAAT Enhancer Binding Protein Beta (C/EBPb), resulting in a significantly higher basal FasL expression, than the T allele, and that increased expression of FasL induces the apoptotic activity of the Fas/FasL pathway [14].

Insight of the above mentioned, the present study was conducted to 1-Investigate the association between -844 T/C (rs763110) polymorphism in FasL with hepatocellular carcinoma in a cohort of Egyptian patients. 2- Explore whether FasL -844T/C polymorphism is associated with the clinical and pathological parameters of the disease.

2. Subjects and methods

2.1. Study subjects

The present study comprised hundred HCC patients (86 males and 14 females) (mean age 59.22 ± 8.0133) diagnosed according to the guidelines formulated by the European Association for the Study of the Liver (EASL) for HCC diagnosis and management [15].

Patients were attending the outpatient clinic or the inpatient wards of the department of Internal medicine, Faculty of Medicine, Cairo University. One hundred, age (mean of 55.30 ± 8.313), sex (86 males and 14 females) and ethnically matched healthy volunteers were included in the current study as a control group. Peripheral blood samples were obtained from all subjects after giving informed consent. The study was approved by the hospital's ethical committee (according to the WMA Declaration of Helsinki).

All patients in the study were subjected to full history taking, careful clinical examination, laboratory investigations, abdominal ultrasonography (US), triphasic helical computed tomography (CT) and/or dynamic magnetic resonance imaging (MRI).

2.2. Methods

2.2.1. Genotyping

Total genomic DNA of patients and healthy controls was extracted from about 2 mL anticoagulated whole blood on EDTA using Qiagen extraction kit (*catalog number 51104, USA*).

The genotyping of FasL gene (rs763110) single nucleotide polymorphism was performed by using a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay. For quality control, genotyping was repeated for random samples in each group to confirm our results.

DNA was amplified by PCR using 5′-CAGCTACTCGGAGGCCAAG-3′ (sense) and 5′-GCTCTGAGGGGAGAGACCAT-3′ (antisense) primers (spanning FasL region containing rs763110 site). (GenBank accession no: AF027385, Z96050).

PCR reactions were carried out in a 25-μL reaction mixture containing 100 ng extracted DNA, 100 μm dNTPs, 20 pmol of each Primer, 1.5 mM $MgCl_2$, 1x PCR buffer with $(NH_4)_2$ SO_4 and 2U Taq DNA polymerase. The PCR reaction tubes were then placed in the thermal cycler *(GeneAmp PCR system 9700, Germany)*. The PCR amplifications cycles were performed as follow: initial denaturation at 95 °C for 2 min followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 62 °C for 1 min and extension at 72 °C for 90 s. A final extension step at 72 °C for 7 min was performed.

PCR products were then digested by 10 U *Bse*MI, using the conditions recommended in the manufacturer's instructions *(Fermentas, Lithuania)* and DNA fragments were separated by electrophoresis in 3% agarose gel stained with ethidium bromide.

The homozygous T/T allele (wild type) does not contain a recognition site for the enzyme *Bse*MI, so the 401 bp amplificon remains unaltered after incubation with *Bse*MI. As for the homozygous C/C allele, it contains a recognition site for the FastDigest *Bse*MI restriction enzyme, so the digestion product of the PCR amplificon having C/C genotype, using *Bse*MI, yields two DNA fragments 233 bp and 168 bp in length. In case of the heterozygous T/C allele, incubation of the PCR amplification product with *Bse*MI yields three DNA fragments 401 bp, 233 bp and 168 bp in length [16].

2.2.2. Statistical analysis

Data were statistically described in terms of mean ± standard deviation (± SD), or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables was done using unpaired T test. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. All p values less than 0.05 were considered statistically significant. In addition, conformity to the Hardy Weinberg law of genetic equilibrium was tested among controls using the chi square test through the assessment of the difference between the frequencies of the observed and the expected genotypes. Odds ratio (OR) and 95% confidence interval (CI) were used for risk estimation. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

3. Results

Genotype distributions of FasL gene (rs763110) single nucleotide polymorphism in the case-control cohort of patients with HCC are shown in Table 1. Genotype distribution of FasL-844 T/C polymorphism in controls was in accordance with Hardy–Weinberg equilibrium ($p > 0.05$).

The HCC patients were classified according to FasL genotypes into 2 groups: T/T group which included 60 (60%) patients and T/C group which included 40 (40%) patients. On the other side, the controls were classified into 3 groups according to the FasL genotypes: T/T group which comprised 42 (42%) subjects, T/C group which comprised 50 (50%) subjects and C/C group which comprised 8 (8%) subjects. These frequencies carry significant difference as regards the susceptibility to HCC ($p = 0.011$), with the TT genotype showing twofold higher in cases than controls.

Table 2
Comparison between FasL genotypes as regards to the clinical data of HCC patients.

Clinical Data		T/T group (n = 60)	T/C group (n = 40)	*p*
Child Class	A	30 (50%)	20 (50%)	1.000
	B	24 (40%)	16 (40%)	
	C	6 (10%)	4 (10%)	
Lymph Node Metastasis	Yes	10 (16.7%)	8 (20%)	0.671
	No	50 (83.3%)	32 (80%)	
Distant Metastasis	Yes	4 (6.7%)	4 (10%)	0.547
	No	56 (93.3%)	36 (90%)	

*$p < 0.05$ is statistically significant.

Table 3
Comparison between FasL genotypes as regards to the laboratory data of HCC patients.

Laboratory Test	T/T group (n = 60)	T/C group (n = 40)	*p*
ALT (U/L)	49.46 ± 17.86	46.80 ± 25.11	0.565
AST (U/L)	93.37 ± 53.31	78.85 ± 37.14	0.138
AFP (ng/ml)	2107.53 ± 6880.29	2681.69 ± 6023.77	0.669

*$p < 0.05$ is statistically significant.

The allele frequencies of the FasL -844 T/C gene polymorphism were also investigated. The T allele of the FasL-844 gene polymorphism was found to be more frequent in patients than in controls (80% and 67%, $p = 0.003$, respectively), while the C allele was established to be more frequent in controls than patients (33% and 20%, $p = 0.003$, respectively).

The 2 groups were compared clinically regarding Child Pugh class, lymph node metastasis and distant metastasis. We did not find any statistically significant differences between the 2 groups as regards all these points of comparison ($p > 0.05$) (Table 2).

Alpha fetoprotein (AFP), alanine transaminase (ALT) and aspartate transaminase (AST) are common clinical and pathological markers of HCC. Our study analyzed the levels of these pathological markers associated with FasL genotypic frequencies. No statistically significant differences were detected between the 2 groups of HCC patients as regards the AST, ALT and the AFP ($p = > 0.05$) (Table 3).

4. Discussion

Identifying the underlying genetic factors of many diseases could help our understanding of their pathogenesis and eventually lead to better outcome of such diseases. Within this context, many researchers studied the role of FAS and FASL gene polymorphisms in the risk to various cancers [17–19]. Of these polymorphisms, attention has been paid to the FasL -844T/C SNP, owing to its proposed role on the FasL expression level and ultimately, the risk to cancer [20].

The burden of HCC in Egypt had urged us to study the role of such potential gene polymorphism in the susceptibility of the disease in a cohort of our patients. The study was conducted on patients submitted to Kasr-AL-aini hospital in Cairo, which is the reference hospital for HCC in Egypt; hence the study was a good representative of the disease in our population.

Our results showed that the FasL gene -844 TT genotype was associated with an increased risk for the development of HCC (OR 2.071 with 95% CI 1.178–3.639, $p < 0.011$) compared with the TC and CC genotypes. In addition, the FasL-844 T haplotype appears to confer a significant risk for the disease (OR 1.970, 95% CI1.250–3.105, $p = 0.003$).

Within this context, Jung et al. constructed a study to determine whether FasL-844 T/C polymorphism is associated with clinical outcome in chronic HBV infection in Korean subjects. However, in contrast to our findings, the study failed to find any association between the SNP and the HBV clearance and development of HCC [21].

The -844C and -844T have different roles in tumor development. In conjunction with previous reports [22,23], our results suggest that the TT genotype and the T allele of the FasL-844 gene polymorphism may decrease the expression of FasL in cytotoxic cells in cancer patients and thus increase the risk of the disease. On the other side, Sung et al. conducted a study on lung tissues of patients with Non-Small Cell Lung Cancer. Their results showed that the FasL -844CC genotype had higher prevalence in those with advanced tumors than in those with early tumors and also show more tendency to tumor relapse. Accordingly they hypothesized that tumor cells with FasL -844CC genotype might have higher FasL expression and hence can counterattack the tumor-infiltrating T cells and thus evade the immune response [24].

Previous studies had shown that the FasL-844T/C gene polymorphism is a risk factor for many cancers as lung cancer [7], cervical cancer [25], bladder cancer [26], pancreatic cancer [19], and breast cancer [27]. Our results are in agreement with previous findings by Ter-Minassian et al., who reported that subjects under the age 60 years with the FasL TT genotype had higher risk for non-small cell lung cancer [22], also Lei et al. found that the -844 TT carriers had significant increased risk of the prevalence of second primary cancer after head and neck squamous cell carcinoma [23]. However, Sun et al. results stand in contrast. They reported a lower risk for developing esophageal squamous-cell carcinoma in individuals with FasL -844 TT genotypes [6]. In accordance with that, a meta-analysis conducted by Zhang et al. showed that the T allele has a possible protective effect on cancer risk while the C allele is a cancer susceptibility marker, this association between the SNP and the cancer risk was obvious among Asians [28].

This significant difference on the role of -844T/C in cancer development may be attributed to many factors. In part the different ethnicity definitely plays a role. This is evidenced in a study done by Hashemi et al. in a cohort of Iranian patients with breast cancer using the tetra-ARMS-PCR method. However the results came contradictory to those of our study cohorts, as authors reported that the rs763110 T haplotypes are associated with decreased risk of breast cancer in their ethnic group [29].

The discrepancies in results could also be allocated to several environmental and other factors that influence the studied population as for example, the genetic background of different patients. Moreover, it is noteworthy that there are several ways of immune evasion that could be adopted by different types of tumors, other than those related to apoptosis, as reduction in the MHC-I expression, impaired antigen presentation, mutations of the antigen, heterogeneous expression of multiple antigens and the expression of immunosuppressive factors by the tumors [30].

Several other defects of apoptosis have been reported in HCC which could also explain the heterogenous results compared to other types of tumors. Of these are mutations in the p53 tumor suppressor gene, misregulation of the transforming growth factor-

beta (TGF-β) family of cytokines, imbalance in members of Bcl-2 family with overexpression of the anti-apoptotic proteins; Bcl-X_L, Mcl-1 and down regulation of pro-apoptotic members of the family, such as Bax or Bcl-X_S. In addition, over activation of survival signals in HCC cells is also described [31].

It is important to bear in mind the relative or absolute suppression of the apoptotic process displayed by the hepatoma cells that may cooperate with the FasL defect associated with -844T. This is verified in a study carried out by Liu et al. to delineate causes behind persistence of hepatitis B virus (HBV) in hepatocytes and hence progression to cirrhosis and ultimately to HCC. Their report demonstrated that hepatitis B viral core protein (HBc) may prevent hepatocytes from Fas-induced apoptosis by dual effects; on one side it represses the proapoptotic p53-dependent Fas-mediated apoptosis of hepatocytes by suppression of both mFas and FasL expression at the transcriptional level, whereas on the other side it increases the antiapoptotics Fas expression by facilitation of Fas alternative splicing [32]. Another study examined liver samples obtained from patients with: chronic hepatitis (CH), cirrhosis and HCC. They reported that the Fas/FasL system expression and apoptosis are up regulated in chronically-damaged liver tissues, reaching a peak in patients with cirrhosis; however the onset of cancer is not associated with a further increase, but rather with a shut-down of this system, supporting the idea that HCC cells are able to adjust the immune response in favor of their survival [33].

In addition, other defects of cell-mediated cytotoxicity are described in association with tumor development. Indeed, loss of apoptosis signaling through Fas (CD95) as a consequence of deregulation of the expression of the Bcl-2 family proteins or inhibitor of apoptosis proteins has been reported to support tumor survival [34]. Also, a study done by Brennan et al. showed that mutations in the pore forming protein; perforin (PRF) are associated with increased frequency of cancers, arguing strongly in favor of a critical role for PRF in the immune surveillance of cancer [35].

In this study, the relations of the gene polymorphisms with clinical and pathological parameters of the disease were detected. There were no significant differences between the gene polymorphism and age, gender, lymph node metastasis, distant metastasis, and child classification.

In conclusion, to the best of our knowledge, this is the first study implicating the FasL gene −844T/C polymorphism in the susceptibility to HCC. This step forward might aid to design targeted therapies in the future in people bearing this risk gene.

Conflict of interest

The authors have no conflicts of interest to declare.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

[1] World Cancer Report, in: B.W. Stewart, P. Kleihues (Eds.), IARC Press, Lyon, 2003.
[2] H. Okano, K. Shiraki, H. Inoue, M. Deguchi, K. Sugimoto, T. Sakai, S. Ohmori, K. Murata, T. Nakano, K. Yamakado, K. Takeda, Treatment of hepatocellular carcinoma and the exacerbation of liver function, Int. J. Oncol. 19 (2001) 1279–1282.
[3] A. El-Zayadi, H.M. Badran, E.M.F. Barakat, M.E. Attia, S. Shawky, M.K. Mohamed, O. Selim, A. Saeid, Hepatocellular carcinoma in Egypt: a single center study over a decade, World J. Gastroenterol. 11 (2005) 5193–5198.
[4] GLOBOCAN database (version 1.2). Available online: http://globocan.iarc.fr, 2008.
[5] H. Guo, H. Liu, J. Wei, Y. Li, H. Yu, X. Guan, W. Li-E, G. Li, M. Erich, E.M. Sturgis, Q. Wei, Z. Liu, Functional single nucleotide polymorphisms of the RASSF3 gene and susceptibility to squamous cell carcinoma of the head and neck, Eur. J. Cancer 50 (2014) 582–592.
[6] T. Sun, X. Miao, X. Zhang, W. Tan, P. Xiong, D. Lin, Polymorphisms of death pathway genes FAS and FASL in esophageal squamous-cell carcinoma, J. Natl. Cancer Inst. 96 (2004) 1030–1036.
[7] X. Zhang, X. Miao, T. Sun, W. Tan, S. Qu, P. Xiong, Y. Zhou, D. Lin, Functional polymorphisms in cell death pathway genes FAS and FASL contribute to risk of lung cancer, J. Med. Genet. 42 (2005) 479–484.
[8] T. Suda, T. Takahashi, P. Golstein, S. Nagata, Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family, Cell 75 (1993) 1169–1178.
[9] N. Itoh, S. Yonehara, A. Ishii, M. Yonehara, S. Mizushima, M. Sameshima, A. Hase, Y. Seto, S. Nagata, The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis, Cell 66 (1991) 233–243.
[10] T.A. Ferguson, T.S. Griffith, A vision of cell death: Fas ligand and immune privilege 10 years later, Immunol. Rev. 213 (2006) 228–238.
[11] K. Schulzeosthoff, D. Ferrari, M. Los, S. Wesselbourg, M.E. Peter, Apoptosis signalling by death receptors, Eur. J. Biochem. 254 (1998) 439–459.
[12] M. Villa-Morales, J. Fernandez-Piqueras, Targeting the Fas/FasL signaling pathway in cancer therapy, Expert. Opin. Ther. Targets 16 (2012) 85–101.
[13] C. Brando, S. Mukhopadhyay, E. Kovacs, R. Medina, P. Patel, T.L. Catina, K.S. Campbell, D. Santoli, Receptors and lytic mediators regulating anti-tumor activity by the leukemic killer T cell line TALL-104, J. Leukoc. Biol. 78 (2005) 359–371.
[14] J. Wu, C. Metz, X. Xu, R. Abe, A.W. Gibson, J.C. Edberg, J. Cooke, F. Xie, G.S. Cooper, R.P. Kimberly, A novel polymorphic CAAT/enhancer-binding protein b element in the FasL gene promoter alters Fas ligand expression: a candidate background gene in African American systemic lupus erythematosus patients, J. Immunol. 170 (2003) 132–138.
[15] J.M. Llovet, M. Ducreux, R. Lencioni, A.M. Di Bisceglie, P.R. Galle, J.F. Dufour, T.F. Greten, E. Raymond, T. Roskams, T. De Baere, M. Ducreux, V. Mazzaferro, M. Bernardi, J. Bruix, M. Colombo, A. Zhu, EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma, J. Hepatol. 56 (2012) 908–943.
[16] S. Yıldır, M. Sezgin, I.O. Barlas, G. Turkoz, H.C. Ankaralı, G. Sahin, M.E. Erdal, Relation of the Fas and FasL gene polymorphisms with susceptibility to and severity of rheumatoid arthritis, Rheumatol. Int. 33 (2013) 2637–2645.
[17] K. Sibley, S. Rollinson, J.M. Allan, A.G. Smith, G.R. Law, P.L. Roddam, C.F. Skibola, M.T. Smith, G.J. Morgan, Functional FAS promoter polymorphisms are associated with increased risk of acute myeloid leukemia, Cancer Res. 63 (2003) 4327–4330.
[18] N.V. Koshkina, E.S. Kleinerman, G. Li, C. Zhao, Q. Wei, E.M. Sturgis, Exploratory analysis of Fas gene polymorphisms in pediatric osteosarcoma patients. Exploratory analysis of Fas gene polymorphisms in pediatric osteosarcoma patients, J. Pediatr. Hematol. Oncol. 29 (2007) 815–821.
[19] M. Yang, T. Sun, L. Wang, D. Yu, X. Zhang, X. Miao, J. Liu, D. Zhao, H. Li, W. Tan, D. Lin, Functional variants in cell death pathway genes and risk of pancreatic cancer, Clin. Cancer Res. 14 (2008) 3230–3236.
[20] H. Zhao, L. Zheng, X. Li, L. Wang, FasL gene -844T/C mutation of esophageal cancer in south China and its clinical significance, Sci. Rep. 4 (2014) 3866.
[21] Y.J. Jung, Y.J. Kim, L.H. Kim, S.O. Lee, B.L. Park, H.D. Shin, H. Lee, Putative association of Fas and FasL gene polymorphisms with clinical outcomes of hepatitis B virus infection, Intervirology 50 (2007) 369–376.
[22] M. Ter-Minassian, R. Zhai, K. Asomaning, L. Su, W. Zhou, G. Liu, R.S. Heist, T.J. Lynch, J.C. Wain, X. Lin, I. DeVivo, D.C. Christiani, Apoptosis gene polymorphisms age, smoking and the risk of non-small cell lung cancer, Carcinogenesis 29 (2008) 2147–2152.
[23] D. Lei, E.M. Sturgis, L.E. Wang, Z. Liu, M.E. Zafereo, Q. Wei, G.I. Li, FAS and FASLG genetic variants and risk for second primary malignancy in patients with squamous cell carcinoma of the head and neck, Cancer Epidemiol. Biomark. Prev. 19 (2010) 1484–1491.
[24] W.W. Sung, Y.C. Wang, Y.W. Cheng, M.C. Lee, K.T. Yeh, J. Wang, C.Y. Chen, H. Lee, A polymorphic -844T/C in FasL promoter predicts survival and relapse in non-Small cell lung cancer, Clin. Cancer Res. 17 (2011) 5991–5999.
[25] T. Sun, Y. Zhou, H. Li, X. Han, Y. Shi, L. Wang, X. Miao, W. Tan, D. Zhao, X. Zhang, Y. Guo, D. Lin, FASL -844C polymorphism is associated with increased activation-induced T cell death and risk of cervical cancer, J. Exp. Med. 202 (2005) 967–974.
[26] C. Li, W. Wu, J. Liu, L. Qian, A. Li, K. Yang, Q. Wei, J. Zhou, Z. Zhang, Functional polymorphisms in the promoter regions of the FAS and FAS ligand genes and risk of bladder cancer in south China, Pharmacogenet. Genom. 16 (2006) 245–251.
[27] W. Wang, Z. Zheng, W. Yu, H. Lin, B. Cui, F. Cao, Polymorphisms of the FAS and FASL genes and risk of breast cancer, Oncol. Lett. 3 (2006) 625–628.
[28] Z. Zhang, L. Qiu, M. Wang, N. Tong, J. Li, Z. Zhang, The FAS ligand promoter polymorphism, rs763110 (-844C/T), contributes to cancer susceptibility: evidence from 19 case-control studies, Eur. J. Hum. Genet. 17 (2009) 1294–1303.
[29] M. Hashemi, A. Fazaeli, S. Ghavami, E. Eskandari-Nasab, F. Arbabi, M.A. Mashhadi, M. Taheri, W. Chaabane, M.V. Jain, M.J. Łos, Functional polymorphisms of FAS and FASL gene and risk of Breast cancer—pilot study of 134 cases, PLoS One 8 (2013) e53075.
[30] F.H. Igney, P.H. Krammer, Immune escape of tumors: apoptosis resistance and tumor counterattack, J. Leukoc. Biol. 71 (2002) 907–920.
[31] I. Fabregat, Dysregulation of apoptosis in hepatocellular carcinoma cells, World J. Gastroenterol. 15 (2009) 513–520.

[32] W. Liu, Y. Lin, X. Yan, Y. Ding, Y. Wu, W. Chen, X. Lin, Hepatitis B virus core protein inhibits Fas-mediated apoptosis of hepatoma cells via regulation of mFas/FasL and sFas expression, FASEB J. (2015), http://dx.doi.org/10.1096/fj.14-263822.
[33] M. Bortolami, A. Kotsafti, R. Cardin, F. Farinati, Fas/FasL system, IL-1b expression and apoptosis in chronic HBV and HCV liver disease, J. Viral Hepat. 15 (2008) 515–522.
[34] M.E. Peter, A. Hadji, A.E. Murmann, S. Brockway, W. Putzbach, A. Pattanayak, P. Ceppi, The role of CD95 and CD95 ligand in cancer, Cell Death Differ. 22 (2015) 549–559.
[35] A.J. Brennan, J. Chia, J.A. Trapani, I. Voskoboinik, Perforin deficiency and susceptibility to cancer, Cell Death Differ. 17 (2010) 607–615.