Department of Hematology[1], The Third Xiangya Hospital of Central South University; Hunan Center for Drug Evaluation and ADR Monitoring[2], Changsha, China

IL-10 promoter polymorphisms affect IL-10 production and associate with susceptibility to acute myeloid leukemia

Yao Chenjiao[1], Fan Zili[1], Chen Haibin[1], Liu Ying[2], Xiao Sheng[1], Huang Lihua[1], Du Wei[1]

Received August 13, 2012, accepted September 14, 2012

Fan Zili, Department of Hematology, The Third Xiangya Hospital of Central South University, Changsha, China fanzl163@yahoo.com.cn

Pharmazie 68: 201–206 (2013) *doi: 10.1691/ph.2013.2737*

We investigated the possible association of Interleukin-10 (IL-10) single nucleotide polymorphisms (SNPs) and susceptibility to acute myeloid leukemia (AML) in 115 AML patients and 137 gender- and age-matched controls. Genetic analysis of IL-10 SNPs at -819 and -592 was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results revealed that the -819AA genotype frequencies and the -819A allele frequencies of AML group were higher than the controls (59.1% vs 40.9%; 75.6% vs 63.9%, respectively); there were remarkable differences in -819T/C and -592A/C gene distribution ($P < 0.05$) and the TA haploid frequencies were higher in AML group (75.6% vs 63.9%, $P < 0.05$). The IL-10 mRNA expression of AML patients and controls with different genotype was detected by Real-time quantitative Polymerase Chain Reaction (RT-PCR). IL-10 mRNA expression in incipient AML patients increased obviously compared with the non-tumor group and remission group ($P < 0.05$). Further analysis suggested that the IL-10 mRNA expression of TA/TA genotype was the lowest and CC/CC genotype was the highest; the haploid TA and genotype TA/TA may be associated with AML. The research suggested the IL-10 SNPs at -819 and -592 sites were associated with AML and may affect the IL-10 mRNA expression in AML patients in Han people of Hunan province.

1. Introduction

Interleukin-10 (IL-10), which is primarily produced by monocytes, has pleiotropic effects in immunoregulation and inflammation and influences many aspects of the immune response (Gibson et al. 2001). The IL-10 gene is located on chromosome 1 and the promoter is polymorphic (Eskdale et al. 1997). Over the past decade, research in IL-10 gene polymorphisms, specially in the promoter region, showed that IL-10 single nucleotide polymorphisms (SNPs) was associated with susceptibility to several diseases, such as cancer, lupus erythematosus, asthma, diabetes, *et al.*; it also suggested that IL-10 SNPs affected the IL-10 expression level and the severity, progress of the disease (Chong et al. 2004; Zhou et al. 2008; Zhuang et al. 2010; Chen et al. 2010; Eder et al. 2007; McCarron et al. 2002; Rosado et al. 2008). SNPs at -819 (C/T) and -592 (C/A) sites have become the research focus in genetic susceptibility and process of cancer in the recent 10 years (Cunningham et al. 2003). They can help us to understand the occurrence and development of human diseases, and the response to drug therapy. Currently, it is known that at least 93% of human genes could present SNPs (Li et al. 2012).

IL-10 has dual biological functions of immune-stimulating (promote cancer potentially) and immuno-suppressive (inhibit cancer potentially) effects and may regulate tumor susceptibility and development (Armando et al. 2012; Garra and Vieira 2007). Recent studies showed that IL-10 SNPs was associated with the non-Hodgkin's lymphoma (Cunningham et al. 2003; Lan et al. 2006). There were few relative researches on the association of IL-10 SNPs and myeloid systemic tumor, acute myeloid leukemia especially and this study investigated the association of the IL-10 SNPs at -819, -592 and the susceptibility to acute myeloid leukemia and the effects of IL-10 SNPs on the mRNA expression.

2. Investigations and results

2.1. Analysis of the SNPs at IL-10 -819, -592 sites

The PCR electrophoregram results revealed that the SNPs at -819 include TT, CC and TC 3 kinds of alleles, while the SNPs at -592 include AA, CC and AC. When the PCR products at -819 site were digested with MslI restriction enzyme, the fragments were 93bp + 116 bp, 209 bp and 209bp + 116bp + 93 bp, respectively, as shown in Fig. 1A; When the PCR product at -592 site was digested with RsaI restriction enzyme, the fragments were 176bp + 236 bp, 412 bp and 412bp + 236bp + 176 bp, respectively, as shown in Fig. 1B.

10% of the PCR samples were examined by sequencing to confirm the digestion results. As shown in Fig. 2, the sequencing results of the SNPs at -819 showed that there was only a single C peak in CC genotype, a single T peak in TT genotype and the overlaping C and T peaks in CT genotypes; similar to SNPs at -592 site, a single A peak in AA genotype, a single C peak in CC genotype and the overlaping A and C peaks in AC genotypes were found.

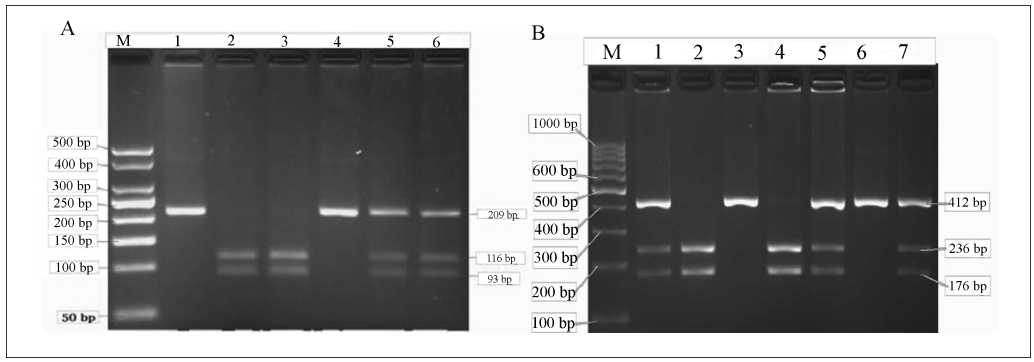

Fig. 1: The PCR electrophoregram of the SNPs of human IL-10 DNA with different restriction enzyme digestion

2.2. Hardy-Weinberg equilibrium test

The allele frequencies of the SNPs at -819 and -592 sites in AML cases and controls were tested in Hardy-Weinberg equilibrium and summarized in Table 1. There were no statistically significant differences in the distribution of alleles between patients and controls ($P > 0.05$). The results suggested that the genotype distribution was in the Hardy-Weinberg equilibrium.

2.3. Allele and genotype distribution of the SNPs at -819 and -592 in AML cases and controls

Three kinds of genotype were found in the IL-10 SNPs and Table 2 shows the allele and genotype distribution of the two SNPs. For SNPs at -819, allele analysis revealed that the T allele frequency was 75.6% in AML patients and 63.9% in controls (P = 0.004). A significant difference was observed in the genotype distribution between cases and controls. TT prevalence was markedly higher in cases than controls (59.1 *vs* 40.9%, P = 0.014). The prevalence risk of the -819 TT was 2.492 times higher than the -819 CC genotype (OR = 2.492; 95% CI: 1.013–5.825). Similar to SNPs at -819, significant differences were observed in the -592AA genotype percentage and -592A allele frequencies between case and control groups ($P < 0.05$). There were significant more individuals with the AA genotype in cases than controls (59.1 *vs* 40.9%, P = 0.014). The A allele frequency was markedly higher in cases than in controls (75.6 *vs* 63.9%, P = 0.004). The prevalence risk of the -592AA genotype was 2.492 times more than the -592 CC genotype (OR = 2.492; 95% CI: 1.013–5.825).

2.4. The linkage disequilibrium and haploid analysis of the IL-10 SNPs at -819 and -592

The SNPStats software (http://bioinfo.iconcologia.net/snpstats/start.htm) was used to analyze the linkage disequilibrium of the IL-10 SNPs at -819 and -592. The results were D' = 1 and r2=1 (D' > 0.8 suggested that the two SNPs had strong linkage and r2 > 1/3 meaned that the two SNPs were linkage disequilibrium), suggesting that -819C and -592C were complete linkage and -819T and -592A were co-inherited. There were only two kinds of haploids consisted of the two sites, which were TA and CC. The results (as in Table 3) suggested that there were significant differences in the IL-10 genotype and haploid frequencies between AML patients and healthy controls. The percentage of TT/AA genotype and TA haploid frequencies were higher in AML patients than healthy controls. We also found that the risk of the one with TA/TA genotype suffered from AML was 2.492 times higher than the CC/CC genotype (OR = 2.492).

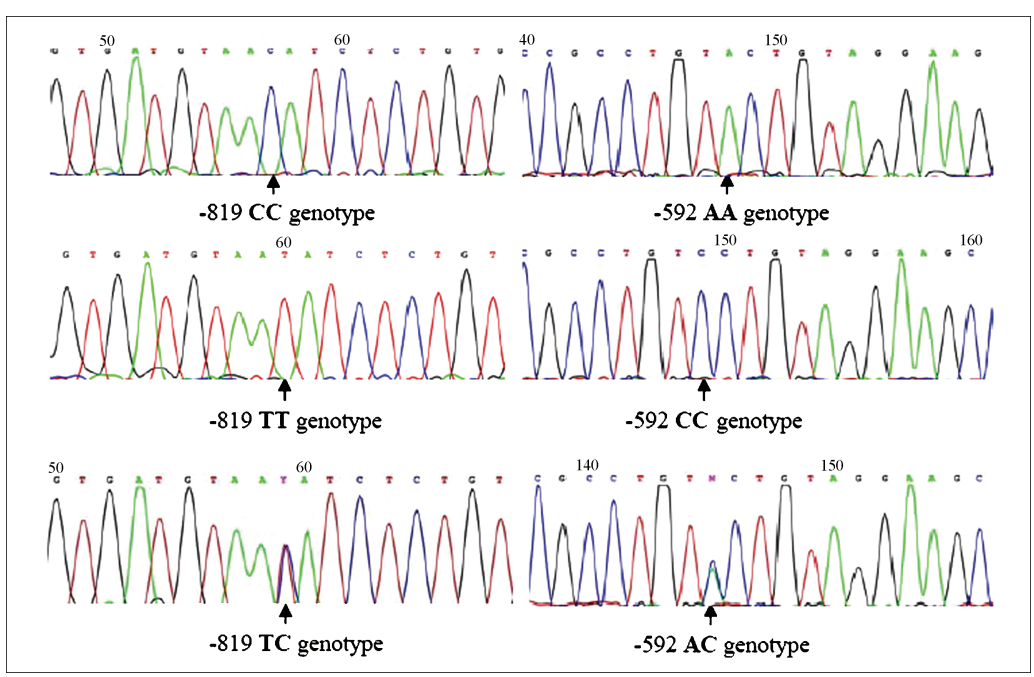

Fig. 2: Sequencing results of the IL-10 SNPs at -819, -592 sites

Table 1: Hardy-Weinberg equilibrium test of the IL-10 SNPs at -819 and -592

Group	819(T/C)			*P*	-592(A/C)			χ^2	*P*
	CC	TT	TC		AA	CC	AC		
AML group									
Actual frequencies	9	68	38	0.269	9	68	38	1.220	0.269
Expected frequencies	6.817	65.817	42.365		6.817	65.817	42.365		
Healthy controls									
Actual frequencies	18	56	63	0.966	18	56	63	0.001	0.966
Expected frequencies	15.445	55.885	63.229		15.445	55.885	63.229		

$P > 0.05$ means the genotype distribution matches Hardy-Weinberg equilibrium.

Table 2: Allele and genotype distribution of the SNPs at -819 and -592 in AML cases and controls

SNPs			Case (n = 115)	Control (n = 137)	χ^2	*P*
-819	Genotype	CC	9(7.8%)	18(13.1%)	8.494	0.014
		TT	68(59.1%)	56(40.9%)		
		TC	38(33.1%)	63(46.0%)		
	Allele	T	169(75.6%)	175(63.9%)	8.153	0.004
		C	61(24.4%)	99(36.1%)		
-592	Genotype	CC	9(7.8%)	18(13.1%)	8.494	0.014
		AA	68(59.1%)	56(40.9%)		
		AC	38(33.1%)	63(46.0%)		
	Allele	A	169(75.6%)	175(63.9%)	8.153	0.004
		C	61(24.4%)	99(36.1%)		

Data are reported as number with percent in parentheses.

2.5. The IL-10 mRNA expression in bone marrow of AML patients

The IL-10 expression were detected using SYBR Green I Quantitative Real-time PCR in the bone marrow of 54 newly diagnosed AML patients, 35 remission patients and 30 non-tumor controls. Ct value was calculated through the fluorescence intensity curves with RT-PCR and $2^{-\Delta Ct}$ represented the expression of IL-10 mRNA in every sample ($\Delta Ct = Ct_{IL\text{-}10} - Ct_{GAPDH}$). If the results did not accord with normal distribution, M (QR) was used as the results. Table 4 reveals that the IL-10 expression in the newly diagnosed AML patients was higher than the non-tumor controls; the expression of the remission patients was lower than the newly diagnosed ones, but still higher than the non-tumor controls ($P < 0.05$).

We also investigated the relation between IL-10 mRNA expression ($2^{-\Delta Ct}$) and the genotypes (CC/CC, TA/TA and TA/CC). Table 5 suggested that the IL-10 mRNA expression in the AML patients with IL-10 TA/TA genotype was low, while it was higher in the CC/CC genotype (in the newly diagnosed AML group). Significant differences were found among the IL-10 expression in the three IL-10 genotype (CC/CC, TA/TA and TA/CC, $P < 0.05$).

3. Discussion

The research focused on the association between IL-10 SNP at -819, -592 and the susceptibility to AML. The observed gene polymorphisms were in Hardy-Weinberg genetic equilibrium with good representative of population.

Mok et al. (1998) found that the allele frequencies of IL-10 SNPs at -819T/C and -592A/C were significantly different in different peoples. Our research suggested that the allele frequencies of healthy Han people controls in Hunan at -819T/C (T 63.9%, C 36.1%) and -592A/C (T 63.9%, C 36.1%) were similar to Guangxi province (-819T 65.9%, C 34.1%; -592A 65.9%, C 34.1%) (Lan et al. 2007).

With the genetic analysis, the remarkable higher value of -819AA and -819A allele frequencies in AML patients and the significant differences in -819T/C and -592A/C gene distribution ($P < 0.05$) indicated that -819T and -592A alleles probably were the inherent predisposing genes of AML. The linkage disequilibrium analysis of -819T/C and -592A/C genotype showed that the two SNP sites were with complete linkage disequilibrium (D' = 1). The higher TA haploid frequencies and TA/TA genotype distribution in AML group ($P < 0.05$) suggested that the TA haploid and TA/TA genotype of IL-10 were associated with the susceptibility of AML in Han people of Hunan province.

Table 3: Genotype and haploid frequencies of IL-10 in AML cases and controls

		Case (n = 115)	Control (n = 137)	χ^2	*P*
Genotype	CC/CC	9(7.8%)	18(13.1%)	8.494	0.014
	TA/TA	68(59.1%)	56(40.9%)		
	TA/CC	38(33.1%)	63(46.0%)		
Haploid frequencies	TA	169(75.6%)	175(63.9%)	8.153	0.004
	CC	61(24.4%)	99(36.1%)		

Data are reported as number with percent in parentheses.

Table 4: The IL-10 mRNA expression in bone marrow of AML patients (M(QR))

Group	Case (n)	$2^{-\triangle Ct}$	d.f.	*P*
Newly diagnosed AML	54	$7.78 \times 10^{-3}(3.31 \times 10^{-3})$		
Remission AML	35	$3.64 \times 10^{-3}(0.98 \times 10^{-3})$	2	<0.05
Non-tumor controls	30	$2.43 \times 10^{-3}(1.22 \times 10^{-3})$		

d.f. = degrees of freedom.

Table 5: The IL-10 mRNA expression in AML patients and non-tumor controls

Group	$2^{-\triangle Ct}$			d.f.	*P*
	CC/CC	TA/TA	TA/CC		
Newly diagnosed AML	$12.48 \times 10^{-3}(4.56 \times 10^{-3})$	$5.71 \times 10^{-3}(3.01 \times 10^{-3})$	$9.54 \times 10^{-3}(2.94 \times 10^{-3})$	2	<0.05
Non-tumor controls	$4.54 \times 10^{-3}(2.52 \times 10^{-3})$	$1.26 \times 10^{-3}(0.64 \times 10^{-3})$	$2.74 \times 10^{-3}(0.97 \times 10^{-3})$	2	<0.05

Helper T lymphocyte subsets are consisted of Th1 and Th2 subsets and IL-10 is mainly secreted by Th2 subset. The recent study pointed out that IL-10 down-regulated the perforin and gran zyme B gene expression, so as to inhibit the function of cytotoxic T lymphocytes. RT-PCR was performed to analyze the IL-10 mRNA expression in the research. Our study showed that the IL-10 mRNA expression of the AML group was remarkable higher than the non-tumor group (7.78×10^{-3} vs 2.43×10^{-3}, $P<0.05$). This indicated the leukemic cells in bone marrow could secret IL-10, as the local secretion of IL-10 in the tumor growth process, may cause the loss of the sensitivity to cytotoxic T lymphocytes and make the growth of tumor cells easier. Similar to the published paper (Yang et al. 2007), IL-10 mRNA of remission group (by chemotherapy) was significantly decreased when compared with the newly diagnosed AML group (3.64×10^{-3} vs 7.78×10^{-3}, $P<0.05$). Maybe the chemotherapy inhibited the growth and activity of the leukemic cells and affected the founction of the patients' immune system (Hsieh et al. 2000).

The mechanism of the IL-10 promoter gene polymorphisms affecting IL-10 expression is not yet clear. Changes of the IL-10 promoter activity may be a possible mechanism for the changes of IL-10 production (Steinke et al. 2004). Brightbill et al. (2000) found that SP1 had positive regulatory function when there was the A allele in the IL-10 promoter gene, while it turned to be inhibitory effect when C allele replaced the A allele. Studies (Steinke et al. 2004; Rees et al. 2002) also suggested that -592 site was located between the two binding sequence of transcription factors (Ets and SP1) and it increased the affinity of SP1, another unclear transcription factors and promoter. The individuals with -592C allele were found with high level of IL-10. Crawley et al. (1999) found that the transcriptional activity of haploid TA was relatively weaker than the haploid CC in rheumatoid arthritis research. They also pointed out that the expression of IL-10 mRNA of the TA/TA genotype was the lowest through a peripheral blood culture study, which supported our conclusion. Our research on the genotype and haploid of IL-10 and the expression of IL-10 mRNA revealed that IL-10 mRNA of the TA/TA genotype was the lowest in the AML patients and non-tumor controls, while the CC/CC genotype was the highest. So the genotype of IL-10 at -819 and -592 sites may affect the expression of IL-10 mRNA.

The present study showed that the percentage of AML patients with TA/TA genotype was higher than that of healthy controls, and the TA/TA genotype expressed low levels of IL-10 mRNA. According to these results, IL-10 mRNA expression of AML patients should be lower than in normal controls, but the study showed that it was much higher in AML patients than in healthy controls. It is possible that the IL-10 promoter genotype may not be the only factor to affect IL-10 mRNA expression. IL-10 promoter SNP is a genetic marker, which is relatively constant in the individual, and high IL-10 expression did not mean the genotype of the patient was the high IL-10 secretion genotype. It was possible that the IL-10 promoter, combined with the other stimulating factors, affected the IL-10 expression in AML patients.

4. Experimental

4.1. Clinical samples

All specimens were obtained from adults diagnosed and/or treated in the Xiangya Hospital, the Second and the Third Xiangya Hospital of Central South University from March 1, 2011 to January 1, 2012. All the participants were Han people in Hunan province. The specimens were collected from the AML patient group, non-tumor patient group and the control group, respectively. A total of 115 AML patients were enrolled in the study, including 54 newly diagnosed AML patients, 35 complete remission patients treated with chemotherapy and 26 relapsing AML patients (without complete remission). All the cases met the AML diagnose standards (Zhang and Shen 2008). These patients did not have any hereditary blood diseases, such as hereditary hemorrhagic telangiectasia or Fanconi anemia, and were not exposed to drugs and poisons potentially causing leukemia. The non-tumor patient group included 30 patients (19 iron-deficiency anemia patients and 11 leukopenia patients). There were 137 gender- and age-matched healthy physical examinees in the control group and they did not have any hereditary diseases (such as diabetes, connective tissue diseases, tumor, *et al.*). The study was approved by the local ethics committee on human research, and informed consent was obtained from all the patients.

Bone marrow (2–5 ml) was collected and treated with erythrocyte lysis solution. Leukocytes were then colletcted with centrifugation (2000 rpm, 2 min) at 4 °C and stored in Trizol (10^7 leukocytes/ml) at −80 °C for RNA extraction.

4.2. Genetic polymorphism analysis

Genomic DNA was extracted from bone marrow using the Qiagen DNA Isolation kit (Qiagen GmbH, Hilden, Germany). IL-10 promoter polymorphisms were identified by polymerase chain reaction amplification and restriction fragment length polymorphism analysis (PCR-RFLP). The SNPs sequence of -819 T/C (rs 1800871) and -592 A/C (rs 1800872) of human IL-10 was searched through the GenBank from the National Center for Biotechnology Information (NCBI). The primer sequence of each SNP was designed by the software Primer 5.0 (Lin et al. 2011) as shown in Table 6.

The genomic DNA of AML patients and healthy controls was used as template and the amplification was performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, USA) at a final volume of 25 μL, containing 5 pmol of each primer, 50 ng genomic DNA and 12.5 μl 2 × Taq Master Mix (Omega, USA). PCR cycles were set as follows:

Table 6: Primers, restriction enzymes and digested fragments of the SNPs at -819 T/C and -592 A/C of human IL-10

	IL-10 -819 T/C	IL-10 -592 A/C
Upstream primer	5′-TCATTCTATGTGCT GGAGATGG-3′	5′-GGTGAGCACTACC TGACTAGC-3ĭ
Downstream primer	5′-TGGGGGAAGTGG GTAAGAGT-3′	5′-CCTAGGTCACAGT GACGTGG-3′
Annealing temperature	59 °C	58 °C
Length of digested fragment	209 bp	412 bp
Restriction enzyme	MslI [5′. . .CAYNN ÑNRTG. . .3ĭ]	RsaI [5′. . .GTÂC. . .3ĭ]
Digested fragment	C: 93bp + 116bp; T: 209bp	A: 176bp + 236bp; C: 412bp

Table 7: Primers of human IL-10 and GAPDH DNA

	IL-10	GAPDH
Upstream primer	5′-GGACTTTAAGGGTTACCTGGGTTGCC-3′	5′-AATCCCATCACCATCTTCC-3′
Downstream primer	5′-GCCTTGATGTCTGGGTCTTGGTTCTC-3′	5′-CATCACGCCACAGTTTCC-3′

94 °C for 5 min, 35 cycles of denaturing at 94 °C for 45 s, annealing at the indicated temperature for 30 s (as shown in Table 1), extension at 72 °C for 45 s, and a single final extension at 72 °C for 7 min. After amplification, all products were digested with restriction enzymes, and separated by 2.5% agarose gel electrophoresis stained with GoldView for visualization. 10% PCR-amplified DNA samples were examined by DNA sequencing by Shanghai biological engineering company to confirm the genotyping results.

4.3. Real-time PCR of IL-10 mRNA

IL-10 mRNA of the isolated bone marrow from AML patients or healthy controls was determined by real-time PCR. The cDNA was used as the template and RT-PCR was carried out with IL-10 primers shown in Table 2. The procedure conditions were: pre-denaturation at 95 °C for 1 min, 45 cycles of denaturation at 95 °C for 15 s, annealing at 65 °C for 30 s, extension at 72 °C for 30 s, and a final cycle of 5 min at 72 °C. Each time, the GAPDH was amplified to detect the cytokine quantity. $2^{-\Delta Ct}$ was used to quantitate the expression of IL-10 mRNA and $\Delta Ct = Ct_{IL\text{-}10} - Ct_{GAPDH}$.

4.4. Statistical analysis

Genotype, allele and haploid frequencies were compared by the χ^2 test to determine whether the genotype distribution of the two SNPs matched the Hardy-Weinberg equilibrium. P value > 0.05 was considered as the SNPs matched the Hardy-Weinberg equilibrium. The quantity of IL-10 mRNA was shown as median (interquartile range, M (QR)) and the Kruskall-Wallis test was used to compare the values. The linkage disequilibrium and haplotype analysis of two SNPs used SNPStats software. All statistical tests were two-sided test and significant level $\alpha = 0.05$. The data were analyzed using SPSS 16.0 software.

References

Armando VS, Chiara R, Deng B, Liu G, Fedor B, Tidball JG (2012) Interleukin-10 reduces the pathology of mdx muscular dystrophy by deactivating M1 macrophages and modulating macrophage phenotype. Hum Mol Genet 20: 790–805.

Brightbill HD, Plevy SE, Modlin RL, Smale ST (2000) A prominent role for Sp1 during lipopolysaccharide-mediated induction of the IL-10 promoter in macrophages. J Immunol 164: 1940–1951.

Chen KF, Li B, Wei YG, Peng CJ (2010) Interleukin-10–819 promoter polymorphism associated with gastric cancer among Asians. J Int Med Res 38: 1–8.

Chong WP, Ip WK, Wong WH, Lau CS, Chan TM, Lau YL (2004) Association of interleukin-10 promoter polymorphisms with systemic lupus erythematosus. Genes Immun 5: 484–492.

Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson L, Woo P (1999) Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum 42: 1101–1108.

Cunningham LM, Chapman C, Dunstan R, Bell MC, Joske DJ (2003) Polymorphisms in the interleukin-10 gene promoter are associated with susceptibility toaggressive non-Hodgkin's lymphoma. Leuk Lymphoma 44: 251–255.

Eder T, Mayer R, Langsenlehner U, Renner W, Krippl P, Wascher TC, Pummer K, Kapp KS (2007) Interleukin-10 [ATA] promoter haplotype and prostate cancer risk: a population-based study. Eur J Cancer 43: 472–475.

Eskdale J, Kube D, Tesch H, Gallaqher G (1997) Mapping of the human IL-10 gene and further characterisation of the 5' flanking sequence. Immunogenetics 46: 120–128.

Garra AO, Vieira P (2007) TH1 cells control themselves by producing interleukin-10. Nature Rev Immunol 7: 425–428.

Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP (2001) Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. J Immunol 166: 3915–3922.

Hsieh CL, Chen DS, Hwang LH (2000) Tumor-induced immunosuppression: a barrier to immunotherapy of large tumors by cytokine-secreting tumor vaccine. Hum Gene Ther 11: 681–692.

Lan Q, Zheng T, Rothman N, Zhang Y, Wang SS, Shen M, Berndt SI, Zahm SH, Holford TR, Leaderer B, Yeager M, Welch R, Boyle P, Zhang B, Zou K, Zhu Y, Chanock S (2006) Cytokine polymorphisms in the Th1/Th2 pathway and susceptibility to non-Hodgkinlymphoma. Blood 107: 4101–4108.

Lan Y, Tan J, Wu J, Tang XS, Tan JM (2007) The association of the IL-10 promoter polymorphisms and the systemic lupus erythematosus of Zhuang People in Guangxi. Chinese J Department Dermatology 40: 555–557.

Li QY, Guo ZZ, Liang J, Zhang W, Xu LM, Gao YQ, Wang XS, Xue DY, Su SB (2012) Interleukin-10 genotype correlated to deficiency syndrome in hepatitis B cirrhosis. Evid Based Complement Alternat Med Epub 2012 May 28.[PubMed: 298925].

Lin WP, Lin JH, Chen XW, Wu CY, Zhang LQ, Huang ZD, Lai JM (2011) Interleukin-10 promoter polymorphisms associated with susceptibility to lumbar disc degeneration in a Chinese cohort. Genet Mol Res 10: 1719–1727.

McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A, Southgate C, Easton DF, Eeles RA, Howell WM (2002) Influence of cytokine gene polymorphisms on the development of prostate cancer. Cancer Res 62: 3369–3372.

Mok CC, Lanchbury JS, Chan DW, Lau CS (1998) Interleukin-10 promoter polymorphisms in Southern Chinese patients with systemic lupus erythematosus. Arthritis Rheum 41: 1090–1095.

Rees LE, Wood NA, Gillespie KM, Lai KN, Gaston K, Mathieson PW (2002) The interleukin-10-1082 G/A polymorphism: allele frequency in different populations and functional significance. Cell Mol Life Sci 59: 560–569.

Rosado S, Rua-Figueroa I, Vargas JA, Garcia-Laorden MI, Losada-Fernandez I, Martin-Donaire T, Perez-Chacon G, Rodriguez-Gallego C, Naranjo-Hernandez A, Ojeda-Bruno S, Citores MJ, Perez-Aciego P

(2008) Interleukin-10 promoter polymorphisms in patients with systemic lupus erythematosus from the Canary Islands. Int J Immunogenet 35: 235–242.

Steinke JW, Barekzi E, Hagman J, Borish L (2004) Functional analysis of −571 IL-10 promoter polymorphism reveals a repressor element controlled by sp1. J Immunol 173: 3215–3222.

Yang Y, Zhang WG, Qiao YC, Cao XM, Chen YZ, He AL, Liu J, Tian W (2007) The quantitate method of serum IFN-γ and IL-10 in AML patients. Chinese J Hematol 7: 371–372.

Zhang ZN, Shen T (2008) Standard of diagnosis and treatment of blood diseases. 3rd ed., China, p. 139–147.

Zhou Y, Li N, Zhuang W, Liu GJ, Wu TX, Yao X, Du L, Wei ML, Wu XT (2008) Interleukin-10–1082 promoter polymorphism associated with gastric cancer among Asians. Eur J Cancer 44: 2648–2654.

Zhuang W, Wu XT, Zhou Y, Liu L, Liu GJ, Wu TX, Yao X, Du L, Wei ML (2010) Interleukin10–592 promoter polymorphism associated with gastric cancer among Asians: a meta-analysis of epidemiologic studies. Dig Dis Sci 55: 1525–1532.