MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2016; 22: 563-568
DOI: 10.12659/MSM.895818

Received: 2015.08.31
Accepted: 2015.09.28
Published: 2016.02.20

Association Between *MIF-AS* rs755622 and Nephrolithiasis Risk in a Chinese Population

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCEF 1,2 Gaoxiang Ma*
BCD 1,2,3 Qinbo Yuan*
BC 4 Qiangdong Wang*
D 1,2 Mulong Du
CE 1,2 Haiyan Chu
BF 4 Zhenjia Dong
BD 4 Xu Xiao
AD 1,2 Meilin Wang
BD 3 Chao Qin
BD 3 Changjun Yin
A 1,2 Zhengdong Zhang
ABF 3 Wei Zhang

1 Department of Environmental Genomics, Jiangsu Key Laboratory of Cancer Biomarkers, Prevention and Treatment, Cancer Center, Nanjing Medical University, Nanjing, Jiangsu, P.R. China
2 Department of Genetic Toxicology, The Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing, Jiangsu, P.R. China
3 Department of Urology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, P.R. China
4 Department of Urology, Huaiyin Hospital of Huai'an City, Huai'an, Jiangsu, P.R. China

* These authors contributed equally to this work

Corresponding Authors: Zhengdong Zhang, e-mail: drzdzhang@gmail.com or Wei Zhang, e-mail: zhangwei@njmu.edu.cn
Source of support: This study was partly supported by Huai'an Science and Technology Support Program (HAS2011037), and the Priority Academic Program for Development of Jiangsu Higher Education Institutions (Public Health and Preventive Medicine)

Background: Single-nucleotide polymorphisms (SNPs) located at lncRNA may affect the stability and splicing processes of mRNA formation, which result in the alteration of its interacting partners. The SNP rs755622 within exon of antisense lncRNA *MIF*- AS and promoter of *MIF* was implicated in renal disease risk.

Material/Methods: In this case-control study, we genotyped the SNP rs755622 in 230 patients diagnosed with nephrolithiasis and 250 controls in a Chinese population.

Results: We found that the rs755622 CG and CC genotypes had a significantly increased nephrolithiasis risk (adjusted OR=1.52, 95% CI=1.03–2.25; OR=2.63, 95% CI=1.21–5.72, *P*=0.015), compared with GG genotype in the additive model. The rs755622 C carriers (GC/CC) had an adjusted OR (95% CI) of 1.65 (1.14–2.39, *P*=0.016), compared with the GG genotype in the dominant model. This hazardous effect was more pronounced in subgroup age >46, BMI >24, hypertension, ever smoking, and ever drinking subjects. Moreover, we found that rs755622 could modulate the function of *MIF-AS* by influencing its folding.

Conclusions: These results indicate that the *MIF-AS* rs755622 polymorphism may have a crucial role in the development of nephrolithiasis.

MeSH Keywords: Nephrolithiasis • Polymorphism, Single Nucleotide • RNA, Long Noncoding

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/895818

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Nephrolithiasis is a worldwide problem and affects almost all ethnicities and populations; most are calcium oxalate (CaOx) kidney stones. The prevalence rate in developed countries is between 4% and 20% [1]. The recurrence rate of nephrolithiasis is as high as 50% within 5 years [2]. Emerging studies have elucidated a series of events leading to formation of kidney stone [3]. Nephrolithiasis is affected by multiple risk factors, including environmental, hormonal, and genetic ones. The individuals with positive family history are predisposed to nephrolithiasis [1], which suggests that genetic factors may play a key role in development of kidney stone formation.

With the development of high-throughput transcriptome analyses, most of the human genome has been identified to be transcribed into noncoding RNAs. Different from the conventional coding genes, the noncoding RNAs play crucial roles in regulation of various physiological processes in the form of RNA, and do not have the ability to encode proteins. Long non-coding RNAs (lncRNAs) are a novel class of recently identified transcripts, which are transcribed pervasively in the genome and are involved in modulation of the epigenome [4]. LncRNAs can be roughly divided into five categories – intronic lncRNAs, sense lncRNAs, antisense lncRNAs, bidirectional lncRNAs, and intergenic lncRNAs – according to their relative position to the adjacent coding genes [5]. Among them, antisense lncRNAs have been reported to regulate function of corresponding coding genes at the post-transcriptional level [6]. However, much of the role of lncRNAs in nephrolithiasis remains unknown.

Located on 22q11.2, the macrophage migration inhibitory factor (*MIF*) gene encodes a multifunctional cytokine, MIF, generated from some types of cells, including epithelial cells [7,8]. It has a key role in many kidney diseases. However, antisense transcript of MIF, named *MIF-AS* in the present study, was a novel unknown lncRNA. Studies on the biological function of *MIF-AS* and its role in nephrolithiasis have not been reported yet. Single-nucleotide polymorphism (SNP) mainly refers to the DNA sequence polymorphism at the genomic level caused by a single-nucleotide variation. Recent evidence has confirmed that SNPs in lncRNAs can affect its biological processes of mRNA formation, which may lead to the aberration of its interacting genes [9–11].

MIF-AS rs755622 has been shown to be associated with renal disease risk [12]. In the present study, we hypothesized that rs755622 located on exon of *MIF-AS* is involved in nephrolithiasis. Briefly, a total of 480 subjects, including 230 patients with nephrolithiasis and 250 healthy controls, were recruited to assess the association between the rs755622 polymorphism and nephrolithiasis risk.

Material and Methods

Study subjects

This present study was approved by the Institutional Review Board of Huaiyin Hospital (Huai-An), and all subjects signed the informed consent form. The experimental protocol was carried out in accordance with the approved guidelines. There were 230 nephrolithiasis cases and 250 controls in this hospital-based case-control study. Briefly, all cases were confirmed to have nephrolithiasis at the time of enrollment in our ongoing study, and were recruited from Huaiyin Hospital (Huai-An) starting from March 2010, of which 91% had calcium oxalate kidney stones. Those people seeking general physical examinations at the outpatient department were recruited as the controls in the same hospital. Controls with renal diseases were excluded. Individuals who smoked daily for >1 year were defined as smokers. The status of body mass index (BMI), hypertension, and diabetes were based on World Health Organization (WHO) standards. Informed consent was obtained from the eligible subjects before recruitment. Through face-to-face interview, individual demographics information was obtained.

Genotyping

Genomic DNA was isolated from peripheral blood lymphocytes from cases and controls. The ABI 7900HT real-time PCR System (Applied Biosystems, Foster City, CA, USA) was used to conduct the TaqMan SNP genotyping assay. Two investigators implemented the genotype analysis independently. Approximately 10% of all the subjects were selected randomly for the verification of accuracy, which were totally consistent with the previous results.

Statistical analysis

The Pearson's χ^2 test and Student's t-test were applied to check the differences in the selected variables and distributions of demographic characteristics between cases and controls. Using adjusted odds ratios (ORs) and 95% confidence intervals (CIs) from unconditional logistic regression, we estimated the association between the nephrolithiasis risk and genotypes. Hardy-Weinberg equilibrium was used to calculate the genotype frequencies among the controls. The statistical analyses were conducted with SAS software (version 9.1, SAS Institute, Inc, Cary, NC, USA) and the differences were considered to be statistically significant when 2-sided $P<0.05$.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 1. The characteristics description of kidney stone and controls.

Variables	Cases n=230 (%)	Controls n=250 (%)	*P**
Age (years)	46.7±12.5	45.9 ±12.5	
≤46	109 (47.4)	134 (53.6)	0.174
>46	121 (52.6)	116 (46.4)	
Sex			
Male	150 (65.2)	166 (66.4)	0.785
Female	80 (34.8)	84 (33.6)	
BMI			
≤24	111 (48.3)	127 (50.8)	0.578
>24	119 (51.7)	123 (49.2)	
Hypertension			
Yes	59 (25.7)	46 (18.4)	0.055
No	171 (74.3)	204 (81.6)	
Diabetes			
Yes	17 (7.4)	19 (7.6)	0.931
No	213 (92.6)	231 (92.4)	
Smoking status			
Ever	103 (44.8)	76 (30.4)	0.001
Never	127 (55.2)	174 (69.6)	
Drinking status			
Ever	88 (38.3)	93 (37.2)	0.811
Never	142 (61.7)	157 (62.8)	

Results

Characteristics of study subjects

Table 1 present the demographic information of cases and controls enrolled in this study. The mean age of the nephrolithiasis patients was 46.7 years compared with 45.9 years in controls, which showed no significant difference (*P*=0.174). The sex distributions were similar (*P*=0.785) among cases and controls. However, there were more smokers among the cases than among the controls (*P*=0.001), suggesting that smoking may have an effect on the etiology of nephrolithiasis. Furthermore, no statistically significant differences were found in the distributions of BMI, hypertension status, diabetes, and drinking status between cases and controls.

Association between *MIF-AS* rs755622 polymorphism and nephrolithiasis risk

The genotype distributions of *MIF-AS* rs755622 in 480 subjects are shown in Table 2. The genotype frequency conformed to Hardy-Weinberg equilibrium in controls (*P*=0.473). The frequency of rs755622 GC/CC genotypes was significantly higher among cases than among controls (52.2% *vs*. 41.2%, *P*=0.016). We evaluated the association between rs755622 and nephrolithiasis risk by unconditional logistic regression. In the additive model, individuals with the CT and CC genotype had significantly increased nephrolithiasis risk (adjusted OR=1.52, 95% CI=1.03–2.25; OR=2.63, 95% CI=1.21–5.72, *P*=0.015) than those with GG genotype, as presented in Table 2. In addition, compared with those carrying GG genotype, the rs755622 C carriers (GC/CC) had an adjusted OR (95% CI) of 1.65 (1.14–2.39, *P*=0.016) in the dominant model.

Furthermore, we conducted stratified analyses of sex, age, BMI, hypertension status, drinking, and smoking status. As presented in Table 3, when the rs755622 GG genotype was regarded as the reference, the increased risk of nephrolithiasis for GC/CC genotypes was also found among subgroup age >46 (adjusted OR=2.12, 95% CI=1.23–3.66, *P*=0.007), male (adjusted OR=1.82, 95% CI=1.15–2.90, *P*=0.001), BMI >24 (adjusted OR=2.50, 95% CI=1.44-4.34, *P*=0.001), hypertension patients

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 2. Genotype and allele frequencies of MIF rs755622 among cases and controls and their associations with kidney stone risk.

MIF rs755622	Cases (n=230)	Controls (n=250)	*P**	OR (95% CI)**
GG	110	147	0.015	1.57 (1.16–2.12)
GC	98	92		1.52 (1.03–2.25)
CC	22	11		2.63 (1.21–5.72)
GC+CC	120	103	0.016	1.65 (1.14–2.39)
GG+GC	208	239		1.00 (ref.)
CC	22	11	0.026	2.21 (1.03–4.71)
Trend			0.005	

* *P* for two-sided χ^2 test; ** Adjusted for age, sex and smoking status in logistic regression model.

Table 3. Stratification analyses of MIF rs755622 SNP association with kidney stone risk.

Characteristics	Cases (n=230)		Controls (n=250)		OR (95% CI)a	*P**	*P***
	GG	GC+CC	GG	GC+CC			
Age (years)							
≤46	55	54	75	59	1.32 (0.78–2.21)	0.300	0.221
>46	55	66	72	44	**2.12 (1.23–3.66)**	**0.007**	
Sex							
Male	67	83	95	71	**1.82 (1.15–2.90)**	**0.001**	0.663
Female	43	37	52	32	1.51 (0.79–2.87)	0.213	
BMI							
≤24	57	54	70	57	1.12 (0.66–1.91)	0.676	0.115
>24	53	66	77	46	**2.50 (1.44–4.34)**	**0.001**	
Hypertension							
Yes	35	24	27	19	**1.94 (1.27–2.97)**	**0.002**	0.163
No	75	96	120	84	1.46 (0.89–3.65)	0.413	
Smoking status							
Ever	48	55	48	28	**1.94 (1.05–3.59)**	**0.034**	0.367
Never	62	65	99	75	1.55 (0.96–2.48)	0.072	
Drinking status							
Ever	35	53	55	38	**2.44 (1.31–4.54)**	**0.005**	0.151
Never	75	67	92	65	1.35 (0.85–2.16)	0.206	

* Adjusted for age, sex and smoking status in logistic regression model; ** *P* for heterogeneity test.

(adjusted OR=1.94, 95% CI=1.27–2.97, *P*=0.002), smoking (adjusted OR=1.94, 95% CI=1.05–5.59, *P*=0.034), and drinking (adjusted OR=2.44, 95% CI=1.31–4.54, *P*=0.005).

In silico analysis of rs755622 on *MIF-AS* folding

It is plausible that rs755622 may disrupt the function of *MIF-AS* by influencing its dimensional folding structure, because the rs755622 locates at the exon region of *MIF-AS*. The local structure change of *MIF-AS* caused by rs755622 was predicted through RNAfold [13] and SNPfold [14] algorithms. Indeed, the SNP rs755622 changed the folding structures of *MIF-AS*, as shown in Figure 1.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

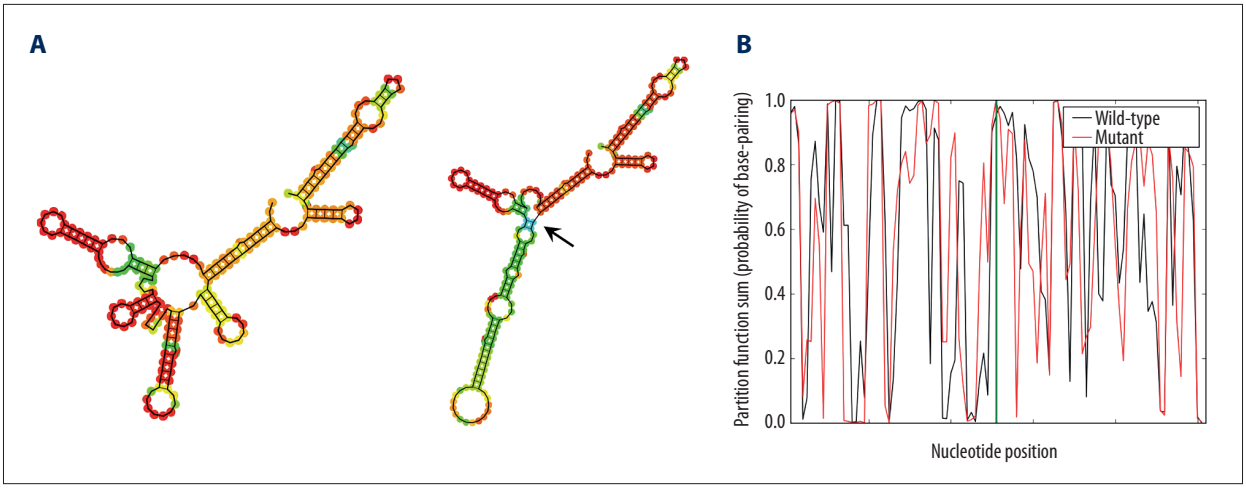

Figure 1. Influence of rs755622 on *MIF-AS* local folding structures. The local structure changes were illustrated by RNAfold (**A**) and SNPfold (**B**), respectively. The arrow (**A**) indicates the position of rs755622. The black line (**B**) represents the SNP rs755622 G allele sequences, whereas the red line represents the C allele. The blue line (**B**) indicates the position of rs755622.

Discussion

Genetic variations are involved in the development and progression of nephrolithiasis [15]. The *MIF-AS* rs755622 was associated with renal disease risk [12], which suggests that it may participate in development of nephrolithiasis. In this study, we hypothesized that rs755622 located on exon of *MIF-AS* is involved in nephrolithiasis. Briefly, a total of 480 subjects, including 230 patients with nephrolithiasis and 250 healthy controls, were recruited to assess the association between the rs755622 polymorphism and nephrolithiasis risk.

The results showed that individuals with the rs755622 genotypes (GC/CC) had a significantly increased nephrolithiasis risk compared with those carrying the GG genotype. In addition, we also confirmed the association between the SNP rs755622 and age >46, male, BMI >24, hypertension, and smoking in the development of nephrolithiasis. Moreover, we identified that the SNP rs755622 may lead to abnormal function of *MIF-AS* by modifying its folding structures.

Studies have found the MIF is a multifunctional cytokine, which is secreted from endothelial cells, macrophages, T lymphocytes, and other inflammatory cells [16–18]. MIF has been identified to participate in a variety of inflammatory and immune response-related conditions, including ankylosing spondylitis [19], ocular inflammation [20], and rheumatoid arthritis [21]. In addition, it is also involved in many kidney diseases. Brown et al. that revealed urine MIF level was correlated with kidney MIF protein and was up-regulated in patients with acute renal rejection [22]. Elevated circulating MIF was detected in end-stage renal disease patients but not in healthy controls [23]. A case-control study showed that patients with end-stage renal disease with *MIF* rs755622 CC genotype had 8-fold higher MIF expression compared with healthy individuals [24], suggesting that rs755622 may have a key role in development of kidney diseases. However, few studies have investigated the role of *MIF-AS* rs755622 and its influence on *MIF-AS* function in the development of nephrolithiasis.

LncRNAs are a crucial class of pervasive genes involved in various important biologic processes [25]. However, how genetic variations in lncRNAs contribute to nephrolithiasis predisposition has not been elucidated. Many studies have reported that variations of the key regulatory locus of an RNA molecule can severely disturb its function [14], which shows that SNPs may be one of the key mechanisms effecting function of lncRNAs. Considering the crucial role of antisense lncRNAs in regulating their neighboring coding genes, we hypothesized that this antisense lncRNA *MIF-AS* may take part in nephrolithiasis through regulating the function of *MIF*. Based on the results of this study, our findings provide a plausible theoretical foundation for our hypothesis *in silico*, as shown in Figure 1. It is biologically plausible that SNP rs755622 further changes the interactions between the *MIF-AS* and *MIF*.

We found that rs755622 is also located on the CpG island of *MIF* promoter by using the public dataset UCSC (*http://genome.ucsc.edu/*). The SNP rs755622G>C changes the DNA sequence GG of *MIF* promoter to CG, a CpG site. The methylation of promoter CpG islands is an important gene regulatory mechanism. Hypermethylation of gene promoter often represses its expression. A genetic variation in *MIF* promoter could affect the combination of transcription factors and modulate its expression. Moreover, the SNPs in the promoter may also eliminate or create CpG sites by which gene expression is suppressed or

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

promoted. As this study indicated, the SNP rs755622G>C created a CpG site, which may influence the methylation of *MIF* promoter. We thus propose that individuals with the rs755622 genotypes (GC/CC) had significantly increased nephrolithiasis risk compared with those carrying the GG genotypes because of aberrant methylation of *MIF* promoter, but his hypothesis requires confirmation in functional studies.

Because kidney stone formation is a complex process, it is impossible that any single SNP or gene exerts a remarkable influence on nephrolithiasis risk. Finally, there was a limitation of our study. Although we demonstrated a dramatic association between the SNP rs755622 and nephrolithiasis risk, how this genetic variation influences the biological function of *MIF-AS* needs to be fully elucidated. According to a previous study by Faghihi et al., antisense lncRNA may regulate the expression of coding genes by increasing mRNA stability [26]. Investigation on this association between *MIF-AS* and *MIF* will be conducted in further studies.

Conclusions

We provided the initial evidence that individuals with the SNP rs755622 GC/CC had a significantly increased nephrolithiasis risk compared to those with the GG genotype, indicating that the C allele has a deleterious effect on nephrolithiasis risk. The results revealed that the *MIF-AS* rs755622 may act as a candidate marker to predict the nephrolithiasis risk in Chinese populations. Lastly, we proposed a hypothesis that rs755622 participates in the development of nephrolithiasis by modulating the function of *MIF* and lncRNA *MIF-AS*. Further large well-designed functional studies in other independent populations are needed.

Conflict of interest

The authors declare no conflicts of interest.

Ethical standard

The present study was approved by the Institutional Review Board of Huaiyin Hospital (Huai-An), and all subjects signed the informed consent form.

References:

1. Lee YH, Huang WC, Tsai JY et al: Epidemiological studies on the prevalence of upper urinary calculi in Taiwan. Urol Int, 2002; 68: 172–77
2. Ljunghall S: Incidence of upper urinary tract stones. Miner Electrolyte Metab, 1987; 13: 220–27
3. Khan SR: Crystal-induced inflammation of the kidneys: results from human studies, animal models, and tissue-culture studies. Clin Exp Nephrol, 2004; 8: 75–88
4. Guttman M, Amit I, Garber M et al: Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature, 2009; 458: 223–27
5. Pang KC, Frith MC, Mattick JS: Rapid evolution of noncoding RNAs: lack of conservation does not mean lack of function. Trends Genet, 2006; 22: 1–5
6. Katayama S, Tomaru Y, Kasukawa T et al: Antisense transcription in the mammalian transcriptome. Science, 2005; 309: 1564–66
7. Calandra T, Bernhagen J, Mitchell RA, Bucala R: The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. J Exp Med, 1994; 179: 1895–902
8. Bacher M, Meinhardt A, Lan HY et al: Migration inhibitory factor expression in experimentally induced endotoxemia. Am J Pathol, 1997; 150: 235–46
9. Zhu Z, Gao X, He Y et al: An insertion/deletion polymorphism within RERT-lncRNA modulates hepatocellular carcinoma risk. Cancer Res, 2012; 72: 6163–72
10. Chung S, Nakagawa H, Uemura M et al: Association of a novel long non-coding RNA in 8q24 with prostate cancer susceptibility. Cancer Sci, 2011; 102: 245–52
11. Li L, Sun R, Liang Y et al: Association between polymorphisms in long non-coding RNA PRNCR1 in 8q24 and risk of colorectal cancer. J Exp Clin Cancer Res, 2013; 32: 104
12. Tong X, He J, Liu S et al: The macrophage migration inhibitory factor –173G/C gene polymorphism increase the risk of renal disease: A meta-analysis. Nephrology (Carlton), 2015; 20(2): 68–76
13. Gruber AR, Lorenz R, Bernhart SH et al: The Vienna RNA websuite. Nucleic Acid Res, 2008; 36: W70–4
14. Halvorsen M, Martin JS, Broadaway S, Laederach A: Disease-associated mutations that alter the RNA structural ensemble. PLoS Genet, 2010; 6: e1001074
15. Safarinejad MR, Shafiei N, Safarinejad S: Association between polymorphisms in osteopontin gene (SPP1) and first episode calcium oxalate urolithiasis. Urolithiasis, 2013; 41: 303–13
16. Metz CN, Bucala R: Role of macrophage migration inhibitory factor in the regulation of the immune response. Adv Immunol, 1997; 66: 197–223
17. Calandra T, Roger T: Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol, 2003; 3: 791–800
18. Nishihira J, Ishibashi T, Fukushima T et al: Macrophage migration inhibitory factor (MIF): Its potential role in tumor growth and tumor-associated angiogenesis. Ann NY Acad Sci, 2003; 995: 171–82
19. Kozaci LD, Sari I, Alacacioglu A et al: Evaluation of inflammation and oxidative stress in ankylosing spondylitis: a role for macrophage migration inhibitory factor. Mod Rheumatol, 2010; 20: 34–39
20. Kotake S, Kitaichi N, Ohno S: Macrophage migration inhibitory factor in uveitis. Int Ophthalmol Clin, 2002; 42: 99–103
21. Leech M, Metz C, Hall P et al: Macrophage migration inhibitory factor in rheumatoid arthritis: evidence of proinflammatory function and regulation by glucocorticoids. Arthritis Rheum, 1999; 42: 1601–8
22. Brown FG, Nikolic-Paterson DJ, Chadban SJ et al: Urine macrophage migration inhibitory factor concentrations as a diagnostic tool in human renal allograft rejection. Transplantation, 2001; 71: 1777–83
23. Bruchfeld A, Carrero JJ, Qureshi AR et al: Elevated serum macrophage migration inhibitory factor (MIF) concentrations in chronic kidney disease (CKD) are associated with markers of oxidative stress and endothelial activation. Mol Med, 2009; 15: 70–75
24. Tripathi G, Borkar M, Akhter A et al: Association of proinflammatory cytokines with end stage renal disease. Cytokine, 2010; 50: 278–83
25. Wang KC, Chang HY: Molecular mechanisms of long noncoding RNAs. Mol Cell, 2011; 43: 904–14
26. Faghihi MA, Modarresi F, Khalil AM et al: Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. Nat Med, 2008; 14: 723–30

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]