RESEARCH ARTICLE

Bioinformatic Prediction of SNPs within miRNA Binding Sites of Inflammatory Genes Associated with Gastric Cancer

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Abstract

Polymorphisms in miRNA binding sites have been shown to affect miRNA binding to target genes, resulting in differential mRNA and protein expression and susceptibility to common diseases. Our purpose was to predict SNPs (single nucleotide polymorphisms) within miRNA binding sites of inflammatory genes in relation to gastric cancer. A complete list of SNPs in the 3'UTR regions of all inflammatory genes associated with gastric cancer was obtained from Pubmed. miRNA target prediction databases (MirSNP, Targetscan Human 6.2, PolymiRTS 3.0, miRNASNP 2.0, and Patrocles) were used to predict miRNA target sites. There were 99 SNPs with MAF>0.05 within the miRNA binding sites of 41 genes among 72 inflammation-related genes associated with gastric cancer. NF-κB and JAK-STAT are the two most important signaling pathways. 47 SNPs of 25 genes with 95 miRNAs were predicted. CCL2 and IL1F5 were found to be the shared target genes of hsa-miRNA-624-3p. Bioinformatic methods could identify a set of SNPs within miRNA binding sites of inflammatory genes, and provide data and direction for subsequent functional verification research.

Keywords: Gastric cancer - inflammatory genes - miRNA - miRNA binding sites - SNP

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Introduction

Gastric cancer is one of the most malicious diseases of the world, and the second most frequent cause of cancer deaths (Hartgrink et al., 2009). Chronic inflammation is a very important factor for gastric cancer, and contributes to about 25% of all gastric cancer cases worldwide (Hussain et al., 2007). It is well established that Helicobacter pylori (Hp) associated chronic gastritis will lead to gastric cancer going through a classic process of "chronic gastritis, intestinal metaplasia, dysphasia, gastric cancer" (Konturek et al., 2009). Hp associated Chronic gastritis will induce high expression of some inflammatory cytokines; studies have verified that inflammation and inflammatory cytokine genes play a very important role in the oncogenesis of gastric cancer (Grivennikov et al., 2010; Schetter et al., 2010). Chemokines are combined with its ligands and receptors, which are downstream of pro-inflammatory cytokines, and the components of the chemokine system can recruit leukocyte, cause neo-angiogenesis, and promote tumor cell growth, proliferation and survival, invasion and metastasis, such as CCL2, CC12, CXCR4 (Gonda et al., 2009; Allavena et al., 2011; Wu et al., 2013).

Bioinformatic and cloning studies have estimated that miRNAs may regulate 30% of all human genes (Lewis et al., 2003). miRNAs can regulate genes by pairing to the 3' untranslated regions (UTRs) of messenger RNAs (mRNAs) of target genes and specifying mRNA cleavage or repression of protein synthesis (Bartel 2009). Complementarity to bases 2-8 of the miRNA (the seed site) is important in miRNA-mRNA binding. However, SNP in 3'UTR of miRNA target genes might create, destroy, or modify a miRNA binding site (Kertesz et al., 2007), then may influence the binding between miRNA and the target 3'UTR, and accordingly lead to the high or low expression of target gene (Kertesz et al., 2007; Wang et al., 2008; Nicoloso et al., 2010).

In the previous genetic studies, researchers have placed more interest on the gene function region, such as gene coding region and promoter region. However, the introns and proximal untranslated regions remain unexplored. The discovery of miRNA makes the study of these untranslated regions an important research (Lheureux et al., 2011). In this study, we will just focus on the miRNA binding sites SNPs located in the 3'UTR of inflammation-related genes by using bioinformatic methods. It will provide data for the follow-up studies and functional verification tests, and build evidence for diagnosis and treatment of gastric cancer.

Materials and Methods

Analysis of candidate inflammation-related genes and their pathways

Published studies that focused on inflammation-

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Table 1. Starting List of Candidate Genes Evaluated for the Presence of Polymorphic miRNA Target Sites

Gene Symbol	Gene Name	Gene Symbol	Gene Name
Pro-inflammatory cytokines, receptors, and related molecules			
CCL2	chemokine (C-C motif) ligand 2	CCL3	chemokine (C-C motif) ligand 3
CCL4	chemokine (C-C motif) ligand 4	CCL5	chemokine (C-C motif) ligand 5
CCL7	chemokine (C-C motif) ligand 7	CCL8	chemokine (C-C motif) ligand 8
CCL17	chemokine (C-C motif) ligand 17	CCL20	chemokine (C-C motif) ligand 20
CCL21	chemokine (C-C motif) ligand 21	CCL22	chemokine (C-C motif) ligand 22
CXCL1	chemokine(C-X-Cmotif) ligand 1	CXCL9	chemokine(C-X-Cmotif) ligand 9
CXCL10	chemokine(C-X-Cmotif) ligand 10	CXCL11	chemokine(C-X-Cmotif) ligand 11
CXCL12	chemokine(C-X-Cmotif) ligand 12	CXCL13	chemokine(C-X-Cmotif) ligand 13
CCR1	chemokine (C-C motif) receptor 1	CCR2	chemokine (C-C motif) receptor 2
CCR3	chemokine (C-C motif) receptor 3	CCR4	chemokine (C-C motif) receptor 4
CCR5	chemokine (C-C motif) receptor 5	CCR6	chemokine (C-C motif) receptor 6
CCR7	chemokine (C-C motif) receptor 7	CX3CR1	chemokine(C-X3-Cmotif) receptor 1
CD40LG	CD40 ligand	CXCR2	chemokine (C-X-C motif) receptor 2
IL1A	interleukin 1, alpha	IL1B	interleukin 1, beta
IL1F5	interleukin 1 family, member 5	IL1R1	interleukin 1 receptor, type I
IL1RN	interleukin 1 receptor antagonist	IL2	interleukin 2
IL2RB	interleukin 2 receptor, beta	IL6	interleukin 6
IL6R	interleukin 6 receptor	IL7R	interleukin 7 receptor
IL8	interleukin 8	IL9	interleukin 9
IL9R	interleukin 5 receptor	IL12B	interleukin 12, beta
IL15	interleukin 15	IL16	interleukin 16
IL17A	interleukin 17A	IL17C	interleukin 17C
IL18RA	interleukin 8 receptor, alpha	IL23R	interleukin 23 receptor
IL32	interleukin 32	IL33	interleukin 33
LTA	lymphotoxin alpha	LTB	lymphotoxin beta
LTB4R	leukotriene B4 receptor	MIF	macrophage migration inhibitory factor
GM-CSF	granulocyte-macrophage colony-stimulating-factor	CEBPB	CCAAT/enhancer binding protein (C/EBP), beta
TNFA	tumor necrosis factor A	TNFB	tumor necrosis factor B
TNFR1	tumor necrosis factor receptor 1	TNFR2	tumor necrosis factor receptor 2
IFNG	interferon, gamma	IFNA2	interferon, alpha, type 2
SPP1	secreted phosphoprotein 1		
Anti-inflammatory cytokines, receptors, and related molecules			
IL4	interleukin4	IL5	interleukin 5
IL5RA	interleukin 5 receptor, alpha	IL10	interleukin 10
IL10RA	interleukin 10 receptor, alpha	IL10RB	interleukin 10 receptor, beta
IL13	interleukin 13	IL13RA1	interleukin 13 receptor, alphal, type I
TGFB1	transforming growth factor, beta 1		
Prostaglandins and nitric oxide			
INOS	inducible nitric oxide synthase	COX2	cycloxygenase-2

related genes and gastric cancer were reviewed from PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Web of knowledge (http://wokinfo.com/), and Ovid (http://www.ovid.com). The following key search terms were used: 'gastric' or 'stomach', 'neoplasm' or 'cancer' or 'carcinoma' or 'tumor', and 'inflammatory gene' or 'inflammation pathway'. Then the full text or abstract were read carefully, and the genes which were reported with a clearly association with gastric cancer were recorded. The pathways of these inflammatory genes from inflammation to gastric cancer were analyzed. PATHWAY MAPS (http://pathwaymaps.com/maps/) and KEGG PATHWAY Database (http://www.genome.jp/kegg/pathway.html) were used to describe module-based network of cancer relevant signaling pathways of these genes.

Search of the 3'UTR polymorphisms of candidate genes

The "database SNP" (dbSNP 138) (http://www.ncbi.nlm.nih.gov/SNP/) was used to have a complete list of SNPs within those genes, and the SNPs in 3'UTR region were selected. The alleles and their frequencies (HapMap-HCB, HapMap-Human China Beijing) of the SNPs were read carefully, and the SNPs with MAF (minor allele frequency) higher than 0.05 in HCB were chosen and recorded.

Computational predictions of miRNA target binding sites

Putative miRNA-binding sites within the 3'UTR SNPs of each inflammation-related gene associated with gastric cancer selected above were identified by means of specialized miRNA target prediction databases: MirSNP (Liu et al., 2012) (http://202.38.126.151/hmdd/mirsnp/search/), TargetScan Human 6.2 (Kumar et al., 2012) (http://www.targetscan.org), PolymiRTS 3.0 (Ziebarth et al., 2012) (http://compbio.uthsc.edu/miRSNP/), miRNASNP 2.0 (Lipchina et al., 2011) (http://www.bioguo.org/miRNASNP/search.php), and Patrocles Targets Database (Hiard et al., 2010) (http://www.patrocles.org/), which are most commonly used with unique algorithms that find MRE (miRNA recognition

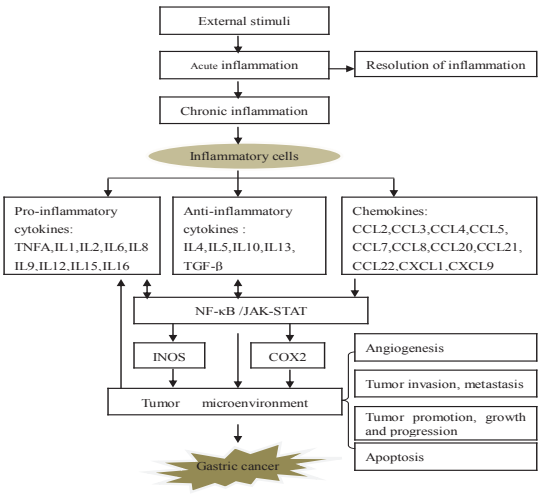

Figure 1. The Pathways from Stomach Inflammation to Gastric Cancer

element) sequences in the 3'UTR of target mRNAs, while miRNA sequences were got from miRBase 18 (http://mirbase.org).

miRNA's function was affected by the SNP in the 3'UTR of target gene. SNPs within the target sites could analogously modulate the miRNA-mRNA interaction and decrease, break, enhance and create a miRNA-mRNA binding site (Liu et al., 2012). 'Create' means that when the allele changes from wild to variant, a new miRNA binding site is created. 'Break' means the original miRNA binding site is broken. 'Enhance' means the ability of miRNA and target gene binding is enhanced, presenting the miRNA binding site with one more base-pair. Meanwhile, 'decrease' means the miRNA binding site was reduced by one base-pair. The function was directly got from MirSNP or by miRNA binding site of the miRNA and SNP sequence.

Assessment of the binding free energy

The sequences of SNPs and miRNAs were respectively got from the database of dbSNP and miRBase.

RNAcofold (http://rna.tbi.univie.ac.at/cgi-bin/RNAcofold.cgi), this database was used to assess the Gibbs binding free energy (△G, expressed in kJ/mol) for the wild and the variant alleles, then the difference of the free energies between the two alleles was computed as "variation of △G" (i.e., △△G) (Landi et al., 2011).

Network analysis of the interaction between miRNAs and mRNAs

Cytoscape software (version 2.8.3, National Institute of General Medical Sciences (NIGMS), U.S.) was used to visualize the network of the target genes and the related miRNAs, and terms with attribute of interest were highlighted (Ross et al., 2013; Song et al., 2013; Spinelli et al., 2013). Specifically, we build a network centered on the inflammation-related genes and the corresponding miRNAs. In the network, each node was an entry, and two nodes were linked by an edge if they had a relation by the above bioinformatic prediction. Node or edge attributes represented entity descriptions and relations annotations.

Table 2. Starting List of Candidate Genes and SNPs with MAFs Higher than 0.05 in HCB

Gene name	dbSNP ID	Variation	MAF
Pro-inflammatory cytokines, receptors, and related molecules			
CCL2	rs13900	C/T	0.419
CCL4	rs1719153	A/T	0.261
CCL11	rs1019109	C/T	0.085
CCL22	rs170360	A/G	0.095
CCR2	rs743660	A/G	0.278
	rs762789	A/G	0.344
CCR3	rs3091312	A/T	0.419
CCR4	rs6770096	C/T	0.233
CX3CR1	rs9826296	A/G	0.278
CXCR2	rs1126580	A/G	0.19
	rs1126579	C/T	0.291
CXCL5	rs3775488	C/T	0.233
CXCL6	rs16850073	C/T	0.314
CXCL9	rs10336	C/T	0.058
	rs3733236	C/T	0.083
CXCL11	rs10017431	C/T	0.058
	rs6532111	C/T	0.049
	rs7436646	G/T	0.115
CXCL12	rs3740085	C/G	0.133
	rs1029153	C/T	0.233
	rs266093	C/G	0.244
	rs1801157	A/G	0.349
CXCL13	rs10022693	C/T	0.229
IL1B	rs2853550	C/T	0.144
IL1F5	rs2515406	C/T	0.056
	rs996879	A/G	0.06
	rs2515404	C/T	0.116
	rs2472188	C/G	0.341
	rs957201	C/T	0.344
	rs768627	C/T	0.349
	rs3180235	A/G	0.349
	rs2515402	A/C	0.349
	rs1800930	A/G	0.349
	rs2515401	C/T	0.349
IL1R1	rs3732131	C/T	0.14
	rs2110726	C/T	0.395
IL1RN	rs9005	A/G	0.412
	rs315951	C/G	0.5
IL2RB	rs228941	C/G	0.367
IL7R	rs9292617	A/T	0.465
	rs10053847	A/G	0.186
	rs7716064	A/G	0.465
	rs9292618	A/G	0.186
	rs6451231	C/T	0.43
	rs6881270	C/T	0.209
	rs13167136	A/G	0.178
	rs6881706	G/T	0.209
	rs10063294	A/G	0.256
	rs700179	A/C/G/T	0.056
IL11	rs1126760	C/T	0.259
IL15	rs10833	A/G	0.107
	rs2291596	C/T	0.407
	rs10519613	A/C	0.489
IL16	rs1131445	C/T	0.211
	rs11325	G/T	0.267
	rs859	A/G	0.442
	rs4778641	C/T	0.465
	rs3726	A/G	0.465
IL17A	rs1974226	A/G	0.06
	rs3748067	A/G	0.221
IL17C	rs4782390	A/T	0.289
IL18RA	rs3732127	C/G	0.089
	rs3771157	G/T	0.14
	rs1420094	A/G	0.179
	rs1420094	A/G	0.179
	rs1135354	G/T	0.36
	rs3732126	G/T	0.36
IL22	rs1182844	A/T	0.433
IL23R	rs10889677	A/C	0.209
IL33	rs1048274	A/G	0.452
MIF	rs2000466	G/T	0.174
	rs2070767	C/T	0.375
SPP1	rs1126772	A/G	0.211
	rs9138	A/C	0.389
TNFR2	rs1061631	A/G	0.116
	rs1061628	C/T	0.276
	rs3397	C/T	0.462
	rs1061624	A/G	0.345
GM-CSF	rs6000495	A/G	0.058
	rs131842	C/T	0.093
LTB4R	rs1046587	A/G	0.122
Anti-inflammatory cytokines, receptors, and related molecules			
IL4R	rs2074570	A/G	0.07
	rs1049631	A/G	0.442
	rs8832	A/G	0.465
	rs1029489	C/T	0.465
IL5RA	rs17659192	C/T	0.081
	rs340832	C/G	0.171
	rs340828	A/G	0.186
	rs6794523	A/C	0.326
	rs340831	C/T	0.395
IL10RA	rs9610	A/G	0.407
IL10RB	rs7281762	A/G	0.302
	rs1058867	A/G	0.337
	rs3171425	A/G	0.337
IL13	rs1295685	C/T	0.291
	rs848	G/T	0.291
	rs847	A/G	0.291
IL13RA1	rs2254672	G/T	0.419
	rs2495636	A/G	0.419

Table 3. Collection of Candidate SNPs and miRNAs Predicted by miRNA Target Prediction Databases

Gene	dbSNP ID	Variation	miRNAs	‖△△G‖ kj/mol	MirSNP	TagetScan	PolymiRTS	miRNASNP	Patrocles	Effect
CCL2	rs13900	C/T	hsa-miR-624-3p	4.41	√		√	√		create
			hsa-miR-374a	2.42	√	√				enhance
			hsa-miR-374b	0.45	√	√				enhance
CCL22	rs170360	A/G	hsa-miR-365b-5p	3.59	√					enhance
			hsa-miR-365a-5p	1.03	√					enhance
			hsa-miR-4658	0.63	√		√			break
			hsa-miR-4314	0.34			√			create
			hsa-miR-4455	0.58			√			create
			hsa-miR-609	1.31			√			create
			hsa-miR-3659	1.99			√			break
			hsa-miR-4303	0.53			√			break
			hsa-miR-574-5p	1.59			√			break
			hsa-miR-4682	4.99			√			break
CCR2	rs743660	A/G	hsa-miR-4786-3p	6.18	√	√				enhance
CXCR2	rs1126580	A/G	hsa-miR-4524b-3p	3.48	√					decrease
			hsa-miR-5096	0.5	√	√				create
	rs1126579	C/T	hsa-miR-5193	6.69	√					enhance
			hsa-miR-516a-3p	6.62	√					break
			hsa-miR-138-1-3p	3.69	√				√	create
CXCL6	rs16850073	C/T	hsa-miR-3913-3p	3.61	√	√				enhance
CXCL9	rs10336	C/T	hsa-miR-4519	3.44	√					enhance
			hsa-miR-4302	1.68	√					enhance
			hsa-miR-4291	0.87	√					enhance
CXCL11	rs10017431	C/T	hsa-miR-613	4.35	√	√			√	create
	rs7436646	G/T	hsa-miR-744-3p	0.15	√		√			break
			hsa-miR-4776-3p	0.26	√		√			break
			hsa-miR-1208	0.08	√		√			break
			hsa-miR-4423-5p	0.03	√		√			break
CXCL12	rs3740085	C/G	hsa-miR-711	6.41	√			√		break
			hsa-miR-4674	5.62	√					enhance
			hsa-miR-767-5p	3.99	√					create
	rs1029153	C/T	hsa-miR-499b-3p	2.01	√			√		create
			hsa-miR-34c-3p	0.64	√			√		create
	rs266093	C/G	hsa-miR-5695	1.96	√					decrease
IL1F5	rs768627	C/T	hsa-miR-1471	2.22	√			√		break
	rs957201	C/T	hsa-miR-5691	2.31	√		√			break
			hsa-miR-632	1.25	√			√		break
			hsa-miR-934	0.51	√			√		break
	rs2515404	C/T	hsa-miR-577	1.24	√			√		create
	rs2472188	C/G	hsa-miR-197-3p	6.33	√			√		create
			hsa-miR-3065-3p	1.99	√			√		break
	rs3180235	A/G	hsa-miR-128	4.17	√			√		create
			hsa-miR-153	3.18	√			√		break
	rs2515402	A/C	hsa-miR-141-3p	4.63	√					create
			hsa-miR-200a-3p	2.22	√					create
	rs1800930	A/G	hsa-miR-1224-3p	1.76	√					break
	rs2515401	C/T	hsa-miR-624-3p	0.13	√			√		break
IL1R1	rs3732131	C/T	hsa-miR-4762-3p	0.62	√					create
	rs2110726	C/T	hsa-miR-4534	4.09	√	√				create
IL1RN	rs9005	A/G	has-miR-92a-2*	0.01					√	decrease
			hsa-miR-3940-3p	1.35	√			√		create
			hsa-miR-4783-3p	0.04	√					create
			hsa-miR-578	0.17	√			√		break
IL2RB	rs228941	C/G	hsa-miR-4716-3p	0.18	√					break
			hsa-miR-4723-5p	0.86	√					break
			hsa-miR-5698	0.3	√					break
			hsa-miR-874	0.22	√					enhance
IL11	rs1126760	C/T	hsa-miR-371a-5p	4.53	√					create
			hsa-miR-4680-5p	0.91	√					break
IL15	rs10833	A/G	hsa-miR-144-3p	0.97	√			√		break
			hsa-miR-340-5p	0.2	√			√		break
	rs10519613	A/C	hsa-miR-203	0.15	√			√	√	create
IL16	rs4778641	C/T	hsa-miR-33a-3p	0.72	√					create
	rs3726	A/G	hsa-miR-1255b-5p	0.11	√					decrease
	rs1131445	C/T	hsa-miR-1301	0.38	√					decrease
	rs11325	G/T	hsa-miR-1913	3.63	√					decrease
IL18RA	rs3732126	G/T	hsa-miR-1307-3p	0.92	√		√	√		break
			hsa-miR-760	0.67	√		√	√		create
	rs3732127	C/G	hsa-miR-2682-3p	0.86	√					break
IL23R	rs10889677	A/C	hsa-miR-1827	4.85	√	√				create
IL33	rs1048274	A/G	hsa-miR-543	0.01	√		√			create
TNFR2	rs3397	C/T	hsa-miR-3126-5p	1.12	√					decrease
			hsa-miR-5581-5p	1.97	√					enhance
			hsa-miR-122-3p	1.58	√					break
			hsa-miR-362-3p	2.56	√					create
GM-CSF	rs6000495	A/G	hsa-miR-4801	0.26	√					decrease
	rs131842	C/T	hsa-miR-4668-5p	1.66	√					enhance
SPP1	rs9138	A/C	hsa-miR-3618	4.79	√			√		create
			has-miR-3977	2.88			√			create
IL4R	rs2074570	A/G	hsa-miR-502-3p	0.23	√					break
	rs1049631	A/G	hsa-miR-4265	0.15	√					break
			hsa-miR-4322	1.03	√					break
			hsa-miR-940	2.9	√	√				enhance
IL10RA	rs9610	A/G	has-miR-922	1.37	√					enhance
IL10RB	rs1058867	A/G	hsa-miR-219-1-3p	0.2	√				√	decrease
			hsa-miR-377-5p	0.11	√					break
	rs3171425	A/G	hsa-miR-328	3.36	√	√				enhance
			hsa-miR-1282	3.04	√		√	√		create
			has-miR-4655-3p	1.52	√		√			create
			has-miR-5707	1.27			√			create
IL13	rs1295685	C/T	hsa-miR-1202	0.67	√		√			break
			hsa-miR-621	3.06	√		√			create
	rs848	G/T	hsa-miR-1343	1.22	√					break
	rs847	A/G	hsa-miR-300	0.2	√		√	√		break
			hsa-miR-381-3P	0.02	√		√	√		break

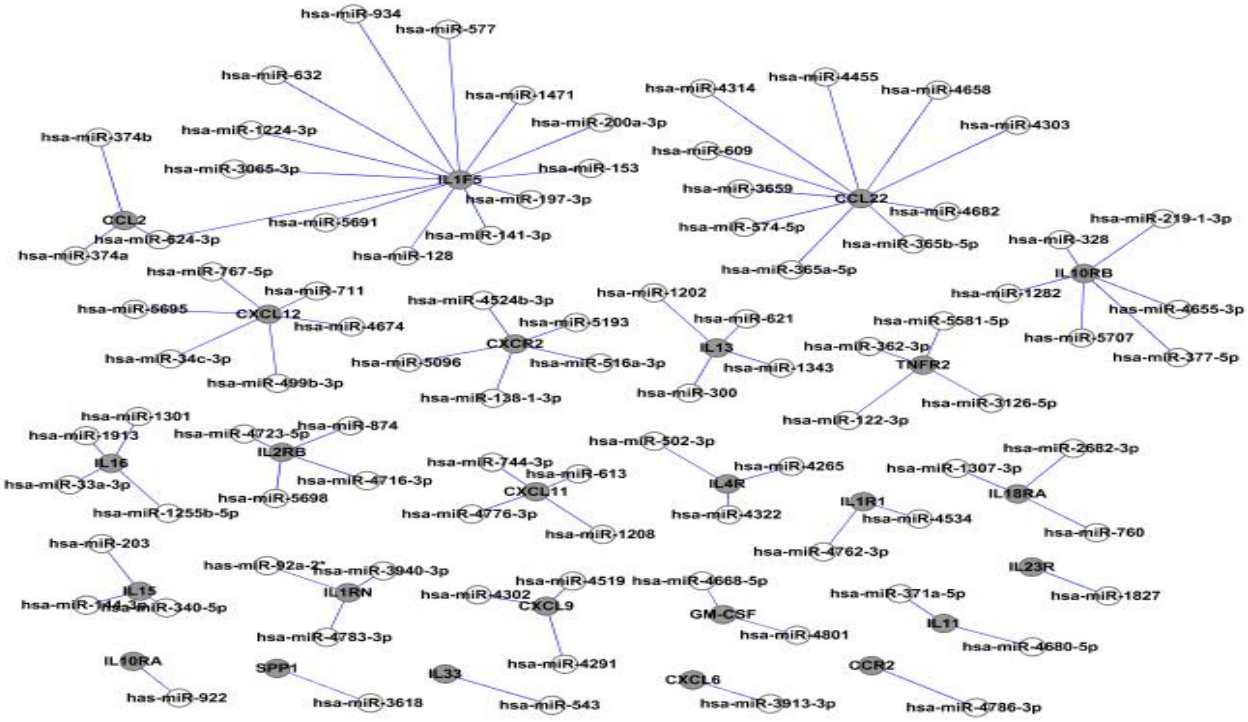

Figure 2. miRNA -mRNA Interaction Network Construction. The inflammation-related genes nodes were shown in gray; and the related miRNAs were shown in white

Results

The inflammatory genes associated with gastric cancer

After searched the data library of PubMed, Web of knowledge, and Ovid, schematic (Figure 1). representation the mechanisms for the involvement of inflammation and inflammatory genes in gastric cancer development. NF-ϰB and JAK-STAT are the two most important signaling pathways.72 inflammation-related genes (Table 1) were found to be related to gastric cancer. They were classified into pro-inflammatory cytokines, receptors, and chemokine genes, anti-inflammatory cytokines, receptors, prostaglandins and nitric oxide. Among these genes, there were 9 anti-inflammatory cytokines, receptors (including IL4, IL5, IL5RA, IL10, IL10RA, IL10RB, IL13, IL13RA1, and TGFB1) and 2 prostaglandins and nitric oxide (COX2 and INOS). Other 61 genes were pro-inflammatory cytokines, receptors, and chemokine genes.

SNPs' information of these inflammatory genes

Among these genes, SNPs without data of HapMap-HCB, or with MAF ≤ 0.05 in HapMap-HCB were all excluded. 99 SNPs of 41 genes in the 3'UTR with MAF>0.05 were selected. There are 35 pro-inflammatory genes with 81 SNPs, and 6 anti-inflammatory genes with 18 SNPs (Table 2). And there are more than one SNP of some genes, such as CCR2, IL1F5, and IL4R.

The results of target SNPs predicted by each miRNA target database

After 99 SNPs with HCB MAF>0.05 locating in 3'UTR of these 41 genes were examined, 47 SNPs of 25 inflammatory genes were found to have the miRNA binding sites. Among these genes, there were 21 pro-inflammatory genes and 4 anti-inflammatory genes. And there were 95 putative miRNAs. 85 miRNAs were obtained by searching in MirSNP, 11 miRNAs were obtained by searching in TargetScan Human 6.2, 25 miRNAs were obtained by searching in PolymiRTS 3.0, 24 miRNAs were obtained by searching in miRNASNP 2.0, and 5 miRNAs were obtained by searching in Patrocles (Table 3).

The functions of the SNPs in the 3'UTR of inflammatory genes to miRNAs were represented in Table 3.

The network of target genes and miRNA interaction

There were 2 disjoint sets of nodes in this graph, mRNA genes (gray circle) and miRNA (white circle). A direct connection placed from a miRNA to an mRNA indicates that the mRNA was predicted to be the target of the miRNA. The length of the line between miRNA and target gene indicated the size of SNPs affecting miRNAs. The shorter the line was, the bigger the effect was. The resulting network had 95 white nodes and 25 gray nodes. CCL2 and IL1F5 were found to have the shared target gene of hsa-miRNA-624-3p.

Discussion

Genetic changes in regulatory regions have been reported to play an important role in susceptibility of common diseases. Variants in 3'UTR of some genes were reported to have relation with higher susceptibility to certain type of cancers (Lheureux et al., 2011; Wang et al., 2012; Iuliano et al., 2013; Skeeles et al., 2013). SNPs within the miRNA binding sites have been shown to affect the ability of miRNAs and target genes binding, resulting in abnormal mRNA and protein expression (Kertesz et al., 2007; Wang et al., 2008; Skeeles et al., 2013), which will influence the susceptibility risk of certain cancers.

Compared with previous genetic studies which focused on the gene coding region, it's also very important to identify a set of SNPs within miRNA binding sites of the inflammatory genes associated with gastric cancer for cancer research.

In consideration of cost-effectiveness, a selection criterion is made in this study that the MAF of the SNPs is higher than 0.05, otherwise it will be excluded (Engels et al., 2007). In this study, although lots of SNPs were searched out in the 3'UTR of 72 inflammatory genes which were found to have association with gastric cancer, at last, most SNPs without data of HapMap-HCB or with MAF≤0.05 were excluded, and there were only 99 SNPs in the 3'UTR of 41 genes selected. Therefore, it's necessary to carry out case-control studies which can provide accurate frequency data of alleles. Then, more susceptibility SNPs will be found out. However, the inferred target SNPs predicted by miRNA target prediction databases were just theoretical results. More evidence from case-control studies and luciferase assays are needed to further validate whether they are the real functional sites.

In general, miRNA could form an actively sTable Watson-Crick base pair with its target mRNA (Bartel 2009). In most occasions, the seed sequence is located at the position 2 to 8 from the 5' end of the miRNA, and acts as an essential scaffold for recognizing the target mRNA by matching with MRE sequences of mRNA (Satoh 2012). Because of thermodynamic rule and the evolutional conservation of MRE sequences, it's possible to accurately predict target mRNAs of miRNAs by computational approaches comparatively. miRNA carries out its function by binding to target gene, so it is crucial to identify its target gene. In recent years, more and more databases have been used to explore the impact of SNPs on miRNA binding sites and are open to public, which respectively are MirSNP, TargetScan Human 6.2, miRNASNP 2.0, Patrocles, and PolymiRTS Database 3.0. They are based on different algorithms, such as sequence complementarily between miRNA and its target gene and the binding energy of the miRNA-target double-stranded, and they will give different predicted results. Therefore, this study listed out all the target SNPs that predicted by these five miRNA target prediction databases. The more databases predicted the SNP, the more likely it would be the true target SNP.

Compared with four other existing databases (TargetScan 6.2, miRNASNP 2.0, PolymiRTS 3.0, and Patrocles), MirSNP prediction was most sensitive. 47 miRNA-related SNPs were identified, and also 85 related miRNAs, which accounted for most of the results. MirSNP is based on information from mirBASE18 and dbSNP135, and it has been developed to identify putative miRNA-related SNPs and miRNAs from single data sets of GWAS (genome-wide association study) or eQTL (expression Quantitative Trait Loci), especially from the newly published datasets (Chenxing Liu et al., 2012). A SNP within the target site could decrease, break, enhance and create a miRNA-mRNA binding site, thus affecting the function of miRNA (Ryan et al., 2010; Liu et al., 2012). A large number of records of SNPs within predicted miRNA target sites are stored in the MirSNP database (Chenxing Liu et al., 2012), and the effects of SNPs on miRNAs could be got directly from MirSNP. So it provides a convenient search platform.

RNAcofold is one of the core programs of the Vienna RNA package (http://www.tbi.univie.ac.at/~ivo/RNA/), which can be used to predict the hybridization energy and base-pairing pattern of two RNA sequences (Gruber et al., 2008; Landi et al., 2011). It is based on concatenating the two RNA sequences and treating the loop containing the concatenation point as an exterior loop. Because of the use of Zuker algorithm, some common interaction motifs such as kissing hairpins can not be predicted (Gruber et al., 2008; Landi et al., 2011).

In this study, the RNAcofold was used to get the $\triangle G$ for the wild and the variant allele of the target SNP, and it was computed as $\triangle\triangle G$ for the difference of the free energies between the two alleles. The absolute values of $\triangle\triangle G$ for each miRNA were listed out. It can be used as parameter for predicting the biological impact of each target SNP. The higher absolute value of $\triangle\triangle G$, the bigger impact SNP on miRNA binding site, and the more product of target gene influenced (Landi et al., 2011; Lipchina et al., 2011). So it is more meaningful for the further experiments of the miRNA function with high absolute value of $\triangle\triangle G$. However, since the inference was based on rules summarized from current uncompleted published data, some exceptions were possible and more experimental data are needed to validate the results.

miRNA exerts function by pairing to the 3' UTR of target genes which lead to mRNA degradation or repression of protein synthesis (Carthew 2006). Owing to their ability to interact with mRNAs, miRNAs can act as oncogenes or tumor-suppressors, depending on the levels of their expression (He et al., 2005). Several miRNAs have been reported in relation to tumorgenesis, while some have the opposite functions of reducing inflammation and inhibiting malignancy in the inflammation pathway (Zabaleta, 2012). For example, hsa-miR-155 inhibited the production of the pro-inflammatory cytokine IL8 by inhibiting the NF-κB pathway (Crone et al., 2012), and the levels of hsa-miR-204 in the gastric mucosa were significantly increased after H. pylori eradication (Shiotani et al., 2012). While Overexpression of miR-150 promoted the proliferation of gastric cancer cells (Wu et al., 2010). Therefore, the identification of cancer related miRNAs and their target genes in the inflammation pathway are important for gastric cancer biology research and its treatment.

In summary, bioinformatic methods could identify a set of SNPs within miRNA binding sites of the inflammatory genes associated with gastric cancer and the corresponding miRNAs. miRNA function was affected by the SNP in the 3'UTR of target gene. This could provide data and direction for subsequent functional verification researches, minimize the costs and narrow the range of experiments. It is very important for gastric cancer biology research. However, the predicted target SNPs and miRNAs were just theoretical. More case-control association studies and function verification experiments such as luciferase report system are needed to carry out.

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