RESEARCH ARTICLE

Inferring Single Nucleotide Polymorphisms in MicroRNA Binding Sites of Lung Cancer-related Inflammatory Genes

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Abstract

Single nucleotide polymorphisms located at microRNA (miRNA)-binding sites are likely to affect the expression of miRNA targets and may contribute to the susceptibility of humans to common diseases. Here 335 candidate lung cancer-related inflammatory genes were selected according to the existing literature and database. We identified putative miRNA-binding sites of 149 genes by specialised algorithms and screened SNPs in the 3'UTRs of these genes. By calculating binding free energy, we sorted 269 SNPs on the basis of the possibility of prediction. The proposed approach could help to easy the identification of functionally relevant SNPs and minimize the workflow and the costs.

Keywords: Algorithms- inflammatory- lung cancer- microrna- polymorphisms- 3'UTR

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Introduction

Since the first noted by the pathologist Rudolph Virchow who found inflammatory cells are present within tumors (Coussens et al., 2002), epidemiological evidences now indicate inflammation mediates oncogenesis and chronic inflammation contributes to about 25% of all human cancers (Hussain et al., 2007). Inflammation induces carcinogenesis maybe by the following mechanism (Ohnishi et al., 2013): reactive oxygen species (ROS), reactive nitrogen species (RNS), harmful endogenous genotoxic substances, generated from inflammatory and epithelial cells result in oxidative and nitrative DNA damage and are involved in the initiation and/or promotion of inflammation-mediated carcinogenesis.

Lung cancer is the most common malignant tumor and the primary reason of global neoplasm deaths (Jemal et al., 2011). Recently, there were also numerous of laboratory and clinical studies have extensively reported the relationship between inflammation and lung cancer, as well as a current review (Cho et al., 2011; Hattar K et al., 2013). Although the exact cause of lung inflammation leading to carcinogenesis is not known, there was the hypothesis (Houghton et al., 2008) demonstrated that chronic airway inflammation alters the bronchial epithelium and lung microenvironment and the expression of oncogenes and tumor suppressor genes might be induced to cause to neoplastic transformation.

Pulmonary inflammation plays a risk role in promoting development of lung cancer. Several kinds of conditions bring about lung inflammation, including tobacco smoke, occupational hazards and pathogen infections. Cigarette smoke contains great amount of carcinogens and modulates inflammation and promotes chronic inflammation in the conducting airways by impairing innate host defense mechanisms (Lee et al., 2009; Lee et al., 2012). Another condition contributes to inflammation is pathogen. Infection triggers the inflammatory response which is a part of normal host defense, preceding tumor development. However, tumorigenic pathogens subvert host immunity and establish persistent infections associated with low-grade but chronic inflammation and then dysregulate inflammatory cytokines and transcription factors (Grivennikov et al., 2010).

Lung cancer cells use inflammatory cytokines and their receptors for tumor growth, invasion, migration and metastasis. MicroRNAs (miRNAs) are endogenous non-coding single-stranded RNAs of about 21–22 nucleotides in length, which can regulate gene translation and modulate gene expression at post-transcriptional level during the most biological and pathological processes (Lagos-Quintana et al., 2001). At present, many researchers (Saunders et al., 2007) considered that single nucleotide polymorphisms (SNPs) in the miRNA seed sequence have higher probability of affecting miRNAs function. Therefore, supposed that SNPs occur in the binding site between miRNAs and mRNAs (usually in 3' untranslated region, UTR) in genes of inflammation signaling pathways, which may weaken or reinforce the expression of miRNA target genes and then, modify

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Table 1. The Evaluation of the miRNA-Binding SNPs in the 3'-UTR of Lung Cancer-related Inflammatory Genes

Gene	*rs*	*MAF*	Common-variation	miRNA	$\|\Delta\Delta G\|_{average}$ (kJ/mol)	Gene	*rs*	*MAF*	Common-variation	miRNA	$\|\Delta\Delta G\|_{average}$ (kJ/mol)
CD4	rs3213427	0.134	T-C	hsa-miR-1272	24.2	CDK6	rs42377	0.116	A-G	hsa-miR-487a	2.97
SARM1	rs118138669	0.05	C-T	hsa-miR-31	9.44					hsa-miR-154	
				hsa-miR-140						hsa-miR-144	
				hsa-miR-3922		NFATC4	rs10362	0.142	G-T	hsa-miR-1207-5p	2.97
				hsa-miR-4708						hsa-miR-486-3p	
				hsa-miR-4753						hsa-miR-625	
				hsa-miR-4701		IFNA1	rs12685904	0.05	T-G	hsa-miR-1911	2.9
				hsa-miR-4696		CXCL12	rs1801157	0.244	G-A	hsa-miR-941	2.9
IRF1	rs6873426	0.153	G-T	hsa-miR-214	9.3					hsa-miR-149	
SMAD3	rs8031627	0.453	A-G	hsa-miR-596	6.5					hsa-miR-631	
CDKN1B	rs4251697	0.085	G-A	hsa-miR-575	6.1	IL1A	rs1304037	0.073	A-G	hsa-miR-298	2.9
KSR1	rs1075952	0.293	A-G	hsa-miR-3136	6					hsa-miR-548	
SLC44A2	rs10948	0.326	T-G	hsa-miR-489	6	SMAD4	rs28403611	0.067	A-G	hsa-miR-4803	2.77
GATA3	rs1058240	0.061	A-G	hsa-miR-95	5.7					hsa-miR-552	
AGT	rs7079	0.098	C-A	hsa-miR-218-2	5.5					hsa-miR-125a-3p	
PPM1A	rs6573305	0.05	G-T	hsa-miR-4789-3p	5	CD4	rs3829972	0.354	G-A	hsa-miR-1206	2.75
CCND1	rs678653	0.091	C-G	hsa-miR-602	5					hsa-miR-663	
GREM1	rs7162202	0.244	C-T	hsa-miR-3921	4.5	GHR	rs2910875	0.321	C-T	hsa-miR-103	2.7
TXNIP	rs7212	0.144	C-G	hsa-miR-296-3p	4.4	MAP2K7	rs3745386	0.085	A-G	hsa-miR-4252	2.68
PRLR	rs392279	0.292	A-G	hsa-miR-3164	4.35					hsa-miR-4488	
				hsa-miR-3118						hsa-miR-4697-5p	
				hsa-miR-134						hsa-miR-4420	
				hsa-miR-4501						hsa-miR-637	
SMAD3	rs1052488	0.314	T-C	hsa-miR-544	4.3					hsa-miR-181a-2	
EDARADD	rs61737025	0.092	T-C	hsa-miR-362-3p	4.23	TLR4	rs11536889	0.216	G-C	hsa-miR-1208	2.65
				hsa-miR-425						hsa-miR-1236	
				hsa-miR-329		COL1A1	rs1061947	0.081	C-T	hsa-miR-1260	2.63
TLR10	rs11466661	0.317	A-C	hsa-miR-3667	4.2					hsa-miR-532-3p	
CFLAR	rs2881929	0.217	G-T	hsa-miR-3678-3p	3.95					hsa-miR-328	
				hsa-miR-1245b-3p		MAPK14	rs6457878	0.19	C-T	hsa-miR-449a/b	2.6
GHR	rs2973016	0.092	A-G	hsa-miR-564	3.9	CDKN1A	rs1059234	0.453	C-T	hsa-miR-3169	2.6
				hsa-miR-4253						hsa-miR-509-5p	
STAT3	rs3744483	0.243	T-C	hsa-miR-1255	3.9					hsa-miR-1288	
TAB2	rs7896	0.1	C-G	hsa-miR-3170	3.85	KRAS	rs8720	0.233	A-G	hsa-miR-374b	2.55
				hsa-miR-4260						hsa-miR-885-5p	
TLR6	rs73236628	0.475	C-T	hsa-miR-494	3.8	PRLR	rs249522	0.233	G-A	hsa-miR-616	2.55
PANX1	rs1046805	0.256	A-G	hsa-miR-10a	3.7					hsa-miR-4289	
				hsa-miR-4732-3p		TNFSF11	rs1054016	0.456	G-T	hsa-miR-3613-3p	2.5
				hsa-miR-212-3p		MAPK11	rs2072878	0.1	A-G	hsa-miR-4300	2.47
ETS2	rs1051476	0.239	C-G	hsa-miR-1293	3.7					hsa-miR-920	
				hsa-miR-323-5p						hsa-miR-939	
GREM1	rs17816260	0.208	C-A	hsa-miR-190	3.6	CCND2	rs3217926	0.105	T-C	hsa-miR-596	2.4
				hsa-miR-299		SOCS5	rs41379147	0.05	C-T	hsa-miR-331-5p	2.4
MAP4K4	rs12622613	0.092	A-G	hsa-miR-4328	3.6	CFLAR	rs7558475	0.016	A-G	hsa-miR-3919	2.4
VEGFA	rs3025053	0.098	G-A	hsa-miR-324	3.45	MAP3K2	rs1129121	0.083	C-T	hsa-miR-15	2.4
				hsa-miR-3130						hsa-miR-195	
CXCL12	rs1029153	0.232	T-C	hsa-miR-499	3.4					hsa-miR-16	
				hsa-miR-34						hsa-miR-497	
ERK2(MAPK1)	rs6928	0.439	G-C	hsa-miR-602	3.35	MAP3K1	rs702688	0.078	T-C	hsa-miR-3682	2.4
			C-G	hsa-miR-602		NFATC4	rs11848279	0.477	A-G	hsa-miR-1285	2.4
TLR4	rs7873784	0.067	G-C	hsa-miR-144	3.3					hsa-miR-3680	
MAPK13	rs2071863	0.389	C-T	hsa-miR-34a	3.3					hsa-miR-3180-5p	
STAT6	rs703817	0.337	G-A	hsa-miR-216a	3.3					hsa-miR-612	
PPM1A	rs2273623	0.134	A-G	hsa-miR-4471	3.25	RELB	rs28372683	0.1	G-T	hsa-miR-1224	2.4
				hsa-miR-1292						hsa-miR-596	
NLRC5	rs27193	0.133	A-G	hsa-miR-490-3p	3.2					hsa-miR-523	
MAPK10	rs1201	0.321	A-G	hsa-miR-147	3.1					hsa-miR-29c	
SMAD3	rs8031440	0.463	G-A	hsa-miR-619	3.03					hsa-miR-138-1	
				hsa-miR-490-3p						hsa-miR-34a	
				hsa-miR-1299		PAK1	rs2844337	0.268	T-G	hsa-miR-4761-5p	2.4
CCND2	rs3217925	0.407	C-T	hsa-miR-3202	3					hsa-miR-320c	
MAPK10	rs3527	0.05	A-G	hsa-miR-466	3	MAP2K1	rs14303	0.108	G-T	hsa-miR-3658	2.4
ETS2	rs1051475	0.225	C-T	hsa-miR-4270	3	JAK3	rs79044512	0.075	A-T	hsa-miR-502-5p	2.37
NR2C2	rs28524664	0.092	A-T	hsa-miR-129	3					hsa-miR-659	
										hsa-miR-4796-3p	

miRNA targeting activities and modulate the level of inflammation in response to various inflammatory stimuli. Many studies evaluated the possible associations between lung cancer and polymorphisms (Chen et al., 2013; Cheng et al., 2013; Zu et al., 2013; Kim et al., 2014).

In this study, we selected lung cancer-related genes that belong to inflammation pathways responding to microorganism and cigarette smoking and hoped to catalogue SNPs, which might affect the expression levels of the target genes. It will provide data for the follow-up studies on susceptibility or prognosis, functional verification and build evidence for diagnosis and treatment of lung cancer.

Materials and Methods

Selection of candidate genes

We focused on the genes which related to lung cancer participated the inflammatory reaction to microorganisms, such as chlamydia pneumonia, mycobacterium tuberculosis or human immunodeficiency virus (HIV) and tobacco smoking. Candidate genes are retrieved according to the

Table 2. The Same Predicted miRNA for the Genes and SNPs of Lung Cancer-related Inflammatory Genes

miRNA	Gene	*rs*	miRNA	Gene	*rs*	miRNA	Gene	*rs*
hsa-miR-103	GHR	rs2910875	hsa-miR-297	FCER1A	rs79965525	hsa-miR-509-5p	CDKN1A	rs1059234
	EGFR	rs884225	hsa-miR-297	CXCL9	rs1050176	hsa-miR-509-5p	PANX1	rs12800562
hsa-miR-106b	PSTPIP1	rs117378779	hsa-miR-299	GREM1	rs17816260	hsa-miR-510	RIPK1	rs17548629
	PRLR	rs371913	hsa-miR-299	IL12A	rs568408	hsa-miR-510	MAPK9	rs34095777
hsa-miR-1197	RIPK1	rs17548629	hsa-miR-30	CCNA2	rs1507987	hsa-miR-512-5p	IL4R	rs2074570
	NLRX1	rs45439091	hsa-miR-30	F2R	rs1801719	hsa-miR-512-5p	MAPK9	rs34095777
	TICAM1	rs1046673	hsa-miR-300	SOCS6	rs7231397	hsa-miR-518a-5p	SMAD3	rs3743342
hsa-miR-1202	EDARADD	rs6428955	hsa-miR-300	ERK2(MAPK1)	rs2276008	hsa-miR-518a-5p	PANX1	rs12800562
	MAPK8IP3	rs2575329	hsa-miR-302	INSR	rs1366600	hsa-miR-519d	XIAP	rs17330644
hsa-miR-1208	TLR4	rs11536889	hsa-miR-302	STAT3	rs1053023	hsa-miR-519d	PRLR	rs371913
hsa-miR-1208	PAK1	rs2729762	hsa-miR-302	KRAS	rs7973450	hsa-miR-520	INSR	rs1366600
hsa-miR-1224	RELB	rs28372683	hsa-miR-302	GREM1	rs33963919		TIRAP	rs8177375
hsa-miR-1224	TOLLIP	rs3168046	hsa-miR-3128	SLC44A2	rs2288902		GREM1	rs33963919
hsa-miR-1233	CFLAR	rs13035714	hsa-miR-3128	GREM1	rs3743104		MMP2	rs7201
hsa-miR-1233	MAPK10	rs958	hsa-miR-3147	PAK1	rs2729762	hsa-miR-527	SMAD3	rs3743342
hsa-miR-1236	TLR4	rs11536889	hsa-miR-3147	TXNIP	rs4755	hsa-miR-527	PANX1	rs12800562
hsa-miR-1236	TIRAP	rs8177375	hsa-miR-3180-5p	NFATC4	rs11848279	hsa-miR-544	SMAD3	rs1052488
hsa-miR-125	IRF1	rs6894655	hsa-miR-3180-5p	NLRC5	rs3751705		KRAS	rs12245
hsa-miR-125	MAPK10	rs958	hsa-miR-32	PANX1	rs12800562		CDK2	rs2069415
hsa-miR-1255	STAT3	rs3744483	hsa-miR-32	FCER1A	rs79965525	hsa-miR-545	CCNA2	rs1507987
hsa-miR-1255	GBP1	rs2624	hsa-miR-329	EDARADD	rs61737025	hsa-miR-545	EDARADD	rs6428955
hsa-miR-1260	COL1A1	rs1061947		EDARADD	rs61736989	hsa-miR-548	IL1A	rs1304037
hsa-miR-1260	TOLLIP	rs3168046		BTK	rs700		NLRP3	rs10802502
hsa-miR-1275	PAX5	rs3739437		MALT1	rs2319974		MMP2	rs7201
hsa-miR-1275	CSF1R	rs2066934	hsa-miR-338-5p	XIAP	rs17334746	hsa-miR-552	SMAD4	rs28403611
hsa-miR-129	NR2C2	rs28524664	hsa-miR-338-5p	NLRP3	rs10754558	hsa-miR-552	SMAD3	rs117707762
hsa-miR-129	BTK	rs1057403	hsa-miR-34a	MAPK13	rs2071863	hsa-miR-561	CASP8	rs13113
hsa-miR-129-5p	COL1A1	rs75713851	hsa-miR-34a	RELB	rs28372683		KRAS	rs9266
hsa-miR-129-5p	TNFSF10	rs11720451	hsa-miR-3609	SMAD3	rs3743342		SMAD3	rs117707762
hsa-miR-132	MAP3K7	rs3734657		XIAP	rs17330644	hsa-miR-570	ECSIT	rs1062958
hsa-miR-132	IL2RA	rs12722604		PRLR	rs371913		INSR	rs1052371
hsa-miR-137	PANX1	rs12800562	hsa-miR-3611	TLR6	rs5743831		IL33	rs1048274
hsa-miR-137	INSR	rs3745550	hsa-miR-3611	PRLR	rs379899		BCL2	rs3744935
hsa-miR-144	TLR4	rs7873784	hsa-miR-3616-5p	XIAP	rs9856	hsa-miR-573	XIAP	rs9856
	CDK6	rs42377		PRLR	rs379899		PRLR	rs379899
	CDK6	rs42035		PRLR	rs371913		PRLR	rs371913
hsa-miR-147	MAPK10	rs1201	hsa-miR-362-3p	EDARADD	rs61737025	hsa-miR-575	CDKN1B	rs4251697
hsa-miR-147	CXCL12	rs1065297	hsa-miR-362-3p	MALT1	rs2319974	hsa-miR-575	VEGFA	rs3025040
hsa-miR-15	MAP3K2	rs1129121	hsa-miR-363	PANX1	rs12800562	hsa-miR-578	BTK	rs1057403
	SARM1	rs739439	hsa-miR-363	FCER1A	rs79965525	hsa-miR-578	CCND2	rs3217929
	CXCL12	rs1804429	hsa-miR-3646	CCND2	rs3217923	hsa-miR-579	NFKBIA	rs8904
hsa-miR-151-5p	COL1A1	rs75713851	hsa-miR-3646	SMAD5	rs3206633	hsa-miR-579	TNFSF10	rs1131535
hsa-miR-151-5p	CCNE1	rs3218073	hsa-miR-3667	TLR10	rs11466661	hsa-miR-591	OSM	rs2070889
hsa-miR-155	CSF1R	rs3828609	hsa-miR-3667	SARM1	rs2239910	hsa-miR-591	VEGFA	rs3025039
hsa-miR-155	SPI1	rs1057233	hsa-miR-367	PANX1	rs12800562	hsa-miR-592	SLC44A2	rs2288902
hsa-miR-16	MAP3K2	rs1129121	hsa-miR-367	FCER1A	rs79965525	hsa-miR-592	ERK2(MAPK1)	rs13515
hsa-miR-16	SARM1	rs739439	hsa-miR-369	IRF1	rs6894655	hsa-miR-596	SMAD3	rs8031627
hsa-miR-17	INSR	rs1366600	hsa-miR-369	CXCL9	rs10337		CCND2	rs3217926
hsa-miR-17	PRLR	rs371913	hsa-miR-374	CCL2	rs13900		RELB	rs28372683
hsa-miR-181	CD4	rs16932921		IRF1	rs6894655	hsa-miR-602	CCND1	rs678653
hsa-miR-181	KRAS	rs9266		ETS2	rs530	hsa-miR-602	ERK2(MAPK1)	rs6928
hsa-miR-182	RAC1	rs9374		CXCL9	rs10337	hsa-miR-603	E2F1	rs3213180
hsa-miR-182	GREM1	rs3743104	hsa-miR-381	SOCS6	rs7231397		MAPK9	rs34095777
hsa-miR-190	GREM1	rs17816260	hsa-miR-381	ERK2(MAPK1)	rs2276008		MALT1	rs2319974
hsa-miR-190	GREM1	rs33963919	hsa-miR-383	IL12A	rs568408	hsa-miR-612	NFATC4	rs11848279
hsa-miR-193	CXCL2	rs9131	hsa-miR-383	IL1R1	rs3732131	hsa-miR-612	TXNIP	rs4755
hsa-miR-193	ERK1(MAPK3)	rs7542	hsa-miR-411	CTSB	rs9009	hsa-miR-625	NFATC4	rs10362
hsa-miR-194	MAP3K7	rs3734657	hsa-miR-411	HSP90AA1	rs1059623	hsa-miR-625	PAX5	rs3739437
	CCND1	rs7177	hsa-miR-424	SARM1	rs739439	hsa-miR-637	MAP2K7	rs3745386
	CDK6	rs42035	hsa-miR-424	CD80	rs17281703		PSTPIP1	rs117378779
hsa-miR-195	MAP3K2	rs1129121	hsa-miR-4253	GHR	rs2973016		NLRP12	rs10410581
hsa-miR-195	SARM1	rs739439	hsa-miR-4253	TNFSF4	rs16845543	hsa-miR-658	CD8A	rs1051386
hsa-miR-195	CD80	rs17281703	hsa-miR-4257	NLRC5	rs3751705	hsa-miR-658	MEFV	rs2741918
hsa-miR-205	CCNA2	rs1507987	hsa-miR-4257	GBP1	rs2296883	hsa-miR-663	CD4	rs3829972
hsa-miR-205	SARM1	rs2239910	hsa-miR-4260	TAB2	rs7896	hsa-miR-663	MAPK8IP3	rs118077547
hsa-miR-205	F2R	rs1801719	hsa-miR-4260	STAT6	rs324015	hsa-miR-665	RIPK1	rs17548629
hsa-miR-20b	CD27	rs1059501	hsa-miR-4266	NFKBIA	rs2273650		STAT5A	rs3198502
hsa-miR-20b	PRLR	rs371913	hsa-miR-4266	CTSB	rs9009		TLR8	rs5744088
hsa-miR-212	MAP3K7	rs3734657	hsa-miR-4270	ETS2	rs1051475	hsa-miR-877	TIRAP	rs8177375
hsa-miR-212	CD80	rs1599795	hsa-miR-4270	OSM	rs2070890		KRAS	rs712
hsa-miR-214	IRF1	rs6873426	hsa-miR-4276	F2R	rs1801719		MYD88	rs7744
	MAP2K2	rs6629	hsa-miR-4276	TAB2	rs2744434	hsa-miR-885-5p	KRAS	rs8720
	SARM1	rs739439	hsa-miR-4327	INSR	rs3745551	hsa-miR-885-5p	CCND3	rs9529
hsa-miR-216a	STAT6	rs703817	hsa-miR-4327	CD4	rs7901	hsa-miR-888	SOCS5	rs4953419
hsa-miR-216a	NLRP4	rs302457	hsa-miR-4658	ERK2(MAPK1)	rs1063311		MAPK10	rs17011312
hsa-miR-216b	SMAD5	rs3206633	hsa-miR-4658	MEFV	rs450021		ATF1	rs829125
	MEFV	rs2741918	hsa-miR-4795-5p	PRLR	rs379899	hsa-miR-92a	PANX1	rs12800562
	MAX	rs4902357	hsa-miR-4795-5p	PRLR	rs371913		FCER1A	rs79965525
hsa-miR-217	RIPK2	rs16900627	hsa-miR-4796-3p	JAK3	rs79044512	hsa-miR-92b	PANX1	rs12800562
	PANX1	rs12800562		XIAP	rs17330644		PSTPIP1	rs117378779
	KSR1	rs2241906		PRLR	rs371913	hsa-miR-93	INSR	rs1366600
hsa-miR-223	CCND1	rs7177	hsa-miR-486-3p	NFATC4	rs10362		XIAP	rs17330644
	FCER1A	rs79965525	hsa-miR-486-3p	EGFR	rs884225		PRLR	rs371913
	BTK	rs700	hsa-miR-492	IKBKE	rs10836	hsa-miR-939	MAPK11	rs2072878
hsa-miR-25	PANX1	rs12800562	hsa-miR-492	CD80	rs57271503		OSM	rs2070890
	FCER1A	rs79965525	hsa-miR-494	TLR6	rs73236628		CCND1	rs7177
hsa-miR-490-3p	NLRC5	rs27193	hsa-miR-494	CREBBP	rs9392		MAPK9	rs1127580
hsa-miR-490-3p	SMAD3	rs8031440	hsa-miR-497	MAP3K2	rs1129121	hsa-miR-96	IL12A	rs568408
hsa-miR-296-3p	CD27	rs1059501	hsa-miR-497	CD80	rs17281703		RAC1	rs9374
hsa-miR-296-3p	TXNIP	rs7212						

information from the website (http://www.sabiosciences.com/Cytokines_Inflammation.php), BioCarta and KEGG pathways which are two common databases that provide displays of gene interactions for human cellular processes (http://cgap.nci.nih.gov/Pathways) and literatures (Shen et al., 2011; Yu et al., 2011; McMillan et al., 2011 ;Lee et al., 2012) commonly acknowledged as important inflammatory genes associated with smoking.

Materials

For this work, we used the followings web sites for prediction the miRNAs binding sites: 1) miRBase (http://www.mirbase.org/); 2) miRanda (http://www.microrna.org/); 3) PicTar (http://pictar.mdc-berlin.de/); 4) Diana-MicroT v3.0 (http://diana.cslab.ece.ntua.gr/microT/); 5) TargetScan Human 6.0 (http://www.targetscan.org/); 6) Patrocles (http://www.patrocles.org/); 7) PolymiRTS Database 3.0 (http://compbio.uthsc.edu/miRSNP/); 8) microRNA-related SNP (http://www.bioguo.org/miRNASNP/).

For selection of 3'UTR: 9) UCSC genome browser (http://www.genome.ucsc.edu).

For searching for SNP in the target sites: 10) SNP database (dbSNP 136; http://www.ncbi.nlm.nih.gov/SNP/).

For calculation of the Gibbs binding free energy: 11) RNAhybrid 2.2 (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/submission.html).

Procedure

For each gene, we proceeded as follows:
1) The 3'UTR was identified according to the UCSC genome browser; 2) Putative miRNA-binding sites within the 3'UTR of each gene were identified by five specialized algorithms approaches as mentioned at materials1.2.3.4.5; 3) The polymorphisms falling within the miRNA-binding sites identified as in stage 2 were searched in SNP database; 4) The polymorphisms were directly predicted by inserting the gene name into the web sites as recited above at materials 6.7.8; 5) The SNPs selection was performed based on frequencies reported for Chinese and the criterion was the SNPs having the minor allele frequency (MAF) lower than 0.05 were excluded. Because in future, we will apply case-control association study on this ethnic group to do the further research; 6) The algorithm of material 11 was run to assess the binding free energy (expressed asΔG (kJ/mol), Gibbs free energy) for both for the common and the variant alleles identified as in stages 3 and 4; 7) The SNPs for their abilities to affect the binding of the miRNAs with their targets were evaluated by calculating as variation of ΔG (i.e., $\Delta\Delta G$), which was expressed as the difference in the energies between the two alleles was computed and used as the parameter for the assessment of the impact that each polymorphism shows for a given miRNA target site.

Results

335 candidate genes that were inflammation-related genes were retrieved from sabiosciences website, BioCarta and KEGG pathways and literatures, which contain the reactive genes under the stimulus of tobacco smoke and microorganisms.

Currently, it is difficult to judge which algorithm produces the most reliable or sensitive target predictions. A union of the result from five algorithm databases was performed for obtaining more reliable genes. Among the 335 candidate genes, 149 genes had miRNA target sequences in their 3'UTR regions, which were predicted by five algorithms. 32 SNPs didn't show any binding free energy at the miRNA target binding site. The remaining 142 genes showed 269 SNPs in the predicted miRNA-binding sites based on criterion (It only list the SNPs, whose $|\Delta\Delta G|_{average} \geq 2.37$ kJ/mol in Table 1 for lack of space) .

For some genes, several miRNAs were predicted as the target sites, and others may be predicted to be targeted by only one miRNA. In order to account for these differences, as parameter for predicting the biological impact of each polymorphism, the average of the absolute values of $\Delta\Delta G$s should be used for each SNP (expressed as $|\Delta\Delta G|$average). In order to give a priority list of SNPs having an impact on miRNA binding, we ranked the values of $|\Delta\Delta G|_{average}$ and classified the SNPs in three groups corresponding to quartile. The first grade ($|\Delta\Delta G|_{average} \geq 2.37$kJ/mol) is composed of SNPs having a predicted high impact on the biology of the miRNA binding sites. The second grade ($0.60 < |\Delta\Delta G|_{average} \leq 2.37$kJ/mol) is composed of SNPs with a predicted mild biological activity, whereas within the last ($|\Delta\Delta G|_{average} < 0.60$kJ/mol) belong SNPs maybe with weakest activity.

For the all 269 SNPs, there were 202 miRNAs predicted for binding more than one SNP (Table 2). CDK6 rs42377 and rs42035 were found to have the shared target gene of hsa-miRNA-144; GREM1 rs17816260 and rs33963919 were found to have the shared target gene of hsa-miRNA-190; EDARADD rs61737025 and rs61736989 were found to have the shared target gene of hsa-miRNA-329; PRLR rs379899 and rs371913 were found to have the shared target gene of hsa-miRNA-3616-5p, hsa-miRNA-4795-5p and hsa-miRNA-573.

Discussion

In this study, we finally identified 269 SNPs within the miRNA-binding sites of 142 genes. Firstly, we chose 335 genes from inflammatory response pathway, including toll-like receptors (TLRs) signaling pathway, JAK/STAT signaling pathway, NF-$\varkappa\beta$ pathway and NOD-like receptors (NLRs) pathway and so on. Such genes play a key role on active immunity and passive immunity and whether in the extrinsic or intrinsic, inflammatory cells and molecules can impact the genomes of cancer cells through a variety of mechanisms. Then by detecting SNPs in miRNA target sites, we got 149 genes. Finally, we obtained 269 SNPs in 142 genes. And we also found that there were 164 SNPs shared the predicted binding miRNAs.

Among these SNPs, some were located in the seen regions of miRNA target sequences, whereas some were in other regions. Since 1993 the first discovery of miRNA, a great number of studies had classified genetic

polymorphisms, which will affect miRNA regulation by various molecular mechanisms into three categories. First are polymorphisms within precursor miRNAs (pre-miRNAs); second are polymorphisms in miRNA-target-mRNA sites and third are variations in miRNA machinery genes (Mishra et al., 2009). It indicated that because SNPs in miRNA-target-mRNA sites are more likely to be under positive selection pressure, they tend to be deleterious, due to differences among various populations and contribute to diseases (Saunders et al., 2007). In current study, we focused SNPs in this region and it will be better for evaluating potential causative SNPs.

Due to the expensive experimental expenses and not suitable approaches, so many researchers conducted bioinformatics prediction and statistical analyses to investigate the diseases associated SNPs in miRNA target sites. Some studies introduced bioinformatics methods to identify a set of SNPs within miRNAs binding sites of genes (Ding et al., 2011; Song et al., 2014) and others verified the specific SNP by molecular experiments (Bhat et al., 2013). It is well-known that the functions of SNPs in miRNA-binding sites were miRNAs regulation and miRNAs or target genes expression. At present, there are many available algorithms and databases to predict miRNA target genes. The commonly rule in the method is that nucleotides 6-8 in the 5' end of the miRNA (called as 'seed' sequences) provide the maximal binding free energy of the miRNA-target duplex and the G:T pairing is admitted in the miRNA-target (John et al., 2004; Tomari et al., 2005). The most studies predict SNPs within miRNA binding sites and assess the potential functions of SNPs in 3'UTR via well-developed algorithms, based on the differences in the alignment scores and variations in binding free energy (Betel et al., 2010; Liu et al., 2012). In addition, some studies investigated the effects of SNPs according to the secondary structures of the miRNA binding sites by using RNAfold (Hariharan et al., 2009). And others employed a linear model to assess the effects of SNPs on the gene expression phenotypes (Richardson et al., 2011; Zhang, 2012).

Using different programmes and databases, we obtained different quantity of lung cancer-related inflammatory genes that have miRNA-binding sites. It is difficult to judge which SNPs or miRNAs are likely to play more roles in lung cancer development without experiments being done, we combined results from eight databases to increase the accuracy of the analysis. In recent years, more and more databases have been used to explore the SNPs in miRNA-binding sites, which including miRanda, PicTar, MirSNP, TargetScan Human, miRNASNP, Patrocles, DIANA-microT and PolymiRTS Database. The more databases predicted the SNP, the more likely it would be the true target SNP (Song et al., 2014).

Currently, there were numbers of studies focus on the association between SNPs in miRNA binding-sites of specific gene and diseases some about susceptibility and others about prognosis. Now genome wide association studies (GWA study, or GWAS) reported scores of diseases related SNPs were in non-coding region. The significance of the association may be brought up by still unknown mechanisms or by linkage disequilibrium (LD) with functional polymorphisms. Thus, the regulation of miRNAs on target genes may work. Some researchers (Richardson et al., 2011) basing on the list of SNPs from GWAS, or in strong LD with a GWAS SNP performed a genome-wide scan of SNPs that abrogate or create miRNA recognition element (MRE) seed sites (MRESS) and identified high priority candidate SNPs for functional studies and for disease risk prediction. Other researchers (Wu et al., 2011) discovered the relationship between specific genes expression or miRNA level and diseases, so they thought that SNPs within miRNA target sites may influence their encoded target-mRNAs and their downstream effectors and they would predict the 3'UTR of these genes contains potential MREs and verify that these mutations maybe as a key in regulating gene expression. At present, all studies the on gene variation in miRNA-binding regions were performing several kinds of arithmetic or getting help from various websites. But there was no uniform standard and procedures in selecting SNPs, the results of predicting not only need evaluate in the association study also confirm by the functional research. So we will do the further investigation to certificate these SNPs.

In our results, we found some SNPs were reported have association with cancer or other disease which may impact function of genes by combing different miRNAs because of changing sequences. This paper provided the basis for a reasoned algorithm-driven selection of SNPs. It is important to address that all the polymorphisms predicted supports future investigations to validate these results in well-characterised populations by functional assays or case-control association studies. The proposed approach could help to ease the identification of functionally relevant SNPs and minimize the workflow and the costs.

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References

Betel D, Koppal A, Agius P, et al (2010). Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome Biol*, **11**, 90.

Bhat IA, Pandith AA, Bhat BA, et al (2013). Lack of association of a common polymorphism in the 3'-UTR of interleukin 8 with non small cell lung cancer in Kashmir. *Asian Pac J Cancer Prev*, **14**, 4403-8.

Chen Z, Xu L, Ye X, et al (2013). Polymorphisms of microRNA sequences or binding sites and lung cancer: a meta-analysis and systematic review. *PLoS One*, **8**, 61008.

Cheng M, Yang L, Yang R, et al (2013). A microRNA-135a/b binding polymorphism in CD133 confers decreased risk and favorable prognosis of lung cancer in Chinese by reducing CD133 expression. *Carcinogenesis*, **34**, 2292-9.

Cho WC, Kwan CK, Yau S, et al (2011). The role of inflammation in the pathogenesis of lung cancer. *Expert Opin Ther Targets*, **15**, 1127-37.

Coussens LM, Werb Z (2002). Inflammation and cancer. *Nature*, **420**, 860-7.

Ding J, Gao Y, He Y, et al (2011). Screening SNPs residing in the microRNA-binding sites of hepatocellular carcinoma related genes. *Int J Data Min Bioinform*, **5**, 1-21.

Grivennikov SI, Greten FR, Karin M (2010). Immunity, inflammation, and cancer. *Cell*, **140**, 883-99.

Hattar K, Savai R, Subtil FS, et al (2013). Endotoxin induces proliferation of NSCLC *in vitro* and *in vivo*: role of COX-2 and EGFR activation. *Cancer Immunol Immunothe*, **62**, 309-20.

Hariharan M, Scaria V, Brahmachari SK. dbSMR: a novel resource of genome-wide SNPs affecting microRNA mediated regulation. *BMC Bioinformatics*, **10**, 108.

Houghton AM, Mouded M, Shapiro SD (2008). Common origins of lung cancer and COPD. *Nat Med*, **14**, 1023-4.

Hussain SP, Harris CC (2007). Inflammation and cancer: an ancient link with novel potentials. *Int J Cancer*, **121**, 2373-80.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

John B, Enright AJ, Aravin A, et al (2004). Human MicroRNA targets. *PLoS Biol*, **2**, 363.

Kim M, Chen X, Chin LJ, et al (2014). Extensive sequence variation in the 3' untranslated region of the KRAS gene in lung and ovarian cancer cases. *Cell Cycle*, **13**.

Lagos-Quintana M, Rauhut R, Lendeckel W, et al (2001). Identification of novel genes coding for small expressed RNAs. *Science*, **294**, 853-8.

Lee G, Walser TC, Dubinett SM (2009). Chronic inflammation, chronic obstructive pulmonary disease, and lung cancer. *Curr Opin Pulm Med*, **15**, 303-7.

Lee J, Taneja V, Vassallo R (2012). Cigarette smoking and inflammation: cellular and molecular mechanisms. *J Dent Res*, **91**, 142-9.

Liu C, Zhang F, Li T, et al (2012). MirSNP, a database of polymorphisms altering miRNA target sites, identifies miRNA-related SNPs in GWAS SNPs and eQTLs. *BMC Genomics*, **13**, 661.

McMillan DH, Baglole CJ, Thatcher TH, et al (2011). Lung-targeted overexpression of the NF-kappaB member RelB inhibits cigarette smoke-induced inflammation. *Am J Pathol*, **179**, 125-33.

Mishra PJ1, Bertino JR (2009). MicroRNA polymorphisms: the future of pharmacogenomics, molecular epidemiology and individualized medicine. *Pharmacogenomics*, **10**, 399-416.

Ohnishi S, Ma N, Thanan R, et al (2013). DNA damage in inflammation-related carcinogenesis and cancer stem cells. *Oxid Med Cell Longev*, 387014.

Richardson K, Lai CQ, Parnell LD, et al (2011). A genome-wide survey for SNPs altering microRNA seed sites identifies functional candidates in GWAS. *BMC Genomics*, **12**, 504.

Saunders MA, Liang H, Li WH (2007). Human polymorphism at microRNAs and microRNA target sites. *Proc Natl Acad Sci USA*, **104**, 3300-5.

Shen N, Gong T, Wang JD, et al (2011). Cigarette smoke-induced pulmonary inflammatory responses are mediated by EGR-1/GGPPS/MAPK signaling. *Am J Pathol*, **178**, 110-8.

Song CQ, Zhang JH, Shi JC, et al (2014). Bioinformatic prediction of SNPs within miRNA binding sites of inflammatory genes associated with gastric cancer. *Asian Pac J Cancer Pre*, **15**, 937-43.

Tomari Y, Zamore PD (2005). Perspective: machines for RNAi. *Genes Dev*, **19**, 517-29.

Wu Y, Xiao Y, Ding X, et al (2011). A miR-200b/200c/429-binding site polymorphism in the 3' untranslated region of the AP-2alpha gene is associated with cisplatin resistance. *PLoS ONE*, **6**, 29043.

Yu H, Zhao H, Wang LE, et al (2011). An analysis of single nucleotide polymorphisms of 125 DNA repair genes in the Texas genome-wide association study of lung cancer with a replication for the XRCC4 SNPs. *DNA Repair*, **10**, 398-407.

Zhang W, Edwards A, Zhu D, et al (2012). miRNA-mediated relationships between Cis-SNP genotypes and transcript intensities in lymphocyte cell lines. *PLoS One*, **7**, 31429.

Zu Y, Ban J, Xia Z, et al (2013). Genetic variation in a miR-335 binding site in BIRC5 alters susceptibility to lung cancer in Chinese Han populations. *Biochem Biophys Res Commun*, **430**, 529-34.