外周血中*KCNMA1*基因甲基化水平与肺癌的发生和发展相关

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摘要:目的 探究外周血中*KCNMA1*基因的甲基化水平与肺癌的相关性。**方法** 应用基质辅助激光解吸电离飞行时间质谱(MALDI-TOF-MS)技术对285例肺癌患者、186例年龄、性别匹配的良性肺结节患者和278例匹配的健康对照外周血中*KCNMA1*基因上4个CpG位点甲基化水平进行半定量检测。使用Logistic回归模型校正协变量分析DNA甲基化水平与肺癌的相关性。使用Mann-Whitney *U*检验分析不同临床特征组别的甲基化水平差异。**结果** 在>55岁和女性人群中,KCNMA1_CpG_5甲基化水平最高四分位Q4 *vs*最低四分位Q1均与肺癌显著相关(>55岁人群:OR=2.60,95% *CI*: 1.25~5.41,*P*=0.011;女性人群:OR=2.09,95% *CI*: 1.03~4.26,*P*=0.042),且KCNMA1_CpG_5甲基化的相关性从Q2到Q4逐渐增强(*P*=0.003,0.038)。与良性患者相比,KCNMA1_CpG_5最高四分位Q4在男性肺癌患者中的OR值为0.35(95% *CI*: 0.16~0.79,*P*=0.012)。浸润性腺癌中KCNMA1_CpG_3甲基化水平显著低于非浸润性腺癌(*P*=0.028),较大肿瘤(T2-4)中KCNMA1_CpG_1甲基化水平显著高于较小肿瘤(T1)(*P*=0.021)。**结论** 外周血中*KCNMA1*甲基化水平的改变可能与肺癌发生和发展相关。
关键词:肺癌;*KCNMA1*;DNA甲基化;外周血;病例对照研究

Peripheral blood *KCNMA1* methylation level is associated with the occurrence and progression of lung cancer

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Abstract: Objective To explore the association of *KCNMA1* gene methylation levels in peripheral blood with lung cancer. **Methods** The methylation levels of 4 CpG sites in *KCNMA1* gene were quantitatively detected in 285 patients with lung cancer, 186 age- and sex-matched patients with benign pulmonary nodules and 278 matched healthy control subjects using mass spectrometry (MALDI-TOF-MS). The association of *KCNMA1* methylation levels with lung cancer was analyzed using logistic regression models adjusted for covariates. The *KCNMA1* methylation levels in different subgroups of lung cancer patients were compared using Mann-Whitney *U* test. **Results** In subjects over 55 years and in female subjects, the highest quartile (Q4) *vs* the lowest quartile (Q1) of KCNMA1_CpG_5 methylation levels were significantly correlated with lung cancer (for subjects over 55 years: OR=2.60, 95% *CI*: 1.25−5.41, *P*=0.011; for female subjects: OR=2.09, 95% *CI*: 1.03−4.26, *P*=0.042). From Q2 to Q4 of KCNMA1_CpG_5 methylation levels, their correlation with lung cancer became gradually stronger (*P*=0.003 and 0.038, respectively). In male subjects, the OR of Q4 of KCNMA1_CpG_5 methylation levels was 0.35 in patients with lung cancer as compared with patients with benign nodules (95% *CI*: 0.16 − 0.79, *P*=0.012). KCNMA1_CpG_3 methylation level was significantly lower in invasive adenocarcinoma than in noninvasive adenocarcinoma (*P*=0.028), and that of KCNMA1_CpG_1 was significantly higher in patients with larger tumors (T2-4) than in those with smaller tumors (T1) (*P*=0.021). **Conclusion** The change of peripheral blood *KCNMA1* methylation level is correlated with the occurrence and development of lung cancer.
Keywords: lung cancer; *KCNMA1*; DNA methylation; peripheral blood; case-control study

2020年癌症最新数据显示,肺癌的新发病例数约为220万,是全球第2大癌症,也是导致人类癌症死亡的主要原因[1,2]。肺癌主要分为非小细胞肺癌(NSCLC)和小细胞肺癌(SCLC)两类,其中NSCLC约占所有肺癌患者的85%以上[3]。肺癌的预后与其诊断时的分期密切相关,有研究显示,I期NSCLC患者的5年生存率可达70%~90%,而晚期患者(III/IV期)的生存率则很低[4,5]。目前很多患者在初次诊断时就已处于肺癌晚期,导致肺癌的治疗困难,预后差,生存期短,因此,肺癌的早期诊断至关重要[6]。目前广泛应用低剂量螺旋计算机断层扫描(LDCT)进行肺癌筛查,可提高肺癌的早期发现率,并可使其死亡率降低20%,但存在假阳性率过高、有辐射风险和过度诊断等问题[7,8],故迫切需要寻找更加准确、高效的生物标志物进行辅助诊断,提高肺癌早期诊断的精度。

DNA甲基化是癌症的早期事件和伴随事件,在癌症的发生和发展中起着重要作用[9,10],可被用作恶性肿瘤的早期诊断生物标志物[11]。既往已有很多研究报道了基因甲基化与肺癌之间的关联性。比如研究发现*HOXA9*、*KRTAP8-1*、*CCND1*和*TULP2*的甲基化水平有

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助于肺腺癌的早期识别[12]。*XXYLT1*在肺癌中呈现高甲基化，表达降低，其甲基化水平可能是肺癌风险增加的潜在生物标志物[13]。Weiss等[14]验证了血浆中*SHOX2*和*PTGER4*甲基化可用于检测肺癌并对非恶性疾病进行鉴别。我们此前的工作表明，外周血中*PNPLA2*、*FYB*、*RAPSN*、*SH3BP5*及*RPTOR*等基因甲基化的改变与早期肺癌显著相关[15-19]。此外，有学者对DNA甲基化作为早期肺癌检测手段的可行性也进行了评估[20]。

*KCNMA1*基因位于人染色体10q22.3上，编码大电导、电压和钙敏感的钾离子通道的成孔α亚基[21]。*KCNMA1*参与多种癌症发生和发展，但所起的作用不一致，可能与不同的癌症类型有关。*KCNMA1*在一些癌症中高表达，促进肿瘤的恶性行为。比如，*KCNMA1*的拷贝数扩增促进人前列腺癌细胞的增殖[22]，在脑转移性乳腺癌中高表达，且在乳腺癌的侵入和向脑转移中是必需的[23]。*KCNMA1*拷贝数在乳腺癌、卵巢癌、子宫内膜癌中扩增，且*KCNMA1*的扩增与乳腺癌的高分期、高分级、高肿瘤增殖性和弱预后相关，其增强表达与乳腺癌的高增殖性和恶性相关，且可导致乳腺癌的高增殖性和恶性[24]。但同样有研究表明，*KCNMA1*抑制癌症的生长。比如，*KCNMA1*经抑制*PTK2*的表达，诱导胃癌细胞的凋亡，抑制胃癌的生长；进一步观察发现，大多数胃癌组织中*KCNMA1*甲基化与缩短的生存显著相关[25]。

到目前为止关于*KCNMA1*基因甲基化与肺癌之间相关性的研究尚未见报道。本研究通过病例对照研究，分析外周血中*KCNMA1*甲基化水平与肺癌的相关性。

1 资料和方法

1.1 研究对象

2019~2020年在上海胸科医院纳入了285例肺癌患者（中位年龄55岁，29~80岁），其中I期患者232例，占81.4%和186名良性患者（中位年龄55岁，25~80岁）。患者的基本信息见表1。所有患者均在接受肺癌相关的治疗和手术之前采集其外周血样本，其肺癌患病情况均经胸外科手术及临床病理确诊。同时在2019~2020年从江苏省中医院体检中心收集年龄、性别匹配的健康对照278例（中位年龄54岁，23~80岁）。对照组无任何癌症或自身免疫疾病史，且血常规检查显示血细胞各亚型的组成均在正常参考范围内。本研究经中国上海市胸科医院伦理委员会批准（伦理审批编号：KS1407）。

表1 肺癌患者的临床特征

Tab.1 Clinical characteristics of lung cancer patients

Characteristics	Type	*n*	%
Age (year)	≤55	148	51.9
	>55	137	48.1
Gender	Male	142	49.8
	Female	143	50.2
Tumor subtype	Adenocarcinoma in situ	67	23.5
	Microinvasive adenocarcinoma	49	17.2
	Invasive adenocarcinoma	135	47.4
	Others	34	11.9
Tumor stage	I	232	81.4
	II	15	5.3
	III	23	8.1
	IV	3	1.0
	Missing	12	4.2
Tumor size	T1	218	76.5
	T2	38	13.3
	T3	14	4.9
	T4	1	0.4
	Missing	14	4.9
Lymph node involvement	pN0	242	84.9
	pN1	7	2.4
	pN2	21	7.4
	Missing	15	5.3
Tumor length (cm)	≤1	100	35.1
	>1	171	60.0
	Missing	14	4.9

1.2 样本处理和亚硫酸氢盐转化

采用含乙二胺四乙酸(EDTA)试管采集病例组和对照组的外周血样本,在−80 ℃下进行保存。使用DNA提取试剂盒(腾辰,南京)从外周血中提取基因组DNA,再用DNA甲基化检测样本前处理试剂盒(腾辰,南京)对DNA进行重亚硫酸氢盐转化处理。CpG位点的非甲基化胞嘧啶(C)碱基经处理后转化为尿嘧啶(U),而甲基化的胞嘧啶保持不变。肺癌患者组、良性患者组和健康对照组的样本均进行平行处理。

1.3 MALDI-TOF质谱分析

应用基质辅助激光解吸电离飞行时间质谱(MALDI-TOF-MS)(Agena Bioscience, San Diego, CA, USA)对外周血中*KCNMA1*基因的甲基化水平进行半定量检测。使用特异性引物对经亚硫酸氢盐转化后的DNA进行目的序列扩增。*KCNMA1*的聚合酶链式反应(PCR)引物对如下:正向引物:TGAGTTTGGTTATTTTGGAGGATT;反向引物:AACAAAAACAATAATAACCCTTCCC。在目的序列引物区域和CpG位点上均没有单核苷酸多态性(SNPs)。PCR产物经虾碱性磷酸酶[26]孵育、T7转录酶剪切和清洁树脂处理后,应用Nanodispenser RS1000(Agena Bioscience)转移到硅基靶板上,并由MassARRAY光谱分析仪检测,再通过EpiTyper v1.3软件进行可视化处理。EpiTyper v1.3软件共检测到*KCNMA1*扩增子中的4个质量峰,每个质量峰包含一个位点,分别代表了KCNMA1_CpG_1、KCNMA1_CpG_2、KCNMA1_CpG_3和KCNMA1_CpG_5的甲基化水平(图1)。

1.4 统计学分析

采用SPSS25.0软件进行数据分析。使用校正过年龄、性别和实验批次的二元Logistic回归模型对每一个CpG位点甲基化水平进行四分位分析,以甲基化值最低的四分位(quartile 1, Q1)一组作为参照,其余三组设虚拟变量进入回归模型;使用Mann-Whitney *U*检验分析肺癌患者同一临床特征经不同分组后的甲基化水平是否存在差异。所有统计分析均采用双侧检验,$P<0.05$时认为差异具有统计学意义。

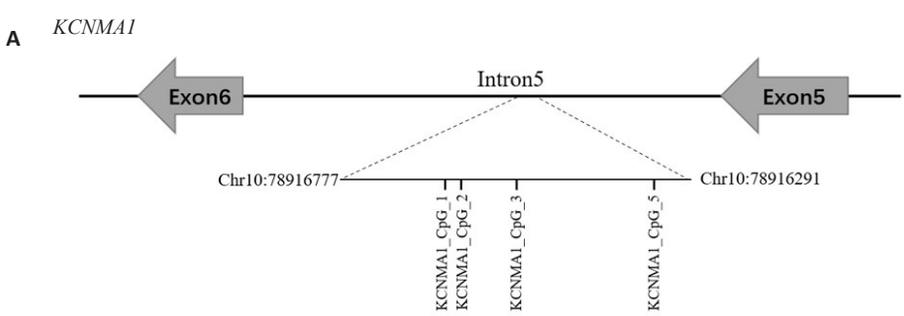
2 结果

2.1 外周血中*KCNMA1*甲基化水平

所有研究对象的*KCNMA1*甲基化水平均应用基质辅助激光解吸电离飞行时间质谱分析技术进行检测,以其中具有代表性的样本展示*KCNMA1*扩增子中CpG位点的质谱峰(图2)。

2.2 肺癌患者与健康对照外周血中*KCNMA1*甲基化水平的四分位分析

以每个CpG位点甲基化水平最低的四分位Q1作



B

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TGAGTCTGGCCACCCTGGAGGACTAGGGTTATCCTTCAAATCAGTCTCCC  50
CAAAGGCTAGGAGGTTAGGAGTTTTTCAGGATGGTTTGGTGGGCAGGGGG  100
CTAGGGAACAGGGAATGTTGATTGCTTGGGGGTAAAACCACAAGCGTATG  150
GAAAACAGTCCACTCGTGTTGAGTCCTCTGGGTGGGGACCACAGGACTGG  200
CTGAGTCATGAGTCTTGGGTCCAGGTGGGGTCAGTCACTCGCCAGAAATG  250
CCAGAAGTCTGAAAAACATCTCCCAGGGCCAGTCTTCAGTTCTACAGTAG  300
TGATGTTATTTACAAGAGTAATTGAGGAAGTTACAAATCTGTGACCTCCA  350
GAACAATGGCTGGTTATCATTTAACTGCGCTTATGTGTTAGCAGAATTCA  400
GGCACCTCTTATAACCCTAACCTTATGGCCTTTCGTTAGTTTTACAAGGA  450
CAATTTAGTTTTGGGAAGGGCTATTACTGTCCTTGCT
```

图1 *KCNMA1*基因的位点分布图和序列

Fig.1 Site map and sequence of *KCNMA1* gene. **A**: Position of *KCNMA1* amplified fragment (487 bp) and the 4 measurable CpG sites. **B**: *KCNMA1* amplicon sequence (chr10:78916291-chr10:78916777) detected by EpiTyper assay, with the 4 measurable CpG sites marked in grey.

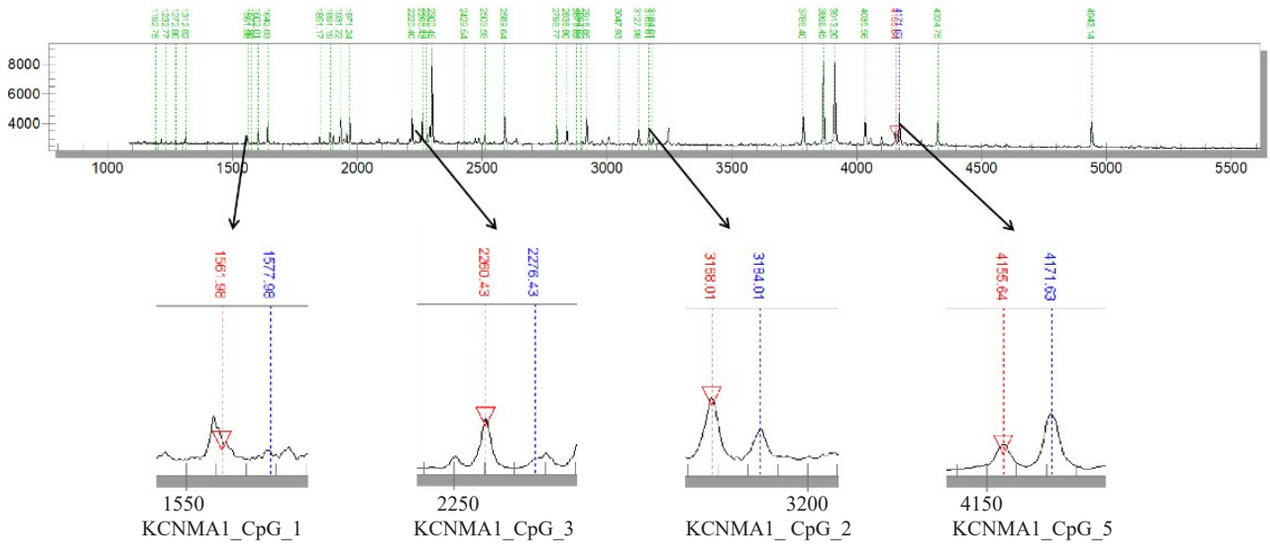

图2 *KCNMA1*扩增子的质谱峰

Fig.2 Mass peaks of *KCNMA1* amplicon. The MassARRAY assay yielded 4 distinguishable peaks. The downward red arrows pointing at the signal peak indicate completely non-methylated CpG site, and the blue line above the signal peak indicates the methylated CpG site. The sample mass of the signal peak is represented at the bottom.

为参照，采用校正过年龄、性别和实验批次的二元logistic回归模型，对甲基化水平较高的四分位（Q2-Q4）进行分析，其中KCNMA1_CpG_1由于甲基化水平整体较高，对其采用三分位分析。结果显示，*KCNMA1*4个CpG位点的甲基化水平与肺癌之间均无显著的相关性（*P*>0.05，表2）。

2.3 经年龄分层的肺癌患者与健康对照外周血中*KCNMA1*甲基化水平的四分位分析

以研究对象中位年龄（55岁）为界限分层，研究结果显示，在>55岁人群中，KCNMA1_CpG_5甲基化Q4 *vs* Q1与肺癌显著相关（OR=2.60，95% *CI*：1.25~5.41，*P*=0.011），且相关性从Q2~Q4逐渐增强，*P*=0.003。而在≤55岁组中，4个位点的甲基化均未显示出与肺癌的相关性（表3）。

2.4 经性别分层的肺癌患者与健康对照外周血中*KCNMA1*甲基化水平的四分位分析

表2 肺癌患者与健康对照外周血中*KCNMA1*甲基化的四分位分析

Tab.2 Interquartile analysis of peripheral blood *KCNMA1* methylation levels in lung cancer patients and healthy control subjects

CpG sites	Quartiles (methylation)	Healthy controls *n* (%)	Lung cancer cases *n* (%)	OR (95% *CI*)*	*P**
KCNMA1_CpG_1	Q1 (≤0.70)	72 (25.9%)	74 (26.0%)	1.00	-
	Q2 (0.71-0.86)	68 (24.5%)	65 (22.8%)	0.93 (0.58-1.49)	0.750
	Q3 (≥0.87)	138 (49.6%)	146 (51.2%)	1.06 (0.68-1.63)	0.807
	P for trend				0.757
KCNMA1_CpG_2	Q1 (≤0.25)	78 (28.1%)	77 (27.0%)	1.00	-
	Q2 (0.26-0.31)	70 (25.2%)	70 (24.6%)	1.01 (0.64-1.59)	0.979
	Q3 (0.32-0.39)	64 (23.0%)	77 (27.0%)	1.19 (0.75-1.88)	0.462
	Q4 (≥0.40)	66 (23.7%)	61 (21.4%)	0.91 (0.57-1.46)	0.696
	P for trend				0.918
KCNMA1_CpG_3	Q1 (≤0.24)	70 (25.2%)	73 (25.6%)	1.00	-
	Q2 (0.25-0.31)	70 (25.2%)	83 (29.1%)	1.14 (0.72-1.79)	0.584
	Q3 (0.32-0.38)	71 (25.5%)	59 (20.7%)	0.79 (0.49-1.28)	0.337
	Q4 (≥0.39)	67 (24.1%)	70 (24.6%)	0.99 (0.62-1.58)	0.951
	P for trend				0.595
KCNMA1_CpG_5	Q1 (≤0.65)	78 (28.0%)	59 (20.7%)	1.00	-
	Q2 (0.66-0.72)	69 (24.8%)	80 (28.1%)	1.58 (0.98-2.53)	0.058
	Q3 (0.73-0.78)	63 (22.7%)	72 (25.2%)	1.59 (0.98-2.59)	0.060
	Q4 (≥0.79)	68 (24.5%)	74 (26.0%)	1.49 (0.92-2.41)	0.105
	P for trend				0.136

*Logistic regression, adjusted for age, gender and different batches.

表3 经年龄分层的肺癌患者与健康对照外周血中*KCNMA1*甲基化的四分位分析

Tab.3 Age-stratified interquartile analysis of peripheral blood *KCNMA1* methylation level in lung cancer patients and healthy control subjects

CpG sites	Quartiles (methylation)	Healthy controls [*n* (%)]	Lung cancer cases [*n* (%)]	OR (95% *CI*)*	*P**
≤55 years old (148 lung cancer cases *vs* 159 healthy controls)					
KCNMA1_CpG_1	Q1 (≤0.67)	41 (25.8%)	27 (18.3%)	1.00	-
	Q2 (0.68-0.83)	40 (25.1%)	35 (23.6%)	1.33 (0.68-2.60)	0.402
	Q3 (≥0.84)	78 (49.1%)	86 (58.1%)	1.77 (0.94-3.31)	0.075
	P for trend				0.072
KCNMA1_CpG_2	Q1 (≤0.25)	49 (30.8%)	46 (31.1%)	1.00	-
	Q2 (0.26-0.30)	37 (23.3%)	32 (21.6%)	0.93 (0.50-1.74)	0.822
	Q3 (0.31-0.39)	37 (23.3%)	42 (28.4%)	1.22 (0.67-2.22)	0.519
	Q4 (≥0.40)	36 (22.6%)	28 (18.9%)	0.84 (0.44-1.60)	0.592
	P for trend				0.876
KCNMA1_CpG_3	Q1 (≤0.25)	44 (27.7%)	43 (29.0%)	1.00	-
	Q2 (0.26-0.31)	38 (23.9%)	37 (25.0%)	1.00 (0.54-1.86)	0.999
	Q3 (0.32-0.37)	38 (23.9%)	26 (17.6%)	0.70 (0.37-1.35)	0.289
	Q4 (≥0.38)	39 (24.5%)	42 (28.4%)	1.11 (0.60-2.04)	0.743
	P for trend				0.992
KCNMA1_CpG_5	Q1 (≤0.68)	43 (27.0%)	47 (31.8%)	1.00	-
	Q2 (0.69-0.74)	40 (25.2%)	43 (29.0%)	0.97 (0.53-1.78)	0.929
	Q3 (0.75-0.80)	47 (29.6%)	39 (26.4%)	0.75 (0.41-1.38)	0.359
	Q4 (≥0.81)	29 (18.2%)	19 (12.8%)	0.60 (0.29-1.23)	0.165
	P for trend				0.127
>55 years old (137 lung cancer cases *vs* 119 healthy controls)					
KCNMA1_CpG_1	Q1 (≤0.75)	31 (26.0%)	49 (35.8%)	1.00	-
	Q2 (0.76-0.90)	34 (28.6%)	32 (23.3%)	0.60 (0.30-1.17)	0.132
	Q3 (≥0.91)	54 (45.4%)	56 (40.9%)	0.66 (0.35-1.25)	0.198
	P for trend				0.225
KCNMA1_CpG_2	Q1 (≤0.26)	34 (28.6%)	37 (27.0%)	1.00	-
	Q2 (0.27-0.32)	27 (22.7%)	35 (25.5%)	1.18 (0.59-2.35)	0.639
	Q3 (0.33-0.40)	30 (25.2%)	33 (24.1%)	0.99 (0.50-1.97)	0.985
	Q4 (≥0.41)	28 (23.5%)	32 (23.4%)	1.06 (0.53-2.13)	0.860
	P for trend				0.974
KCNMA1_CpG_3	Q1 (≤0.24)	32 (26.9%)	35 (25.5%)	1.00	-
	Q2 (0.25-0.32)	30 (25.2%)	43 (31.4%)	1.28 (0.66-2.51)	0.468
	Q3 (0.33-0.39)	28 (23.5%)	32 (23.4%)	1.05 (0.52-2.11)	0.895
	Q4 (≥0.40)	29 (24.4%)	27 (19.7%)	0.85 (0.42-1.73)	0.653
	P for trend				0.567
KCNMA1_CpG_5	Q1 (≤0.62)	31 (26.0%)	23 (16.8%)	1.00	-
	Q2 (0.63-0.70)	34 (28.6%)	27 (19.7%)	1.17 (0.55-2.49)	0.685
	Q3 (0.71-0.76)	25 (21.0%)	36 (26.3%)	2.11 (0.99-4.52)	0.054
	Q4 (≥0.77)	29 (24.4%)	51 (37.2%)	2.60 (1.25-5.41)	**0.011**
	P for trend				**0.003**

*Logistic regression, adjusted for age, gender and different batches.

在女性中，KCNMA1_CpG_5甲基化值Q4 *vs* Q1在肺癌患者和健康对照之间具有显著差异（OR=2.09，95% *CI*：1.03~4.26，*P*=0.042），且甲基化水平升高与肺癌相关（*P*=0.038）。而在男性中，4个位点的甲基化均未观察到与肺癌的相关性（表4）。

2.5 肺癌患者与良性患者外周血中*KCNMA1*甲基化水平的四分位分析

以甲基化值最低的四分位Q1为参照，使用校正了年龄、性别和实验批次的二元logistic回归模型分析结果显示，各CpG位点甲基化水平在肺癌患者及良性患者间无显著差异（表5）。

2.6 经年龄、性别分层的肺癌患者与良性患者外周血中*KCNMA1*甲基化水平的四分位分析

分别对年龄和性别进行分层，结果显示，在≤55岁和>55岁人群中均未观察到和肺癌相关的*KCNMA1*位点（表6）。在男性中，以最低四分位Q1为参照，KCNMA1_CpG_5的最高四分位Q4的OR值为0.35（95% *CI*: 0.16~0.79，*P*=0.012），而在女性人群中4个位

表4 经性别分层的肺癌患者与健康对照外周血中*KCNMA1*甲基化的四分位分析

Tab.4 Gender-stratified interquartile analysis of peripheral blood *KCNMA1* methylation level in lung cancer patients and healthy control subjects

CpG sites	Quartiles (methylation)	Healthy controls [*n* (%)]	Lung cancer cases [*n* (%)]	OR (95% *CI*)*	*P**
Female (143 lung cancer cases *vs* 136 healthy controls)					
KCNMA1_CpG_1	Q1 (≤0.70)	35 (25.7%)	36 (25.2%)	1.00	-
	Q2 (0.71-0.90)	39 (28.7%)	52 (36.4%)	1.23 (0.65-2.31)	0.522
	Q3 (≥0.91)	62 (45.6%)	55 (38.4%)	0.79 (0.42-1.49)	0.470
	P for trend				0.390
KCNMA1_CpG_2	Q1 (≤0.26)	39 (28.7%)	42 (29.3%)	1.00	-
	Q2 (0.27-0.31)	29 (21.3%)	30 (21.0%)	0.95 (0.49-1.87)	0.889
	Q3 (0.32-0.40)	35 (25.7%)	41 (28.7%)	1.04 (0.55-1.96)	0.901
	Q4 (≥0.41)	33 (24.3%)	30 (21.0%)	0.82 (0.42-1.59)	0.550
	P for trend				0.652
KCNMA1_CpG_3	Q1 (≤0.24)	36 (26.5%)	31 (21.7%)	1.00	-
	Q2 (0.25-0.32)	35 (25.7%)	51 (35.6%)	1.68 (0.88-3.22)	0.114
	Q3 (0.33-0.39)	32 (23.5%)	31 (21.7%)	1.11 (0.55-2.21)	0.774
	Q4 (≥0.40)	33 (24.3%)	30 (21.0%)	1.01 (0.50-2.03)	0.975
	P for trend				0.690
KCNMA1_CpG_5	Q1 (≤0.65)	34 (25.0%)	25 (17.5%)	1.00	-
	Q2 (0.66-0.72)	38 (27.9%)	37 (25.9%)	1.46 (0.72-2.94)	0.290
	Q3 (0.73-0.79)	34 (25.0%)	39 (27.3%)	1.76 (0.86-3.57)	0.119
	Q4 (≥0.80)	30 (22.1%)	42 (29.3%)	2.09 (1.03-4.26)	**0.042**
	P for trend				**0.038**
Male (142 lung cancer cases *vs* 142 healthy controls)					
KCNMA1_CpG_1	Q1 (≤0.70)	37 (26.1%)	38 (26.8%)	1.00	-
	Q2 (0.71-0.85)	34 (23.9%)	24 (16.9%)	0.72 (0.36-1.45)	0.351
	Q3 (≥0.86)	71 (50.0%)	80 (56.3%)	1.21 (0.64-2.28)	0.550
	P for trend				0.442
KCNMA1_CpG_2	Q1 (≤0.24)	38 (26.8%)	36 (25.4%)	1.00	-
	Q2 (0.25-0.30)	35 (24.6%)	36 (25.4%)	1.10 (0.57-2.11)	0.784
	Q3 (0.31-0.37)	35 (24.6%)	36 (25.4%)	1.09 (0.57-2.10)	0.792
	Q4 (≥0.38)	34 (24.0%)	34 (23.8%)	1.07 (0.55-2.09)	0.846
	P for trend				0.851
KCNMA1_CpG_3	Q1 (≤0.25)	42 (29.6%)	46 (32.4%)	1.00	-
	Q2 (0.26-0.30)	31 (21.8%)	29 (20.4%)	0.85 (0.44-1.64)	0.623
	Q3 (0.31-0.37)	34 (23.9%)	30 (21.1%)	0.81 (0.42-1.55)	0.522
	Q4 (≥0.38)	35 (24.7%)	37 (26.1%)	0.97 (0.52-1.82)	0.926
	P for trend				0.857
KCNMA1_CpG_5	Q1 (≤0.64)	36 (25.4%)	27 (19.0%)	1.00	-
	Q2 (0.65-0.72)	39 (27.5%)	50 (35.2%)	1.78 (0.92-3.48)	0.089
	Q3 (0.73-0.78)	34 (23.9%)	37 (26.1%)	1.52 (0.75-3.06)	0.245
	Q4 (≥0.79)	33 (23.2%)	28 (19.7%)	1.18 (0.57-2.42)	0.658
	P for trend				0.878

*Logistic regression, adjusted for age and different batches.

点与肺癌均无相关性(表7)。

2.7 *KCNMA1*甲基化与肺癌临床特征之间的相关性

结果显示,浸润性腺癌中KCNMA1_CpG_3甲基化水平显著低于非浸润性腺癌(中位数:0.29 *vs* 0.31,*P*=0.028)。与在较小肿瘤(T1)中相比,KCNMA1_CpG_1在较大肿瘤(T2-4)中的甲基化水平显著升高(T2-4 0.95 *vs* T1 0.86,*P*=0.021)。所有位点甲基化水平与其他临床特征(肿瘤长度、肿瘤分期和淋巴转移)均未显现相关性(表8)。

表5 肺癌患者与良性患者外周血中*KCNMA1*甲基化的四分位分析

Tab.5 Interquartile analysis of peripheral blood *KCNMA1* methylation level in patients with lung cancer (*n*=285) and benign pulmonary nodules (*n*=186)

CpG sites	Quartiles (methylation)	Benign cases [*n* (%)]	Lung cancer cases [*n* (%)]	OR (95% *CI*)*	*P**
KCNMA1_CpG_1	Q1 (≤0.66)	46 (24.7%)	61 (21.4%)	1.00	-
	Q2 (0.67-0.88)	50 (26.9%)	96 (33.7%)	1.48 (0.88-2.49)	0.142
	Q3 (≥0.89)	90 (48.4%)	128 (44.9%)	1.12 (0.67-1.87)	0.668
	P for trend				0.864
KCNMA1_CpG_2	Q1 (≤0.25)	49 (26.3%)	77 (27.0%)	1.00	-
	Q2 (0.26-0.32)	47 (25.3%)	80 (28.1%)	1.07 (0.64-1.78)	0.798
	Q3 (0.33-0.38)	44 (23.7%)	61 (21.4%)	0.87 (0.51-1.48)	0.612
	Q4 (≥0.39)	46 (24.7%)	67 (23.5%)	0.91 (0.54-1.54)	0.733
	P for trend				0.575
KCNMA1_CpG_3	Q1 (≤0.24)	47 (25.3%)	73 (25.6%)	1.00	-
	Q2 (0.25-0.31)	53 (28.5%)	83 (29.1%)	0.99 (0.59-1.64)	0.954
	Q3 (0.32-0.38)	41 (22.0%)	59 (20.7%)	0.91 (0.53-1.56)	0.723
	Q4 (≥0.39)	45 (24.2%)	70 (24.6%)	1.00 (0.59-1.69)	0.997
	P for trend				0.923
KCNMA1_CpG_5	Q1 (≤0.67)	50 (26.9%)	80 (28.1%)	1.00	-
	Q2 (0.68-0.73)	49 (26.3%)	75 (26.3%)	0.96 (0.58-1.59)	0.878
	Q3 (0.74-0.80)	41 (22.1%)	83 (29.1%)	1.27 (0.76-2.14)	0.363
	Q4 (≥0.81)	46 (24.7%)	47 (16.5%)	0.63 (0.36-1.09)	0.098
	P for trend				0.293

*Logistic regression, adjusted for age, gender and different batches.

3 讨论

目前尚无关于*KCNMA1*甲基化与肺癌之间相关性的研究报道。本研究对肺癌患者*vs*年龄、性别匹配的健康对照的分析表明，在>55岁以及在女性人群中，KCNMA1_CpG_5甲基化水平与肺癌显著相关。癌症的早筛和相应的早治疗对于提高癌症(特别是预后较差的肺癌)的疗效和改善预后具有重要的意义。为此，本研究收集和分析了高比例(占肺癌病例数的81.4%)的I期肺癌病例。本研究结果将为基于外周血DNA甲基化筛查早期肺癌提供实验依据。

癌症的发生与衰老相关[27]。高龄相关的衰老和癌症均会发生甲基化改变等表观遗传学事件[28, 29]。研究报道发现癌症相关的DNA甲基化并未表现出明显的高甲基化或低甲基化趋势，而衰老相关的变化则更倾向于DNA高甲基化[29]。在本研究中，与健康对照组相比，KCNMA1_CpG_5的高甲基化在>55岁的人群中与肺癌显著相关，而在≤55岁人群中并未表现出相关性，我们的结果与Pérez等[29]的研究中所描述的现象一致。我们此前的一些研究中发现，*RAPSN*低甲基化与肺癌风险增加相关，且在年龄较大的人群中更为明显；*SH3BP5*甲基化水平升高与肺癌患者年龄增加相关；*PNPLA2*低甲基化与肺癌的关联仅表现在年轻人群中，表明年龄是影响人外周血中DNA甲基化水平及其与肺癌相关性的重要因素[15-18]，我们推测不同基因由于功能不同其甲基化水平变化受年龄的影响也存在差异，本研究的结果进一步验证了与衰老相关的较高年龄可能对DNA甲基化与肺癌相关性产生影响，但其具体机制还有待后续更深入的研究。

本研究中，与健康对照组相比，KCNMA1_CpG_5的高甲基化只在女性中呈现出与肺癌的相关性，而与良性患者相比，我们发现KCNMA1_CpG_5最高四分位甲基化在男性中与肺癌相关。这可能与女性、男性中独特的性激素(雌激素/雄激素，及其调控的信号通路)等和肺癌的相关性以及对相关基因的甲基化的影响有关。比如，多项研究表明，女性激素(雌激素和孕激素)参与肺癌的发生和发展[30-32]；研究显示雌激素信号传导在肺上皮和间充质中都起着重要作用，并且雌激素可能潜在地促进肺癌[31]。在不吸烟者的肺癌中，一些与雌激素功能和*MAPK/PI3K*信号通路相关的蛋白等在不吸烟女性和男性肺癌进展中表现出不同的影响[33]，可能影响到肺癌相关基因的甲基化和相应的功能。研究报道发现某些基因的甲基化水平与吸烟密切相关，并可能影响基因的表达[34, 35]，因此我们推测*KCNMA1*甲基化水平在男性和女性之间的差异也可能是与其生活习惯(如吸烟、饮酒等行为)的不同有关，但由于本研究样本来源于医院，缺少吸烟史等生活方式的数据，未来需要在更大样本前

表6 经年龄分层的肺癌患者与良性患者外周血中*KCNMA1*甲基化的四分位分析

Tab.6 Age-stratified interquartile analysis of peripheral blood *KCNMA1* methylation level in patients with lung cancer and benign pulmonary nodules

CpG sites	Quartiles (methylation)	Benign cases [*n* (%)]	Lung cancer cases [*n* (%)]	OR (95% *CI*)*	*P**
≤55 years old (148 lung cancer cases and 95 benign cases)					
KCNMA1_CpG_1	Q1 (≤0.70)	24 (25.3%)	33 (22.3%)	1.00	-
	Q2 (0.71-0.88)	24 (25.3%)	47 (31.8%)	1.42 (0.69-2.95)	0.344
	Q3 (≥0.89)	47 (49.4%)	68 (45.9%)	1.06 (0.53-2.11)	0.871
	P for trend				0.984
KCNMA1_CpG_2	Q1 (≤0.25)	24 (25.3%)	46 (31.1%)	1.00	-
	Q2 (0.26-0.33)	28 (29.5%)	49 (33.1%)	0.89 (0.45-1.76)	0.738
	Q3 (0.34-0.38)	20 (21.0%)	23 (15.5%)	0.59 (0.27-1.30)	0.190
	Q4 (≥0.39)	23 (24.2%)	30 (20.3%)	0.66 (0.31-1.38)	0.269
	P for trend				0.167
KCNMA1_CpG_3	Q1 (≤0.25)	26 (27.4%)	43 (29.1%)	1.00	-
	Q2 (0.26-0.31)	24 (25.3%)	37 (25.0%)	0.91 (0.45-1.87)	0.805
	Q3 (0.32-0.40)	23 (24.2%)	37 (25.0%)	0.97 (0.47-1.98)	0.928
	Q4 (≥0.41)	22 (23.1%)	31 (20.9%)	0.82 (0.39-1.71)	0.595
	P for trend				0.657
KCNMA1_CpG_5	Q1 (≤0.67)	25 (26.3%)	41 (27.7%)	1.00	-
	Q2 (0.68-0.73)	24 (25.3%)	43 (29.0%)	1.08 (0.53-2.20)	0.827
	Q3 (0.74-0.81)	26 (27.4%)	47 (31.8%)	1.09 (0.54-2.19)	0.811
	Q4 (≥0.82)	20 (21.0%)	17 (11.5%)	0.50 (0.22-1.15)	0.103
	P for trend				0.219
>55 years old (137 lung cancer cases and 91 benign cases)					
KCNMA1_CpG_1	Q1 (≤0.65)	24 (26.4%)	34 (24.8%)	1.00	-
	Q2 (0.66-0.88)	24 (26.4%)	43 (31.4%)	1.23 (0.59-2.59)	0.582
	Q3 (≥0.89)	43 (47.2%)	60 (43.8%)	1.02 (0.49-2.15)	0.954
	P for trend				0.990
KCNMA1_CpG_2	Q1 (≤0.25)	25 (27.5%)	31 (22.6%)	1.00	-
	Q2 (0.26-0.31)	22 (24.2%)	33 (24.1%)	1.19 (0.56-2.55)	0.648
	Q3 (0.32-0.39)	24 (26.4%)	40 (29.2%)	1.30 (0.62-2.70)	0.489
	Q4 (≥0.40)	20 (22.0%)	33 (24.1%)	1.33 (0.62-2.86)	0.469
	P for trend				0.442
KCNMA1_CpG_3	Q1 (≤0.24)	25 (27.5%)	35 (25.5%)	1.00	-
	Q2 (0.25-0.30)	21 (23.1%)	37 (27.0%)	1.25 (0.59-2.66)	0.555
	Q3 (0.31-0.36)	25 (27.5%)	30 (21.9%)	0.86 (0.41-1.82)	0.700
	Q4 (≥0.37)	20 (22.0%)	35 (25.5%)	1.29 (0.61-2.76)	0.507
	P for trend				0.753
KCNMA1_CpG_5	Q1 (≤0.67)	25 (27.5%)	39 (28.5%)	1.00	-
	Q2 (0.68-0.72)	22 (24.2%)	25 (18.2%)	0.71 (0.33-1.53)	0.381
	Q3 (0.73-0.80)	23 (25.3%)	45 (32.8%)	1.35 (0.66-2.80)	0.413
	Q4 (≥0.81)	21 (23.1%)	28 (20.4%)	0.89 (0.41-1.92)	0.759
	P for trend				0.821

*Logistic regression, adjusted for age, gender and different batches.

瞻性队列中进一步研究。

在既往有关*KCNMA1*甲基化与多种癌症的研究中，并未关注到其与临床特征之间的联系，本研究针对临床特征进行比较，发现*KCNMA1*一些位点甲基化水平在不同亚型腺癌和不同大小的肿瘤中存在差异。KCNMA1_CpG_3在浸润性腺癌中甲基化水平显著低于在非浸润性腺癌中的水平，KCNMA1_CpG_1在较大肿瘤（T2-4）中的甲基化水平显著高于较小肿瘤中的水平，提示*KCNMA1*的一些位点的甲基化水平的改变可能有助于进一步区分浸润性和非浸润性腺癌以及较大和较小的肺癌，在肺癌早筛中提供更完整更有价值的信息。同样需要在前瞻性大样本量的临床研究中进一步

表7 经性别分层的肺癌患者与良性患者外周血中*KCNMA1*甲基化的四分位分析

Tab.7 Gender-stratified interquartile analysis of peripheral blood *KCNMA1* methylation level in patients with lung cancer and benign pulmonary nodules

CpG sites	Quartiles (methylation)	Benign cases [*n* (%)]	Lung cancer cases [*n* (%)]	OR (95% *CI*) *	*P**
Female (143 lung cancer cases and 86 benign cases)					
KCNMA1_CpG_1	Q1 (≤0.64)	21 (24.4%)	26 (18.2%)	1.00	-
	Q2 (0.65-0.89)	24 (27.9%)	59 (41.3%)	1.90 (0.89-4.06)	0.096
	Q3 (≥0.90)	41 (47.7%)	58 (40.6%)	1.06 (0.49-2.31)	0.881
	P for trend				0.857
KCNMA1_CpG_2	Q1 (≤0.26)	24 (27.9%)	42 (29.4%)	1.00	-
	Q2 (0.27-0.32)	20 (23.3%)	36 (25.2%)	0.98 (0.46-2.07)	0.957
	Q3 (0.33-0.38)	21(24.4%)	30 (21.0%)	0.81 (0.38-1.73)	0.590
	Q4 (≥0.39)	21(24.4%)	35 (24.5%)	0.92 (0.44-1.93)	0.828
	P for trend				0.719
KCNMA1_CpG_3	Q1 (≤0.25)	23 (26.7%)	37 (25.9%)	1.00	-
	Q2 (0.26-0.30)	21 (24.4%)	33 (23.1%)	1.01 (0.47-2.18)	0.971
	Q3 (0.31-0.36)	22 (25.6%)	32 (22.4%)	0.92 (0.43-1.97)	0.840
	Q4 (≥0.37)	20 (23.3%)	41 (28.7%)	1.31 (0.62-2.78)	0.478
	P for trend				0.545
KCNMA1_CpG_5	Q1 (≤0.67)	25 (29.1%)	34 (23.8%)	1.00	-
	Q2 (0.68-0.71)	18 (20.9%)	20 (14.0%)	0.83 (0.36-1.89)	0.659
	Q3 (0.72-0.80)	22 (25.6%)	58 (40.6%)	2.02 (0.97-4.18)	0.059
	Q4 (≥0.81)	21 (24.4%)	31 (21.7%)	1.10 (0.51-2.36)	0.814
	P for trend				0.335
Male (142 lung cancer cases and 100 benign cases)					
KCNMA1_CpG_1	Q1 (≤0.71)	25 (25.0%)	38 (26.8%)	1.00	-
	Q2 (0.72-0.87)	25 (25.0%)	31 (21.8%)	0.85 (0.41-1.80)	0.678
	Q3 (≥0.88)	50 (50.0%)	73 (51.4%)	1.09 (0.54-2.18)	0.816
	P for trend				0.750
KCNMA1_CpG_2	Q1 (≤0.25)	29 (29.0%)	40 (28.2%)	1.00	-
	Q2 (0.26-0.31)	22 (22.0%)	35 (24.6%)	1.16 (0.56-2.37)	0.692
	Q3 (0.32-0.38)	24 (24.0%)	35 (24.6%)	1.06 (0.52-2.16)	0.864
	Q4 (≥0.39)	25 (25.0%)	32 (22.5%)	0.95 (0.46-1.94)	0.885
	P for trend				0.865
KCNMA1_CpG_3	Q1 (≤0.24)	29 (29.0%)	42 (29.6%)	1.00	-
	Q2 (0.25-0.31)	24 (24.0%)	37 (26.1%)	1.05 (0.52-2.12)	0.886
	Q3 (0.32-0.39)	24 (24.0%)	33 (23.2%)	0.95 (0.47-1.93)	0.893
	Q4 (≥0.40)	23 (23.0%)	30 (21.1%)	0.90 (0.44-1.86)	0.785
	P for trend				0.745
KCNMA1_CpG_5	Q1 (≤0.67)	25 (25.0%)	46 (32.4%)	1.00	-
	Q2 (0.68-0.73)	28 (28.0%)	40 (28.2%)	0.78 (0.39-1.55)	0.479
	Q3 (0.74-0.80)	22 (22.0%)	40 (28.2%)	0.99 (0.48-2.03)	0.980
	Q4 (≥0.81)	25 (25.0%)	16 (11.3%)	0.35 (0.16-0.79)	**0.012**
	P for trend				0.050

*Logistic regression, adjusted for age and different batches.

验证这个结果。

综上所述，本研究表明在>55岁以及在女性人群中，外周血中KCNMA1_CpG_5甲基化水平与肺癌显著相关，为开发基于外周血中DNA甲基化的肺癌诊断的生物标志物提供实验证据，但*KCNMA1*基因甲基化与肺癌间相关关系的具体机制还有待后续的进一步研究。

表8 *KCNMA1*甲基化与肺癌患者临床特征的关联分析

Tab.8 Correlation between *KCNMA1* methylation and the clinical characteristics of lung cancer patients

Clinical characteristics	Group (*n*)	Median of methylation levels				
		KCNMA1_CpG_1	KCNMA1_CpG_2	KCNMA1_CpG_3	KCNMA1_CpG_4	KCNMA1_CpG_5
Adenocarcinoma subtypes	Noninvasive adenocarcinoma (*n*=116)	0.88	0.30	0.31	0.74	0.74
	Invasive adenocarcinoma (*n*=135)	0.87	0.32	0.29	0.65	0.72
	*P**	0.878	0.698	**0.028**	0.226	0.058
Tumor size	T1 (*n*=218)	0.86	0.31	0.30	0.74	0.73
	T2 & T3 & T4 (*n*=53)	0.95	0.31	0.32	0.65	0.72
	*P**	**0.021**	0.762	0.635	0.484	0.880
Tumor length (cm)	≤1 (*n*=100)	0.87	0.32	0.31	0.74	0.73
	>1 (*n*=171)	0.88	0.30	0.30	0.65	0.73
	*P**	0.320	0.182	0.381	0.383	0.447
Tumor stage	Stage I (*n*=232)	0.87	0.31	0.30	0.74	0.73
	Stage II & III & IV (*n*=41)	0.87	0.31	0.32	0.65	0.73
	*P**	0.661	0.734	0.634	0.486	0.908
Lymph node (LN) involvement	pN0 (*n*=242)	0.87	0.31	0.30	0.74	0.73
	pN1 & pN2 (*n*=28)	0.90	0.30	0.31	0.65	0.74
	*P**	0.466	0.653	0.998	0.660	0.885

*Mann-Whitney *U* test.

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