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·临床研究·

青岛地区汉族人群中lncRNA H19的多态性与胃癌和EBV相关胃癌易感性的关系

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[摘 要] **目的**:探讨青岛地区汉族人群lncRNA H19单核苷酸多态性(SNP)与胃癌和EBV相关胃癌(EBVaGC)易感性的关系。**方法**:收集青岛地区汉族人群2015年1月至2018年10月青岛大学附属医院经病理科确诊为胃癌的新鲜组织或陈旧的石蜡包埋胃癌组织病理标本共225例,为胃癌组;依据原位杂交法对EBV编码的小分子非多聚腺苷酸(EBER1)转录检测结果再将胃癌组分为2亚组:EBVaGC组70例,EBVnGC组155例;同时选择青岛大学附属医院门诊健康体检者200例为对照组。提取EBVaGC、EBVnGC组织及健康人群外周血标本的DNA,根据HaploView软件常规设置原则(MAF>0.05;r^2>0.8)筛选出rs217727、rs2735971、rs2839698和rs3741216四个H19的TagSNPs。利用Taq-Man MGB等位基因分型试剂盒对各SNP位点基因进行基因分型,并进行基因多态性检测。**结果**:所取标本的H19 SNPs均符合Hardy-Weinberg平衡。与对照组比较,胃癌组H19 rs217727位点TT基因型的发病风险显著增加(χ^2=9.073, *P*=0.003, *OR*=1.999, 95% CI=1.271～3.143),等位基因T的分布也明显增高(χ^2=13.475, *P*=0.001, *OR*=1.661, 95% CI=1.266～2.180);H19 rs2839698位点TC、CC基因型人群可显著增加胃癌的发病风险(χ^2=9.407, *P*=0.002; χ^2=6.517, *P*=0.011),携带C等位基因人群罹患胃癌的风险明显增加(χ^2=6.163, *P*=0.013, *OR*=1.417, 95% CI=1.076~1.867; χ^2=9.542, *P*=0.02, *OR*=2.070, 95% CI=1.298~3.302)。但胃癌组H19 rs2735971和rs3741216位点基因多态性与对照组比较差异不明显(均*P*>0.05);EBVaGC和EBVnGC组中H19的4个位点基因多态性分布差异均无统计学意义(均*P*>0.05)。**结论**:H19 rs217727、rs2839698基因多态性可能与胃癌发病风险有关,携带TT基因型C等位基因和人群胃癌的发病风险明显升高;H19 SNP的多态性与EBVaGC的发病风险无明显相关。

[关键词] 胃癌;长链非编码RNA;H19;EB病毒;单核苷酸多态性;易感性
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Association between lncRNA H19 polymorphisms and susceptibility to gastric carcinoma and EBV-associated gastric carcinoma in Han population in Qingdao

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[Abstract] Objective: To evaluate the potential associations between the single nucleotide polymorphisms (SNPs) of lncRNA H19 genes and the susceptibility to gastric cancer (GC), especially to EBV-associated gastric cancer (EBVaGC) in Han population in Qingdao. **Methods:** 225 cases of pathologically confirmed fresh gastric cancer tissues or paraffin embedded gastric cancer tissues during January 2015 and October 2018 were collected from Affiliated Hospital of Qingdao University, as GC group; in the meanwhile, 200 healthy people underwent physical examination at Outpatient were collected as control. The 225 cases of cancer tissues were assigned into two groups according to the transcription result of EBV-encoded small molecule non-polyadenylation (EBER 1) by In situ hybridization: EBVaGC group (70 cases) and EBVnGC group (155 cases). DNA was extracted from the tissues of two groups and peripheral blood of healthy participants. According to the general setting of HaploView software (MAF>0.05, r^2>0.8), four TagSNPs (rs217727, rs2735971, rs2839698 and rs3741216) of H19 were screened. Genotyping of each SNP locus was carried out by using Taq-Man MGB allele typing kit, and the gene polymorphisms were examined. **Results:** H19 SNPs of collected tissue samples were in accordance with the Hardy-Weinberg equilibrium. Compared with control group, patients carrying TT genotype of H19 rs217727 loci had significantly increased

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susceptibility to gastric cancer (χ^2=9.073, *P*=0.003, *OR*=1.999, 95% CI=1.271-3.143), so did the T allele (χ^2=13.475, *P*=0.001, *OR*=1.661, 95% CI=1.266-2.180). In contrast, patients carrying TC and CC genotype of rs2839698 loci had significantly increased susceptibility to gastric cancer (χ^2=9.407, *P*=0.002; χ^2=6.517, *P*=0.011), and patients carrying C allele had obviously increased susceptibility to GC (χ^2=6.163, *P*=0.013, *OR*=1.417, 95%CI=1.076-1.867; χ^2=9.542, *P*=0.02, *OR*=2.070, 95%CI=1.298-3.302). As for the rs2735971 and rs3741216, there was no significant difference between the GC group and the control group (all *P*>0.05). The distribution of 4 H19 SNPs showed no statistical difference in both EBVaGC group and EBVnGC group (all *P*>0.05). **Conclusion:** There was an association between H19 gene polymorphism (rs217727 and rs2839698) and gastric carcinoma; patients carrying the TT genotype of rs217727 and C allele of rs2839698 may increase the risk of gastric carcinoma. No significant association was observed between H19 SNP polymorphism and risk of EBVaGC.

[Key words] gastric carcinoma; long non-coding RNA; H19; EBV; single nucleotide polymorphism(SNP); susceptibility

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胃癌在中国恶性肿瘤死因中居第3位，其病死率为消化系统肿瘤之首[1]。多因素、多步骤参与是导致胃癌发病过程极其复杂的主要原因。长链非编码RNA(long non-coding RNA，lncRNA)的失调涉足人类多种疾病，也越来越成为人们关注的热点[2-4]。研究[5-7]显示,lncRNA H19在多种肿瘤中高表达，且在众多恶性肿瘤的发生发展中发挥重要作用。H19单核苷酸多态性(single nucleotide polymorphism，SNP)可致其表达和功能发生变化，继而导致肿瘤易感性和预后发生变化[8]，然而其对胃癌的易感性的影响尚未见报道。近年有关EBV相关胃癌(EBV-associated gastric carcinoma，EBVaGC)致病机制中EBV所发挥的作用，关注较多的是EBV通过诱导CpG岛的异常甲基化或通过干扰miRNA实现调控宿主细胞蛋白质编码基因的异常表达[9-11]。目前EBV作用于lncRNA的相关研究报道甚少。本研究检测EBVaGC、EBVnGC(EBV-negative gastric carcinoma)两组胃癌组织中H19 SNP，为进一步澄清该SNP与EBVaGC的关系，同时为深入阐明EBV感染参与H19 SNP及胃癌的先天性免疫机制的作用提供新思路。

1 材料与方法

1.1 研究对象

收集2015年1月至2018年10月青岛大学附属医院经病理科确诊为胃癌的新鲜组织或陈旧的石蜡包埋胃癌组织病理标本共225例，为胃癌组。依据原位杂交法对EBV编码的小分子非多聚腺苷酸(EBER1)转录的情况实施检测，依据监测结果将胃癌组分为2亚组，EBVaGC组70例、EBVnGC组155例。2组病例在年龄、性别、病理特征、胃癌分期等方面相匹配，并提取其DNA。对照组：选择青岛大学附属医院门诊健康体检者200例，经空腹采集抗凝血5 ml，并分离其外周血单个核细胞，并提取其DNA。参与研究的所有病例均属于汉族人群，籍贯为青岛地区。

1.2 主要试剂和仪器

QIAamp DNA FFPE试剂盒(德国Hilden Qiagen公司)，等位基因分型采用TaqMan MGB分型试剂盒(美国ABI公司)，7900HT高通量荧光定量PCR仪(Ap-plied Biosystems公司)。

1.3 H19 SNP的筛选

根据“HapMap计划”中北京汉族人群(CHB)数据库中对人类染色体11p15. 5区域提供的数据信息，并利用4.2版Haploview (Cambridge, MA, USA)软件对数据进行分析。考虑入选SNP位点的预测功能，同时连锁不平衡设置为r^2>0.8，且最小等位基因的频率(MAF)> 5%，最终筛选出H19的4个SNPs，分别为rs217727、rs2735971、rs2839698和rs3741216。

1.4 组织细胞DNA提取及基因多态性检测

所有标本的DNA提取方法按照QIAamp DNA FFPE试剂盒说明书进行。基因分型选择纯度和完整性较好的DNA，最终选定TaqMan MGB等位基因分型试剂盒于PCR仪上进行qPCR检测。利用SDS软件(2.4版本)对最终结果进行分析。所有标本基因分型由2位研究人员以独立双盲的方式完成，且每板均设对照组。最终随机选取10%的样本进行重复检测，以确保结果的准确性。H19 SNP基因分型的引物及探针序列见表1。

1.5 统计学处理

应用SPSS18.0统计学软件，各位点基因多态性与EBVaGC和EBVnGC罹患率的关系用*P*值、*OR*值及95%CI来描述，采用Hardy-Weinberg平衡检验H19 SNP位点于分布情况，对H19 SNP在各组的基因型、等位基因频率等分布差异采用χ^2检验。以*P*<0.05或*P*<0.01表示差异有统计学意义。

2 结 果

2.1 Hardy-Weinberg平衡检验2组标本的群体代表性

H19的4个SNPs位点在对照组和胃癌组中的分布情况均采用Hardy-Weinberg平衡进行检验，结果均

为*P*>0.05，表明本研究所选胃癌病例及健康对照标本均具有群体代表性。见表2。

表1 H19 SNP编码基因的引物序列及探针序列

Tab.1 Primer sequence and probe sequence of H19 SNP coding gene

Gene	Primer sequence（5′-3′）	Probe sequence
rs217727	F: CAAAGAGACAGAAGGATGAAAAAGAA	T: FAM-TCAACCGTCCGCCG-MGB
	R: CGGCGACTCCATCTTCATG	C:FAM-TCAACCGTCCACCGC-MGB
rs2735971	F: CACCTCCGATTCCACAACTACA	T: FAM-CCAATTCTGTGCCATC-MGB
	R: GAGGCTTCCCCTTCAGTCTCA	C: FAM-CAATTCCGTGCCATC-MGB
rs2839698	F: CATCGTCCCCAGCTGATGTC	T: FAM-CTGGGCGCCTACT-MGB
	R: GGAGTGATGACGGGTGGAG	C: FAM-CCTGGGCACCTAC-MGB
rs3741216	F: GCCTCCACGACTCTGTTTCC	T: FAM-CCCTTCTGAATTTTAT-MGB
	R: CACAACTCCAACCAGTGCAAA	A: HEX-CCCTTCTGAATTTAAT-MGB

表2 H19 SNP在胃癌组和对照组中的分布情况HW检验结果

Tab. 2 HW test for the distribution of H19 SNP in gastric cancer and the control group

Genotype	GC group (*n*=225)		χ^2	*P*	Control group (*n*=200)		χ^2	*P*
	Actual value	Expected value			Actual value	Expected value		
rs217727								
CC	88.00	68.02	1.11	0. 5	63.00	36.13	4.56	0.10
TC	72.00	111.38			44.00	97.75		
TT	65.00	45.56			93.00	66.13		
rs2735971								
CC	60.00	44.76	1.05	0. 5	48.00	31.21	5.82	0.06
TC	81.00	110.99			62.00	95.59		
TT	84.00	68.81			90.00	73.21		
rs3741216								
AA	79.00	68.81	2.30	0.25	70.00	59.41	2.52	0.25
AT	91.00	110.99			78.00	99.19		
TT	55.00	44.76			52.00	41.41		
rs2839698								
CC	90.00	68.34	5.12	0.06	88.00	80.47	4.89	0.10
TC	68.00	111.32			78.00	92.71		
TT	67.00	45.34			34.00	26.65		

2.2　胃癌组H19 rs217727位点各基因型、等位基因频率明显提高

基因多态性检测结果（表3）显示，与对照组比较，H19 rs217727位点在胃癌组基因型为TT的携带者人群的胃癌的发病风险显著增加(χ^2=9.073，*P*=0.003，*OR*=1.999，95% CI=1.271～3.143)，等位基因T在胃癌组的分布也明显高于对照组(χ^2=13.475，*P*=0.001，*OR*=1.661，95%CI=1.266～2.180)。但胃癌组H19 rs2735971位点基因多态性（基因型、等位基因频率及携带等位基因频率）与对照组比较差异不明显（*P*>0.05）。

2.3　EBVaGC和EBVnGC组H19 rs217727、rs2735971位点基因多态性比较无明显差异

EBVaGC和EBVnGC组中H19 rs217727、rs2735971位点基因多态性（基因型、等位基因频率及携带等位基因频率）检测结果（表4）显示，差异均无统计学意义（*P*>0.05）。

2.4　胃癌组H19 rs2839698位点各基因型、等位基因频率明显提高

基因多态性检测结果（表5）显示，与对照组比较，胃癌组中H19 rs2839698位点在基因型、等位基因分布频率均有统计学差异（*P*<0.05）。与对照组比较，胃癌组携带TC、CC基因型人群可显著增加胃癌的发病风险(χ^2=9.407，*P*=0.002；χ^2=6.517，*P*=0.011)；表明

胃癌的风险基因可能是C等位基因，携带C等位基因人群罹患胃癌的风险明显增加(χ^2=6.163，*P*=0.013，*OR*=1.417，95% CI=1.076~1.867；χ^2=9.542，*P*=0.02，*OR*=2.070，95% CI=1.298~3.302)。但胃癌组H19 rs3741216位点基因多态性（基因型、等位基因频率及携带等位基因频率）与对照组比较差异无统计学意义（*P*>0.05）。

表3 胃癌组与对照组H19 rs217727、rs2735971基因多态性的比较

Tab.3 Comparison of H19 rs217727 and rs2735971 gene polymorphisms between GC and control group

H19 SNP	Genotype Allele	GC (*n*=225)	Control (*n*=200)	χ^2	*P*	*OR*(95%CI)
rs217727	CC	88(39.1)	63(31.5)	-	-	1
	TC	72(32.0)	44(22.0)	0.393	0.531	0.854(0.520-1.401)
	TT	65(28.9)	93(46.5)	9.073	0.003	1.999(1.271-3.143)
	C	248(55.1)	170(42.5)	-	-	1
	T	202(44.9)	230(57.5)	13.475	<0.001	1.661(1.266-2.180)
	TC+ CC	160(71.1)	107(53.5)	14.061	<0.001	0.467(0.313-0.698)
	TC+ TT	137(60.9)	137(68.5)	2.678	0.102	1.397(0.935-2.086)
rs2735971	TT	84(37.3)	90(45.0)	-	-	1
	TC	81(36.0)	62(31.0)	2.202	0.138	0.714(0.458-1.115)
	CC	60(26.7)	48(24.0)	1.413	0.235	0.747(0.416-1.209)
	T	249(55.3)	242(60.5)	-	-	1
	C	201(44.7)	158(39.5)	2.317	0.128	0.809(0.615-1.063)
	TC+ CC	141(62.7)	110(55.0)	2.574	0.109	0.728(0.494-1.073)
	TC+ TT	165(73.3)	152(76.0)	0.397	0.529	1.152(0.742-1.786)

表4 EBVaGC组与EBVnGC组H19 rs217727、rs2735971基因多态性的比较

Tab. 4 Comparison of H19 rs217727 and rs2735971 gene polymorphisms between EBVaGC and EBVnGC group

H19 SNP	Genotype Allele	EBVaGC (*n*=70)	EBVnGC (*n*=155)	χ^2	*P*	*OR*(95%CI)
rs217727	TT	23(32.9)	65(41.9)	-	-	1
	TC	25(35.7)	47(30.3)	1.390	0.238	0.665(0.337-1.312)
	CC	22(31.4)	43(27.7)	1.070	0.301	0.692(0.343-1.393)
	C	71(50.7)	177(57.1)	-	-	1
	T	69(49.3)	133(42.9)	1.588	0.208	0.773(0.518-1.154)
	TC+ CC	48(68.6)	112(72.3)	0.319	0.572	1.194(0.645-2.208)
	TC+ TT	47(67.1)	90(58.1)	1.669	0.196	0.678(0.375-1.225)
rs2735971	TT	30(42.9)	54(34.8)	-	-	1
	TC	19(27.1)	62(40.0)	2.968	0.085	1.813(0.918-3.581)
	CC	21(30.0)	39(25.2)	0.008	0.930	1.032(0.516-2.064)
	T	79(56.4)	170(54.8)	-	-	1
	C	61(43.6)	140(45.2)	0.099	0.753	1.067(0.713-1.594)
	TC+ CC	40(57.1)	101(65.2)	1.325	0.250	1.403(0.788-2.499)
	TC+ TT	49(70.0)	116(74.8)	0.577	0.447	1.275(0.681-2.386)

2.5 EBVaGC和EBVnGC组H19 rs2839698、rs3741216位点基因多态性比较无明显差异

基因多态性检测结果（表6）显示，EBVaGC和EBVnGC组中H19 rs2839698、rs3741216位点基因多态性（基因型、等位基因频率及携带等位基因频率）的差异无统计学意义（*P*>0.05）。

表5 胃癌组与对照组H19 rs2839698、rs3741216基因多态性的比较

Tab. 5 Comparison of H19 rs2839698 and rs3741216 gene polymorphisms between GC and control group

H19 SNP	Genotype Allele	GC (*n*=225)	Control (*n*=200)	χ^2	*P*	*OR* (95% CI)
rs2839698	TT	67(29.8)	34(17.0)	-	-	1
	TC	68(30.2)	78(39.0)	9.407	0.002	2.260(1.337-3.823)
	CC	90(40.0)	88(44.0)	6.517	0.011	1.927(1.161-3.198)
	T	202(44.9)	146(36.5)	-	-	1
	C	248(55.1)	254(63.5)	6.163	0.013	1.417(1.076-1.867)
	TC+ CC	158(70.2)	166(83.0)	9.542	0.02	2.070(1.298-3.302)
	TC+ TT	135(60.0)	112(56.0)	0.696	0.404	0.848(0.577-1.248)
rs3741216	AA	79(35.1)	70(35.0)	-	-	1
	AT	91(40.4)	78(39.0)	0.022	0.883	0.967(0.622-1.504)
	TT	55(24.4)	52(26.0)	0.065	0.798	1.067(0.649-1.754)
	A	249(55.3)	218(54.5)	-	-	1
	T	201(44.7)	182(45.5)	0.059	0.807	1.034(0.789-1.356)
	AT+ TT	146(64.9)	130(65.0)	0.001	0.981	1.005(0.674-1.498)
	AA+ AT	170(75.6)	148 (74.0)	0.136	0.712	0.921(0.594-1.428)

表6 EBVaGC与EBVnGC组H19 rs2839698、rs3741216基因多态性的比较

Tab. 6 Comparison of H19 rs2839698 and rs3741216 gene polymorphisms between EBVaGC and EBVnGC group

H19 SNP	Genotype Allele	EBVaGC (*n*=70)	EBVnGC (*n*=155)	χ^2	*P*	*OR*(95%CI)
rs2839698	TT	20(28.6)	47(30.3)	-	-	1
	TC	28(40.0)	40(25.8)	1.889	0.169	0.608(0.298-1.239)
	CC	22(31.4)	68(43.9)	0.573	0.449	1.315(0.646-2.677)
	T	68(48.6)	134(43.2)	-	-	1
	C	72(51.4)	176(56.8)	1.114	0.291	1.240(0.831-1.851)
	TC+ CC	50(71.4)	108(34.8)	0.071	0.790	0.919(0.494-1.711)
	TC+ TT	48(68.6)	87(28.1)	3.111	0.078	0.586(0.323-1.064)
rs3741216	AA	24(34.3)	50(32.3)	-	-	1
	AT	32(45.7)	59(38.1)	0.136	0.712	0.885(0.462-1.695)
	TT	14(20.0)	46(29.7)	1.350	0.245	1.577(0.729-3.410)
	A	80(57.1)	159(51.3)	-	-	1
	T	60(42.9)	151(48.7)	1.327	0.249	1.266(0.847-1.893)
	AT+ TT	46(65.7)	105(67.7)	0.090	0.764	1.096(0.603-1.991)
	AA+ AT	56(80.0)	109(70.3)	2.309	0.129	0.592(0.300-1.169)

3 讨　论

lncRNA作为近年来研究的热点，其在细胞发育、增殖和凋亡等病理过程中均作为抑癌或促癌因子，通过不同的调控方式干预肿瘤的形成过程。与蛋白编码基因SNP相似，lncRNA的SNP也被证明具有此功能，如调节基因表达及疾病易感性等[13]。有关lncRNA多态性研究结果[14]表明，lncRNA多态性与疾病风险有关。lncRNA H19是最早发现的、与肿瘤关系密切lncRNA，其基因全长约2.33 kb。由5个外显子和4个内含子组成，在染色体上位于11p15.5。有研究[15-16]显示，H19能够通过印记缺失或通过调节CPT1C蛋白表达等机制参与肿瘤形成，对胃癌的发生发展、侵袭迁移等能力的影响发挥重要的作用。LI等[17]研究显示，通过直接上调ISM1或通过miR-675间接抑制CALN1，H19可进一步发挥其对胃癌形成的关键作用。

本研究共选取H19基因不同功能区的4个SNPs，探讨H19的SNP引起的折叠结构改变导致对其功能的影响。到目前，大量研究已充分显示H19基因多态性与肿瘤的发生率之间关系密切。有研究[18]显示，H19 rs2839689位点的突变可使该lncRNA的结

构和功能受到影响，携带该突变基因的中国人群罹患结直肠癌的风险明显增加。LIN等[19]研究发现，中国东南地区汉族人群中H19（rs217727和rs2839698）的多态性与乳腺癌的患病风险关系密切。YANG等[20]采用竞争性等位基因特异性PCR（KASP）法研究H19相关SNP的多态性与肝癌发病风险的关系，结果表明，rs2839698位点SNP有可能成为肝癌的发病及预后的预测因子。VERHAEGH等[21]报道，H19的遗传变异可降低欧洲白种人群患膀胱癌的风险。以上结果均提示H19遗传变异与恶性肿瘤的发病风险之间存在着较大的相关性。

本研究结果显示，胃癌组与健康对照组中H19 rs217727与rs2839698基因多态性存在显著差异。rs217727位点多态性基因型为TT的人群，其胃癌的发病风险显著增加（χ^2=9.073，*P*=0.003，*OR*=1.999，95%CI=1.271～3.143）；胃癌组T等位基因频率也显著高于对照组。rs2839698位点基因多态性结果表明，携带TC、CC基因型人群可显著增加胃癌的发病风险（χ^2=9.407，*P*=0.002；χ^2=6.517，*P*=0.011）；推测认为C等位基因是胃癌的风险基因，携带C等位基因人群的胃癌发病风险大大增加。但H19 rs2735971及rs3741216位点的多态性与胃癌的发病风险无明显相关。本研究进一步证实了基因多态性因种族、地区、肿瘤的不同而不同，特别是lncRNA的SNP对其功能和结构产生影响的同时还会受环境因素的影响。加之胃癌的诱因复杂，如遗传因素、慢性炎症刺激、幽门螺杆菌感染、一般饮食与生活习惯等众多因素均可对胃癌易感性造成影响。本研究的不足之处为未考虑环境因素对H19遗传变异及胃癌发病的影响。

EBV通过干扰宿主细胞的基因表达，影响其相应的生物学行为，从而发挥致癌作用。一项有关EBV的感染如何调控lncRNA H19对胃癌的发生机制的研究[22]表明，H19在EBV阳性细胞系（胃癌细胞系及鼻咽癌细胞系）中的表达水平较EBV阴性细胞系低，而且EBV感染通过调控H19启动子区高甲基化，进而调控lncRNA H19的表达水平[23]。EBV通过诱导CpG岛区甲基化异常，促使宿主细胞蛋白质编码基因的异常表达；也可通过干扰非编码RNA中miRNA的表达，最终诱导EBVaGC的发生[24]。本研究结果显示，H19的4个SNPs在EBVaGC组中表达无明显差异，表明H19 SNPs与EBVaGC的易感性无明显相关，提示EBVaGC发病机制具有独特性和复杂性，且SNP对lncRNA功能和结构的影响受多因素干扰。

综上所述，H19 rs217727、rs2839698基因多态性与胃癌易感性密切相关，其中携带风险基因的人群胃癌发病风险明显升高，对胃癌易感人群的筛查具有一定价值。

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