

# 川东北地区食管鳞状细胞癌血清肿瘤标志物的诊断效能

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**【摘要】目的:** 探讨川东北地区食管鳞状细胞癌(ESCC)血清肿瘤标志物的诊断效能。**方法:** RT-qPCR检测46例ESCC患者(观察组)与46名健康体检志愿者(对照组)食管组织和血清中miR-222-5p的表达差异,分析ESCC患者血清miR-222-5p的表达水平与不同临床病理特征的相关性及其对ESCC的诊断效能。**结果:** 观察组患者血清miR-222-5p水平高于对照组[(1.76±0.99) vs. (1.24±1.08),  $t = -2.396, P = 0.019$ ]。ESCC I期患者血清miR-222-5p水平低于ESCC II期和ESCC III期[(1.01±0.19) vs. (1.78±0.31) vs. (2.59±0.44),  $F = 44.793, P < 0.001$ ];远处转移组ESCC患者血清miR-222-5p水平高于无远处转移组[(1.02±0.21) vs. (0.60±0.34),  $t = 2.090, P = 0.043$ ];淋巴结转移组ESCC患者血清miR-222-5p水平高于无淋巴结转移组[(1.04±0.32) vs. (0.79±0.39),  $t = 2.223, P = 0.032$ ];男性组和女性组ESCC患者血清miR-222-5p水平比较,差异无统计学意义[(1.01±1.33) vs. (1.07±1.61),  $t = -0.123, P = 0.903$ ];≤65岁组和>65岁组ESCC患者血清miR-222-5p水平比较,差异无统计学意义[(1.09±0.53) vs. (1.06±0.67),  $t = 0.145, P = 0.885$ ];ESCC治疗前组患者血清miR-222-5p水平高于ESCC治疗后组[(2.46±1.65) vs. (1.85±0.99),  $t = 2.141, P = 0.035$ ]。ROC曲线分析显示,血清miR-222-5p为0.393时诊断ESCC效能最高,此时的敏感度和特异度分别为58.70%、80.60%。肿瘤组织miR-222-5p为0.435时诊断ESCC效能最高,此时敏感度和特异度分别为65.20%、78.30%。**结论:** 川东北地区ESCC中miR-222-5p对肿瘤的发生和发展有一定预测作用,有较好的诊断效能。

**【关键词】** 食管鳞状细胞癌;miR-222-5p;川东北地区;诊断效能

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## Diagnostic efficacy of serum tumor markers for esophageal squamous cell carcinoma in northeast Sichuan province

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**【Abstract】 Objective:** To investigate the diagnostic efficacy of serum tumor markers for esophageal squamous cell carcinoma (ESCC) in northeast Sichuan province. **Methods:** The expression of miR-222-5p in esophageal tissues (observation group) and serum of 46 ESCC patients and 46 healthy subjects (control group) was detected by fluorescent quantitative PCR, and the relationship between the expression of serum miR-222-5p in ESCC and clinicopathological features was analyzed. Differential expression of miR-222-5p in tumor tissues and serum samples was analyzed by constructing receiver operating characteristic (ROC) curves to assess its diagnostic value for ESCC. **Results:** The serum level of miR-222-5p in the observation group was higher than that in the control group [(1.76 ± 0.99) vs. (1.24 ± 1.08),  $t = -2.396, P = 0.019$ ]. The serum miR-222-5p level in ESCC stage I was lower than that in ESCC stage II and ESCC stage III [(1.01 ± 0.19) vs. (1.78 ± 0.31) vs. (2.59 ± 0.44),  $F = 44.793, P < 0.001$ ]. The level of serum miR-222-5p in ESCC patients with distant metastasis was higher than that in ESCC patients without distant metastasis [(1.02 ± 0.21) vs. (0.60 ± 0.34),  $t = 2.090, P = 0.043$ ]. The serum miR-222-5p level of ESCC patients with lymph node metastasis was higher than that of ESCC patients without lymph node metastasis [(1.04 ± 0.32) vs. (0.79 ± 0.39),  $t = 2.223, P = 0.032$ ]. There was no significant difference in serum miR-222-5p levels between male and female ESCC patients [(1.01 ± 1.33) vs. (1.07 ± 1.61),  $t = -0.123, P = 0.903$ ]. There was no significant difference in serum miR-222-5p levels between ≤65 years old and >65 years old ESCC patients [(1.09 ± 0.53) vs. (1.06 ± 0.67),  $t = 0.145, P = 0.885$ ]. The serum miR-222-5p level in the pre-ESCC group was higher than that in the post-ESCC group [(2.46 ± 1.65) vs. (1.85 ± 0.99),  $t = 2.141, P = 0.035$ ]. ROC curve showed that when serum miR-222-5p was 0.393, the diagnostic efficacy of ESCC was the highest, the sensitivity was 58.70%, and the specificity was 80.60%. When miR-222-5p of tumor tissue was 0.435, the diagnostic efficacy of ESCC was the highest, and the sensitivity was 65.20% and the specificity was

78.30%。**Conclusion:** miR-222-5p has a certain predictive effect on the occurrence and development of ESCC in northeast Sichuan province, and shows moderate diagnostic efficacy.

**【Key words】** Esophageal squamous cell carcinoma; miR-222-5p; Northeast Sichuan; Diagnostic efficacy

食管鳞状细胞癌(esophageal squamous cell carcinoma, ESCC)构成了食管恶性肿瘤的主要组织学类型,特点是鳞状细胞的异常增殖<sup>[1-2]</sup>。由于侵袭性强、治疗困难,ESCC在全球癌症发病率中排名第八,在癌症死亡率中排名第六<sup>[3-4]</sup>。尽管内外科治疗技术取得了明显进步,但大多数ESCC患者往往在晚期才被确诊,导致预后不佳,五年总体生存率为15%~25%<sup>[5-7]</sup>。早期发现原发性肿瘤可提供有效的治疗和及时的干预,进而改善预后。癌胚抗原、鳞状细胞癌抗原和p53等已被临床用作ESCC肿瘤标志物,但均不足以识别早期肿瘤亚临床患者和预测疾病复发<sup>[8-10]</sup>。非编码RNA如microRNA在许多疾病尤其是癌症的发生发展中发挥着重要的调控作用<sup>[11-13]</sup>。TCGA数据库分析发现miR-222-5p在食管癌中高表达,但四川省东北地区对miR-222-5p可作为ESCC诊断标志物的研究尚无。本研究旨在探讨川东北地区miR-222-5p作为ESCC血清肿瘤标志物的诊断效能。

## 1 资料与方法

### 1.1 一般资料

选取2022年1月至2022年12月川北医学院附属医院收治的46例接受手术治疗的ESCC患者为观察组;46名同期健康体检志愿者为对照组。46例ESCC患者中,男性36例,女性10例,≤65岁14例,>65岁31例,ESCC I期5例,ESCC II期22例,ESCC III期15例,远处转移3例,无远处转移39例,淋巴结转移17例,无淋巴结转移26例。本研究经川北医学院附属医院伦理委员会批准(2022ER555-1)。纳入标准:(1)年龄≥18岁;(2)经病理科确诊为食管鳞状上皮细胞癌;(3)所有患者未行化疗、放疗或手术治疗;(4)所有患者心脏功能无异常,肝肾功能无异常。排除标准:(1)在入院前3个月接受过输血、使用抗凝或促凝药物治疗的患者;(2)有脑血栓、肺栓塞、冠心病、弥散性血管内凝血、肝硬化或糖尿病;(3)妊娠或哺乳期妇女。

### 1.2 方法

1.2.1 外周血的收集及处理 采集所有参与者空腹外周静脉5 mL,4℃ 2 000 r/min离心15 min取上清液,-80℃保存备用。

1.2.2 RT-qPCR检测miR-222-5p表达水平 取肿瘤组织和血清样本,加入Trizol试剂提取总RNA提取,使用无RNA酶水溶解后测定RNA纯度和浓度。

使用反转录试剂盒将RNA反转录成cDNA,利用PCR仪(美国ThermoFisher公司)扩增cDNA进行实时定量聚合酶链反应。Cell/Tissue Total RNA Kit购于YEASEN公司,Bulge-Loop™ miRNA qRT-PCR Starter Kit购于锐博生物。cDNA扩增反应条件如下:95℃预变性10 min→95℃变性20 s→58℃下退火30 s→72℃延长45 s,共40个循环。以U6作为内参照基因,使用PCR仪测定Ct值。通过 $2^{-\Delta\Delta Ct}$ 方法计算miR-222-5p的相对表达水平。见表1。

表1 引物序列

引物	序列	长度(bp)
miR-222-5p		39
正向	5'-CGCGCTCAGTAGCCAGTGT-3'	
反向	5'-AGTGCAGGGTCCGAGGTATT-3'	
U6		40
正向	5'-CGCTTCGGCAGCACATATAAC-3'	
反向	5'-CACGAATTTGCGTGTCAATCC-3'	

### 1.3 统计学分析

采用SPSS 17.0软件对数据进行处理与分析。计量资料符合正态分布且方差齐性,以 $(\bar{x} \pm s)$ 表示,组间比较行独立样本t检验,多组间比较行单因素方差分析;诊断效能采用受试者特征工作(ROC)曲线分析。 $P < 0.05$ 为差异有统计学意义。

## 2 结果

### 2.1 观察组与对照组血清miR-222-5p水平比较

观察组患者血清中miR-222-5p水平高于对照组( $P < 0.05$ )。见表2。

表2 观察组与对照组血清miR-222-5p水平比较( $\bar{x} \pm s$ )

基因	观察组	对照组	t值	P值
miR-222-5p	1.76 ± 0.99	1.24 ± 1.08	-2.396	0.019

### 2.2 不同ESCC分期患者miR-222-5p水平比较

ESCC I期、ESCC II期、ESCC III期患者miR-222-5p水平比较,组间差异有统计学意义( $P < 0.001$ )。见表3。

表3 不同ESCC分期患者miR-222-5p水平比较( $\bar{x} \pm s$ )

分期	miR-222-5p
ESCC I期	1.01 ± 0.19
ESCC II期	1.78 ± 0.31
ESCC III期	2.59 ± 0.44
F值	44.793
P值	0.000

### 2.3 远处转移组与无远处转移组 ESCC 患者 miR-222-5p 水平比较

远处转移组 ESCC 患者血清 miR-222-5p 水平高于无远处转移组 ( $P < 0.05$ )。见表 4。

表 4 远处转移组与无远处转移组 ESCC 患者血清 miR-222-5p 水平比较 ( $\bar{x} \pm s$ )

基因	远处转移组	无远处转移组	t 值	P 值
miR-222-5p	1.02 ± 0.21	0.60 ± 0.34	2.090	0.043

### 2.4 淋巴结转移组与无淋巴结转移组 ESCC 患者 miR-222-5p 水平比较

淋巴结转移组 ESCC 患者血清 miR-222-5p 水平高于无淋巴结转移组 ( $P < 0.05$ )。见表 5。

表 5 淋巴结转移组与无淋巴结转移组 ESCC 患者血清 miR-222-5p 水平比较 ( $\bar{x} \pm s$ )

基因	淋巴结转移组	无淋巴结转移组	t 值	P 值
miR-222-5p	1.04 ± 0.32	0.79 ± 0.39	2.223	0.032

### 2.5 不同性别 ESCC 患者 miR-222-5p 水平比较

男性组和女性组 ESCC 患者血清 miR-222-5p 水平比较,差异无统计学意义 ( $P > 0.05$ )。见表 6。

表 6 不同性别 ESCC 患者血清 miR-222-5p 水平比较 ( $\bar{x} \pm s$ )

基因	男性组	女性组	t 值	P 值
miR-222-5p	1.01 ± 1.33	1.07 ± 1.61	-0.123	0.903

### 2.6 不同年龄 ESCC 患者 miR-222-5p 水平比较

≤65 岁组和 >65 岁组 ESCC 患者血清 miR-222-5p 水平比较,差异无统计学意义 ( $P > 0.05$ )。见表 7。

表 7 不同年龄 ESCC 患者血清 miR-222-5p 水平比较 ( $\bar{x} \pm s$ )

基因	≤65 岁组	>65 岁组	t 值	P 值
miR-222-5p	1.09 ± 0.53	1.06 ± 0.67	0.145	0.885

### 2.7 ESCC 患者治疗前后 miR-222-5p 水平比较

ESCC 治疗前组患者血清 miR-222-5p 水平高于 ESCC 治疗后组 ( $P < 0.05$ )。见表 8。

表 8 ESCC 患者治疗前后血清 miR-222-5p 水平比较 ( $\bar{x} \pm s$ )

基因	ESCC 治疗前组	ESCC 治疗后组	t 值	P 值
miR-222-5p	2.46 ± 1.65	1.85 ± 0.99	2.141	0.035

### 2.8 miR-222-5p 对 ESCC 的诊断效能

ROC 曲线分析显示,血清 miR-222-5p 诊断 ESCC 的 ROC 曲线下面积 (AUC) 为 0.704 (95% CI: 0.584 ~ 0.823), 诊断参考性中等;血清 miR-222-5p 截断值为 0.393 时,诊断的敏感度为 58.70%, 特异度为 80.60%。肿瘤组织 miR-222-5p 诊断 ESCC 的

AUC 为 0.717 (95% CI: 0.610 ~ 0.825), 诊断参考性中等;肿瘤组织 miR-222-5p 截断值为 0.435 时,诊断的敏感度为 65.20%, 特异度为 78.30%。

## 3 讨论

本研究探讨川东北地区 miR-222-5p 对 ESCC 的诊断效能。个体遗传学因素在 ESCC 的发病机制中扮演着越来越重要的角色,基因的突变、缺失或多态性已成为当前研究的焦点<sup>[14]</sup>。MicroRNAs (miRNAs) 是一类不编码蛋白质的基因,但能够通过碱基互补配对原则,靶向调控数千个编码蛋白质的基因表达,参与人体重要的生理和病理活动。近期研究<sup>[15]</sup>发现,miRNAs 在肿瘤发展过程中的关键作用,通过调节自身的表达量或存在状态,对癌细胞的行为和分化路径产生深远影响。研究<sup>[16]</sup>表明,miR-301a-3p 通过抑制 PTEN 促进 ESCC 细胞增殖。MiR-130b 在 ESCC 肿瘤组织和细胞中上调,充当肿瘤启动子,通过抑制作为抗癌基因的 SASH1,证明其对 ESCC 细胞生长和侵袭有刺激作用<sup>[17]</sup>。近期研究<sup>[18]</sup>发现,肿瘤相关 miRNAs 在肿瘤患者的血清中表达水平发生明显变化,这种变化与肿瘤的发生和发展过程紧密相连。在恶性肿瘤的研究领域,血液中的 miRNAs 显示出成为新型早期肿瘤诊断标志物的巨大潜力<sup>[19]</sup>。在肺癌、肝癌、前列腺癌、卵巢癌等多种人类恶性肿瘤患者的血液中,均检测到了具有特异性的 miRNAs 表达模式<sup>[20-23]</sup>。

miR-222 是近期被识别出与肿瘤发展有关的 miRNA<sup>[24-26]</sup>。通过对癌症基因组图谱 (The Cancer Genome Atlas, TCGA) 数据库的分析,发现 miR-222-5p 在 ESCC 的肿瘤组织中呈现高表达状态。本研究探索血清 miR-222-5p 在 ESCC 患者中的诊断价值,结果表明,ESCC 患者血清中 miR-222-5p 水平增加 ( $P < 0.05$ );ESCC III 期血清中 miR-222-5p 表达高于 ESCC II 期 ( $P < 0.05$ );远处转移 ESCC 患者血清 miR-222-5p 表达高于无远处转移者 ( $P < 0.05$ );淋巴结转移 ESCC 患者血清 miR-222-5p 表达高于无淋巴结转移者 ( $P < 0.05$ );ESCC 患者血清 miR-222-5p 水平在性别和年龄比较,差异无统计学意义 ( $P > 0.05$ )。ROC 曲线验证了 miR-222-5p 对 ESCC 的诊断效能。

综上,川东北地区 miR-222-5p 在 ESCC 血清中的表达与肿瘤的 TNM 分期、远处转移、和淋巴结转移相关,miR-222-5p 对 ESCC 有一定的诊断效能,有望成为 ESCC 临床治疗的新靶点。

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