

· 论著 ·

血清miRNA-21-5p和miRNA-574-5p在良恶性肺结节鉴别诊断中的价值*

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【摘要】目的 探究血清miRNA-21-5p 和 miRNA-574-5p 在良恶性肺结节鉴别诊断中的价值。方法 本院收治肺结节患者的57例, 据病理结果分为良性结节组(n=12)和恶性结节组(n=45)。收集临床资料, RT-PCR检测血清miRNA-21-5p 和 miRNA-574-5p 水平。结果 两组在性别、年龄、不同直径、肿瘤家族史、不同密度结节、吸烟史等方面无差异($P>0.05$); 两组在分布上有显著性差异($P<0.05$), 其中恶性结节组在右肺上叶的发生率最高(48.89%)。两组miRNA-21-5p和miRNA-574-5p水平比较, $P<0.05$, 其中恶性结节组更高。据ROC曲线分析, miRNA-21-5p和miRNA-574-5p诊断恶性肺结节的AUC分别为0.690和0.711, 联合AUC为0.819。结论 血清miRNA-21-5p 和 miRNA-574-5p 在良恶性肺结节鉴别中诊断, 具有较高的价值, 有成为潜在的血清生物标志物的可能性。

【关键词】miRNA-21-5p; miRNA-574-5p; 良恶性肺结节; 鉴别诊断

【中图分类号】R44; R563

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Value of Serum miRNA-21-5p and miRNA-574-5p in Differential Diagnosis of Benign and Malignant Pulmonary Nodules*

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Abstract: *Objective* To explore the value of serum miRNA-21-5p and miRNA-574-5p in the differential diagnosis of benign and malignant pulmonary nodules. *Methods* The 57 patients with pulmonary nodules admitted to our hospital were divided into benign nodule group (n=12) and malignant nodule group (n=45) according to the pathological results. The clinical data of the patients were collected, and the levels of miRNA-21-5p and miRNA-574-5p were detected by RT-PCR. *Results* There was no difference in the distribution of gender, age, smoking history, tumor family history, nodules with different diameters and densities between benign nodules and malignant nodules ($P>0.05$). There was a significant difference in the distribution between benign nodules and malignant nodules ($P<0.05$), and the frequency of malignant nodules in the upper lobe of the right lung was the highest (48.89%). The levels of miRNA-21-5p and miRNA-574-5p were significantly different between benign and malignant nodules ($P<0.05$), and the levels of serum miRNA-21-5p and miRNA-574-5p in malignant nodules were higher. According to ROC curve analysis, the AUC of miRNA-21-5p and miRNA-574-5p in the diagnosis of malignant pulmonary nodules were 0.690 and 0.711, respectively, and the AUC of combined detection was 0.819. *Conclusion* The serum levels of miRNA-21-5p and miRNA-574-5p in patients with malignant nodules were significantly higher than those in patients with benign nodules, which may become potential serum biomarkers.

Keywords: miRNA-21-5p; miRNA-574-5p; Benign and Malignant Pulmonary Nodules; Differential Diagnosis

肺癌具有发病率高、死亡率高的特点^[1]。由于早期阶段临床症状无特异性, 大部分患者确诊后, 便已处于晚期, 导致5年总体生存率较差^[2-3]。目前主要通过计算机断层扫描(CT)筛查肺结节高危人群, 近年来随着高分辨率CT的广泛应用^[4-5]。判断结节的良恶性是肺癌早期发现的关键^[6]。肺结节可分为实性和亚实性结节, 形态特征明显不同, 难以诊断^[7]。研究报道, miRNAs、自身抗体、循环肿瘤细胞、DNA甲基化等有助于肺结节诊断^[8-9]。因此, 本文探讨了血清miRNA-21-5p 和 miRNA-574-5p 在良恶性肺结节鉴别诊断中的价值, 旨在为今后临床控制和预防提供思路。

1 资料与方法

1.1 研究对象 选2020年1月至2022年1月本院收治肺结节患者的57例。

纳入标准: 受试者在CT扫描中检测到肺结节; 参与者的临床资料应完整。排除标准: 具有肺癌疾病史; 肺部存在急性感染或活动性肺结核; 心、肝、肾功能异常; 合并其他恶性肿瘤。依据病理结果进行分组, 即良性结节组(12例)和恶性结节组, 其中恶性结节组(45例)。

1.2 资料收集 收集性别、年龄、吸烟史、结节大小及分布等临床资料。吸烟被定义为吸烟超过6个月, 包括接触二手烟。

1.3 血清miRNA-21-5p 和 miRNA-574-5p水平检测 将储存的血清样品解冻, 将300μl 添加到900μl Trizol 试剂(Invitrogen,

San Diego, CA, USA)中的250fmol标准cel-miRNA-39 30μl中。根据制造商的说明提取总RNA。对于miRNA-21-5p、miRNA-574-5p和cel-miRNA-39的定量实时PCR, 使用了miRNA特异性TaqMan MicroRNA Assays(Applied Biosystems, Foster City, CA, USA)。miRNA-21-5p和miRNA-574-5p的特异性逆转录引物由TAKARA Biotechnology提供(miRNA-21-5p: 5'-UAG CUU AUCAGACU GAUGUUGA-3'; miRNA-574-5p: 5'-UGAGUGUGUGUGUGUGUGU GAG UGUG U-3')。逆转录的总反应体积为20μl, 包括总RNA, 50 ng-5 μg; GSP(基因特异性引物), 2pmol; 2×ES 反应混合物, 10μl; Easy Script RT/RI 酶混合物, 1μl; 和足够的无RNase 水至终体积为20μl。逆转录反应在42℃下进行15分钟, 然后在85℃下热灭活5秒。实时PCR反应也使用20μl的体积, 每个样品一式三份测定, 包括cDNA, 3μl; 正向引物(10μM), 0.4μl; 反向引物(10μM), 0.4μl; 2×TransStart Top Green qPCR SuperMix, 10μl; 被动参考染料(50×)(可选), 0.4μl; 和足量的蒸馏去离子水至最终体积为20μl。反应在ABI Prism 7500qPCR机器上进行, 反应条件为94℃变性35s, 60℃退火34s, 72℃延伸10s, 反应40个循环。然后在85℃下热灭活5秒。实时PCR反应也使用20μl的体积, 每个样品一式三份测定, 包括cDNA, 3μl; 正向引物(10μM), 0.4μl; 反向引物(10μM), 0.4μl; 2×TransStart Top Green qPCR SuperMix, 10μl; 被动参考染料(50×)(可选),

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0.4 μ l;和足量的蒸馏去离子水至最终体积为20 μ l。反应在ABI Prism 7500 qPCR机器上进行,反应条件为94 $^{\circ}$ C变性35s,60 $^{\circ}$ C退火34s,72 $^{\circ}$ C延伸10s,反应40个循环。然后在85 $^{\circ}$ C下热灭活5秒。实时PCR反应也使用20 μ l的体积,每个样品一式三份测定,包括cDNA,3 μ l;正向引物(10 μ M),0.4 μ l;反向引物(10 μ M),0.4 μ l;2 $\times$ TransStart Top Green qPCR SuperMix,10 μ l;被动参考染料(50 $\times$)(可选),0.4 μ l;和足量的蒸馏去离子水至最终体积为20 μ l。反应在ABI Prism 7500 qPCR机器上进行,反应条件为94 $^{\circ}$ C变性35s,60 $^{\circ}$ C退火34s,72 $^{\circ}$ C延伸10s,反应40个循环。被动参考染料(50 $\times$)(可选),0.4 μ l;和足量的蒸馏去离子水至最终体积为20 μ l。反应在ABI Prism 7500 qPCR机器上进行,反应条件为94 $^{\circ}$ C变性35s,60 $^{\circ}$ C退火34s,72 $^{\circ}$ C延伸10s,反应40个循环。被动参考染料(50 $\times$)(可选),0.4 μ l;和足量的蒸馏去离子水至最终体积为20 μ l。反应在ABI Prism 7500 qPCR机器上进行,反应条件为94 $^{\circ}$ C变性35s,60 $^{\circ}$ C退火34s,72 $^{\circ}$ C延伸10s,反应40个循环。用线虫 cel-miRNA-39 外源内参对待检测的靶基因进行标准化。miRNA表达水平通过 $\Delta\Delta Ct$ 法计算: $\Delta Ct = \text{平均值} Ct(\text{感兴趣的miRNA}) - \text{平均值} Ct(\text{参考miR-39})$,在这种情况下 $\Delta\Delta Ct = \text{平均值} Ct(\text{恶性结节的miRNA}) - \text{平均值} Ct(\text{良性结节的miRNA})$ 。每个感兴趣的miRNA的相对表达对应于 $2^{-\Delta\Delta Ct}$ 值。

1.4 统计学分析 经SPSS 21.0分析,计量资料采用($\bar{x} \pm s$)表示,t检验,计数资料以例数或率表示,两组比较采用 χ^2 检验,采用受试者工作特征曲线(ROC)评估miRNA-21-5p和miRNA-574-5p的诊断价值, $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 一般临床资料 两组在性别、不同直径、年龄、肿瘤家族史、吸烟史和不同密度结节中的分布方面比较, $P > 0.05$;两组在分布中方面比较, $P < 0.05$,见表1。

表1 一般临床资料

临床病理参数	良性结节组(n=12)	恶性结节组(n=45)	χ^2	P
年龄(岁)			3.211	0.073
<65	8	17		
≥ 65	4	28		
性别			0.0955	0.757
男	7	24		
女	5	21		
吸烟史			0.702	0.402
是	7	25		
否	3	20		
结节大小			3.643	0.162
$\leq 10\text{mm}$	3	4		
11mm~19mm	6	18		
20mm~30mm	3	23		
分布			10.474	0.033
右肺上叶	1	22		
右肺中叶	2	3		
右肺下叶	3	10		
左肺上叶	2	7		
左肺下叶	4	3		

2.2 血清miRNA-21-5p和miRNA-574-5p水平 两组miRNA-21-5p和miRNA-574-5p水平比较, $P < 0.05$,见表2。

2.3 miRNA-21-5p和miRNA-574-5p诊断价值 据ROC曲线分析可知,miRNA-21-5p和miRNA-574-5p诊断肺部恶性结节AUC分别是0.690,0.711,联合检测AUC为0.819,具体见表3及图1。

表2 血清miRNA-21-5p和miRNA-574-5p水平

指标	良性结节组(n=12)	恶性结节组(n=45)	t	P
miRNA-21-5p	2.99 $\pm$ 0.80	16.63 $\pm$ 5.40	8.669	<0.001
miRNA-574-5p	5.59 $\pm$ 1.92	86.75 $\pm$ 15.25	18.278	<0.001

表3 miRNA-21-5p和miRNA-574-5p诊断价值

指标	曲线下面积(AUC)	灵敏度(%)	特异度(%)	95%CI	P值
miRNA-21-5p	0.690	68.84	67.15	0.612~0.876	<0.001
miRNA-574-5p	0.711	70.32	69.54	0.643~0.912	<0.001
联合检测	0.819	78.65	77.29	0.705~0.987	<0.001

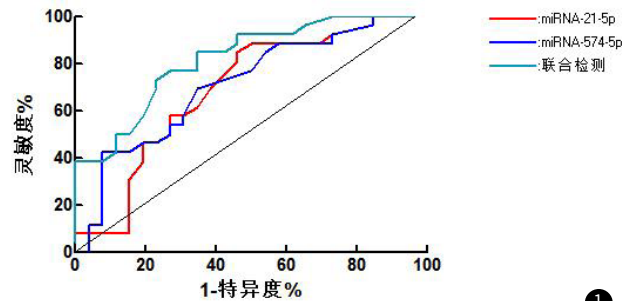


图1 miRNA-21-5p和miRNA-574-5p表达以及联合检测对肺部恶性结节的诊断价值ROC曲线。

3 讨论

随着低剂量胸部CT的普及,肺部结节的检出率急剧上升,尽管这些结节大多是良性的。为了实现恶性结节的早期诊断并避免对良性结节进行侵入性评估,临床医生需要准确区分良恶性结节^[10]。在临床实践中,临床医生使用各种方法来诊断或跟踪肺结节。首先,他们可能会跟踪患有肺结节的患者,以监测结节的变化。或者,可能需要通过经支气管镜活检或手术活检获得病理学诊断,特别是对于那些可能是恶性的结节。在做出这些决定时,临床方法需要考虑患者的合并症以及患者的意愿^[11-12]。多数早期肺癌是无症状的,并且在被发现时已处于晚期阶段^[13]。如果肺癌能够早期临床诊断,患者的预后可以得到显著改善。肺癌各个阶段的5年生存率仅为15~19%。早期诊断的生存率可能会得到改善,特别是对于I期,5年生存率可以提高到81~85%^[14]。因此,在早期阶段改善肺癌筛查似乎是合理的。低剂量计算机断层扫描(LDCT)属于临床上常用的早期检测筛查工具^[15]。随着对肺结节影像学特征和血清肿瘤生物标志物认识的不断深入,有利于寻找一种比现有影像学模型或生物标志物具有更高特异性和敏感性的非侵入性方法,实现对肺结节的可靠早期诊断。恶性肿瘤。本研究的研究旨在寻找更有效的新肿瘤标志物,并利用血清肿瘤标志物以及临床特征和影像学特征建立预测模型,以提高肺癌的早期诊断率^[16]。

对于放射学检查结果,临床医生通常使用Swensen模型和VA模型来区分良性和恶性肺结节。在Swensen模型中,年龄、吸烟史、其他癌症病史、结节的直径、毛刺和位置等因素对判断结节的良恶性有重要意义。本研究发现年龄、吸烟史、肺气肿、直径、毛刺和血管征在鉴别结节良恶性方面比较, $P > 0.05$ 。但有研究表明,年龄较大且吸烟史较长的患者更容易出现恶性结节^[17-18]。miRNA可以通过结合mRNA 3' UTR在转录后水平调节基因表达,导致mRNA降解或转录抑制^[19]。多项研究表明,miRNA的异常表达与肿瘤细胞的增殖、转移、侵袭和血管生成有关^[20]。miRNA可以在生理或病理条件下由正常细胞分泌到体液中,也可以由凋亡和坏死细胞分泌到体液中^[21]。最近也研究了miRNA作为判断肺结节性质的生物标志物^[22]。近年研究表明,miRNA作为生物标志物区分恶性肺结节的敏感性为54.8%~92.9%,特异性为69.2%~90.9%^[23]。血清miRNA是一种新型的血清肿瘤标志物,具有非侵入性、易获取、灵敏度高等特点。已证实miRNA可参与神经母细胞瘤、甲状腺癌、乳腺癌等疾病的发生和发展^[24-26]。miRNA-21是700多种不同miRNA中特别重要的miRNA分子,因为它在乳腺癌、结肠癌、肺癌和其他肿瘤中高度表达。Foss等人的研究表明miRNA-574-5p在非小细胞肺癌的早期诊断中具有重要意义。当miRNA-574-5p和miRNA-1254一起使用时,肺癌早期诊断的敏感性和特异性分别为82%和77%^[27]。有研究

证实证明miRNA-21、miRNA-210和miRNA-15在肺癌患者血清中高表达,其中以miRNA-21最为突出。其研究表明血浆miRNA-21可作为循环肿瘤生物标志物用于早期诊断非小细胞肺癌^[28-29]。另外有研究表明miRNA-574-5p水平可用于非小细胞肺癌的早期诊断^[30]。本研究中两组在分布中存在显著差异,与大多数研究一致。有研究发现肺结节多位于右上叶(37.5%),恶性结节最多位于右上叶(41.4%)^[31]。本研究中良性结节组和恶性结节组之间miRNA-21-5p和miRNA-574-5p水平差异显著($P<0.05$),其中恶性结节组血清miRNA-21-5p和miRNA-574-5p水平更高。根据ROC曲线分析可知,miRNA-21-5p和miRNA-574-5p诊断肺部恶性结节AUC分别是0.690, 0.711,联合检测AUC为0.819。结果表明,联合检测的诊断价值高于miRNA-21-5p和miRNA-574-5p单独检测的诊断价值,表明miRNA-21-5p和miRNA-574-5p联合检测有望有助于肺结节性质的诊断,对肺癌的诊断有一定价值。

综上所述,本研究发现恶性结节患者的血清miRNA-21-5p和miRNA-574-5p水平显著高于良性结节患者,是潜在的血清生物标志物。然而,本研究样本量较小,标本采集具有地域限制;且迄今为止的检测方法在临床上的应用有限。因此,需要进行大规模的临床研究来验证这些标志物的重要性和实用性。本研究将通过扩大样本量、收集更多不同区域的肺结节患者以及延长随访时间的进一步研究来验证结果的准确性。

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