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非小细胞肺癌患者血清 hsa-miR-107 低表达的潜在临床价值

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[摘　要]　**目的：**评估非小细胞肺癌(non-small cell lung cancer，NSCLC)患者血清hsa-miR-107表达水平及临床价值，探究其潜在靶向调控机制。**方法：**基于全球NSCLC血清miRNA和组织mRNA基因芯片及测序数据集，评估hsa-miR-107综合表达水平，计算标准化平均差(standardized mean difference，SMD)，鉴定差异表达miRNA、mRNA。以Kaplan-Meier生存分析评价hsa-miR-107的预后价值。参考萤光素酶报告基因测定和降解组测序结果对hsa-miR-107靶向mRNA进行鉴定，并对hsa-miR-107候选靶基因、NSCLC差异表达基因的交集进行功能注释、蛋白质交互分析。绘制汇总受试者操作特征曲线、Pearson相关系数散点图初步验证hsa-miR-107的靶向枢纽基因。**结果：**与155份正常人血清样本相比，hsa-miR-107在全球145份NSCLC患者血清样本中表达水平明显降低[SMD=−0.49(−0.73~−0.24)]，且对NSCLC有中等区分能力[曲线下面积(area under the curve，AUC)=0.79，95%CI：0.75~0.82]。然而，血清hsa-miR-107高水平表达预示NSCLC患者预后较差(P<0.05，样本数：67)。hsa-miR-107靶基因主要富集于细胞周期通路、DNA复制通路、p53信号通路及细胞衰老通路，其中*CCNE1*、*CDK1*基因与hsa-miR-107呈显著负相关且均在NSCLC中呈高表达，被鉴定为hsa-miR-107靶向通路枢纽基因。**结论：**hsa-miR-107在NSCLC患者血清中低表达且其表达可预示NSCLC患者不良预后。低表达hsa-miR-107可能通过调控细胞周期通路基因*CCNE1*、*CDK1*促进NSCLC进展。

[关键词]　非小细胞肺癌；血清微小RNA；hsa-miR-107；临床意义；潜在机制

Potential clinical value of low expression of serum hsa-miR-107 in patients with non-small cell lung cancer

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Abstract **Objective:** To evaluate the expression level and clinical value of serum hsa-miR-107 in patients with non-small cell lung cancer (NSCLC), and to explore its potential targeted regulation mechanisms. **Methods:** Based on the global NSCLC serum miRNA and tissue mRNA gene chips and sequencing data sets, we assessed the comprehensive expression level of hsa-miR-107 and identified differentially expressed miRNA and mRNA by calculating the standardized mean difference (SMD). The prognostic value of hsa-miR-107 was appraised by Kaplan-Meier survival analysis. The mRNA targets of hsa-miR-107 were identified by referring to the luciferase reporter gene assay and degradome sequencing results. Functional annotation and protein interaction analysis were performed by the intersection genes of hsa-miR-107 candidate targeted genes and NSCLC differentially expressed genes. Summary receiver operating characteristic curve and Pearson correlation coefficient scatter plots were drawn to preliminarily verify the targeted hub genes of hsa-miR-107. **Results:** Hsa-miR-107 was significantly downregulated in 145 serum samples of global NSCLC patients when compared with 155 normal human serum samples [SMD=−0.49 (−0.73 to −0.24)], and had a moderate discriminatory ability [area under the curve (AUC)=0.79, 95%CI 0.75 to 0.82] between NSCLC and normal individuals. However, higher expression level of hsa-miR-107 predicted poor prognosis of NSCLC patients (P<0.05, sample sizes: 67). Hsa-miR-107 targeted genes were mainly enriched in cell cycle pathway, DNA replication pathway, p53 signaling pathway, and cell senescence pathway. Among them, *CCNE1* and *CDK1* were significantly negatively correlated with hsa-miR-107 and were highly expressed in NSCLC sample tissues. Therefore, *CCNE1* and *CDK1* were identified as 2 hub genes in the targeted pathway of hsa-miR-107. **Conclusion:** hsa-miR-107 is lowly expressed in the serum samples of NSCLC patients and its expression level could predict the poor prognosis of lung adenocarcinoma patients. Lowly expressed hsa-miR-107 may promote NSCLC progression by regulating cell cycle pathway genes (i.e., *CCNE1* and *CDK1*).

Keywords non-small cell lung cancer; serum microRNA; hsa-miR-107; clinical significance; potential mechanism

肺癌是当今世界上最为常见的恶性肿瘤之一，其预后普遍较差[1-3]。据2021年世界最新癌症数据报告[4]，2020年全世界估计新增220万肺癌病例和180万肺癌死亡病例，肺癌已成为第2大常见癌症，也是2020年癌症相关死亡的元凶，约占新诊断癌症的1/10和癌症相关死亡人数的1/5。尽管全世界约有2/3的肺癌死亡可归因于吸烟，但迄今为止肺癌的发病机制仍未知晓[5]。2010至2014年多个国家确诊的肺癌患者中，大多数患者在确诊后的5年存活率仅为10%~20%，预后极差[6]。目前，手术和化疗仍是肺癌患者的主要治疗手段[7-8]，近年来各种靶向免疫治疗也为晚期肺癌患者带来福音[9]。然而，临床上有近80%的肺癌患者被诊断时已处于晚期，错失了最佳治疗时机。因此，如何实现早期诊断、早期治疗，这对改善肺癌患者预后至关重要。

微RNA(micro RNA，miRNA)属于非编码RNA，普遍存在于人体中，具有一定的组织特异性，是癌症诊断、治疗的研究热点之一[10-11]。miRNA不仅可在细胞质中参与靶基因转录后调控[12]，还可通过核激活miRNA机制精确调节抑癌基因表达水平[13]。其中，hsa-miR-107(亦作MIRN107、miR-107)与喉癌[14]、卵巢癌[15]、肝细胞癌[16]等多种癌症的发生、进展有密切联系。hsa-miR-107在非小细胞肺癌(non-small cell lung cancer，NSCLC)组织和细胞中显著下调[17-18]，且组织中低表达的hsa-miR-107预示着NSCLC患者不良预后[19]。不仅如此，一项单中心研究[20]提示hsa-miR-107在NSCLC患者血清中同样呈低表达；但遗憾的是，其样本量不足20例，缺乏说服力。迄今为止，血清hsa-miR-107表达在NSCLC患者中的临床价值仍不明确，还有待大样本、多中心研究进行评估和印证；其靶向机制亦有待探究。

本研究旨在印证NSCLC血清hsa-miR-107表达水平并充分评估其临床价值，进一步探究其潜在的靶向调控分子机制。

1 资料与方法

1.1 NSCLC 血清 miRNA 及组织 mRNA 基因芯片及测序数据

通过基因表达数据库(Gene Expression Omnibus，

GEO)、ArrayExpress、癌症基因组图谱(The Cancer Genome Atlas，TCGA)、Oncomine、Sequence Read Archive对肺癌血清及组织miRNA、mRNA数据集进行全数据库检索。纳入标准：1)物种为人类；2)疾病为原发性肺癌；3)标本为组织或血清；4)样本数≥3且必须有正常对照；5)数据类型为miRNA或mRNA表达谱。排除标准：1)已接受治疗(药物治疗、免疫治疗等)；2)样本量不足3例或缺乏对照；3)hsa-miR-107表达值缺失(仅针对miRNA谱而言)。最终纳入的NSCLC miRNA数据集仅包含肺鳞状细胞癌(lung squamous cell carcinoma，LUSC)及肺腺癌(lung adenocarcinoma，LUAD)样本。对所得miRNA、mRNA数据集分别进行对数转换、平台矩阵合并、平台内批次效应移除处理。

1.2 全球 NSCLC 血清及组织差异表达基因鉴定

基于循证理念，本研究从移除批次效应的矩阵中计算获取miRNA、mRNA表达值标准化平均差(standard mean difference，SMD)，并鉴定差异表达miRNA(differentially expressed miRNA，DEmiRNA)及差异表达mRNA(differentially expressed mRNA，DEmRNA)，标准为：|SMD|>0，且其95%CI不包含0。

1.3 NSCLC 患者血清 hsa-miR-107 预后分析

为评估hsa-miR-107在NSCLC患者中的预后性能，本研究利用上述数据库及miRactDB、Kaplan-Meier plotter在线分析平台搜集NSCLC血清miRNA预后表达谱，利用survminer及survival程序包寻找最佳截断值对NSCLC患者进行分组，对hsa-miR-107展开Kaplan-Meier生存分析。

1.4 NSCLC hsa-miR-107 靶基因预测

鉴于miRNA发挥功能得益于其基因调控作用，本研究利用mirecords、mirtarbase、tarbase数据库对hsa-miR-107候选靶基因进行预测，其验证类型包括紫外交联免疫沉淀结合高通量测序(high-throughput sequencing with crosslinking immunoprecipitation，HITS-CLIP)、光活性增强的核糖核苷交联和免疫共沉淀(photoactivatable-ribonucleoside-enhanced crosslinking immunoprecipitation，PAR-CLIP)、萤光素酶报告基因测定、降解组测序等。

1.5 NSCLC hsa-miR-107 在细胞质中介导的靶向调控机制

MiRNA可通过碱基互补配对识别靶基因3'端非翻译区诱导靶基因翻译阻滞[21]，这是miRNA发挥功能的常见机制。本课题组前期研究发现hsa-miR-107在NSCLC中显著下调，故对hsa-miR-107候选靶基因、NSCLC上调基因取交集，所得基因被定义为NSCLC细胞质hsa-miR-107靶基因。

1.6 Hsa-miR-107 靶基因功能注释及蛋白质交互分析

通过基因本体论(Gene Ontology，GO)、京都基因与基因组百科全书(Kyoto Encyclopedia of Genes and Genomes，KEGG)功能注释初步分析NSCLC细胞质hsa-miR-107靶基因潜在分子生物学机制。本研究运用STING v11.5构建蛋白质-蛋白质交互(protein-protein interaction，PPI)网络并基于Cytoscape v3.9.0分析其枢纽基因。

1.7 NSCLC hsa-miR-107 靶基因初步验证

由于hsa-miR-107调控靶标在NSCLC组织的表达水平已通过计算SMD进行初步筛选，故本研究进一步为其靶向枢纽基因绘制汇总受试者操作特征(summary receiver operating characteristic curve，sROC)曲线，分析hsa-miR-107靶标表达水平对NSCLC及正常肺组织的区分能力。分别通过计算TCGA-LUSC及TCGA-LUAD组织样本中hsa-miR-107与调控靶标的Pearson相关系数，用于评价相关性。运用RNA相互作用百科全书预测hsa-miR-107及靶基因匹配序列。

1.8 统计学处理

本研究所有数据分析皆在R v4.0.4、Stata v12.0完成。选取Wilcoxon检验检测NSCLC血清hsa-miR-107表达差异。根据异质性检验结果选取SMD计算所需模型：I^2≤50%，选固定效应模型；I^2>50%，选随机效应模型。绘制sROC曲线图以评估血清hsa-miR-107表达水平对NSCLC的区别能力[曲线下面积(area under the curve，AUC)<0.7，0.7~<0.9，≥0.9分别提示区别能力较弱、中等、较强]。P<0.05为差异有统计学意义。

2 结果

2.1 Hsa-miR-107 在 NSCLC 血清中低表达

经检索，共获取NSCLC血清miRNA数据集4个，分别来自巴西、美国和中国(表1)。图1展示了hsa-miR-107在各数据集中表达下调的趋势以及血清中hsa-miR-107表达水平对NSCLC患者的区别能力。经整合分析发现：与155份正常人血清样本相

比，145份NSCLC患者血清样本中hsa-miR-107表达水平明显降低(图2A)。进一步亚组分析表明：hsa-miR-107在LUAD、LUSC患者血清中均呈低表达，但其下调程度差异无统计学意义(图2B)。sROC曲线(图3A)揭示血清hsa-miR-107对NSCLC具备中等区分能力(AUC=0.79，95%CI：0.75~0.82)，其敏感度、特异度分别达到0.60(95%CI：0.43~0.75)、0.82(95%CI：0.67~0.91)，阳性、阴性似然比分别为3.32(95%CI：1.68~6.57)、0.49(95%CI：0.32~0.75)(图3B、3C)。因此，检测血清hsa-miR-107表达水平对NSCLC有一定的区分价值。

2.2 hsa-miR-107 表达预示 NSCLC 患者预后不佳

GSE198958 NSCLC患者血清miRNA预后分析结果表明：hsa-miR-107在NSCLC患者血清中低表达，预示NSCLC患者预后较好(P<0.05，样本数：67；图4~11)。然而，血清hsa-miR-107表达水平对LUSC患者的预后预测能力并不显著(P>0.05，样本数：21；结果未示)。

2.3 NSCLC hsa-miR-107 靶基因的获取及潜在 miRNA-mRNA 机制

共识别出hsa-miR-107候选靶基因3 859个，经与LUAD、LUSC上调基因交集，共获取NSCLC细胞质hsa-miR-107靶基因195个(图12A)。hsa-miR-107在NSCLC中下调后，其靶基因功能上调，且主要富集于细胞周期通路、DNA复制通路、p53信号通路及细胞衰老通路(表2，图12B、12C)。本研究对细胞周期通路、p53信号通路及细胞衰老通路PPI进行深入探究(图12D~12F)，其中共鉴定出通路枢纽基因15个(*BUB1*、*CCNA2*、*CCNB1*、*CCNB2*、*CCNE1*、*CDC20*、*CDC6*、*CDK1*、*E2F3*、*FOXM1*、*MCM2*、*MCM4*、*MYBL2*、*ORC1*、*RRM2*)。

表1 NSCLC患者血清hsa-miR-107表达数据集纳入信息汇总

Table 1 Summary of inclusion information for serum hsa-miR-107 expression datasets in patients with NSCLC

登录号	LUAD	LUSC	Normal	作者	国家	日期	PMID
GSE152702	21	17	7	Tainara Francini Felix	Brazil	8-Sep-20	32726984
GSE171517	3	3	6	Xiaoxia Zhu	China	6-Apr-21	34306018
GSE27486	22	—	13	Santosh Kumar Patnaik	USA	20-May-11	23029380
GSE40738	45	34	58	Santosh Kumar Patnaik	USA	11-Sep-12	28742859

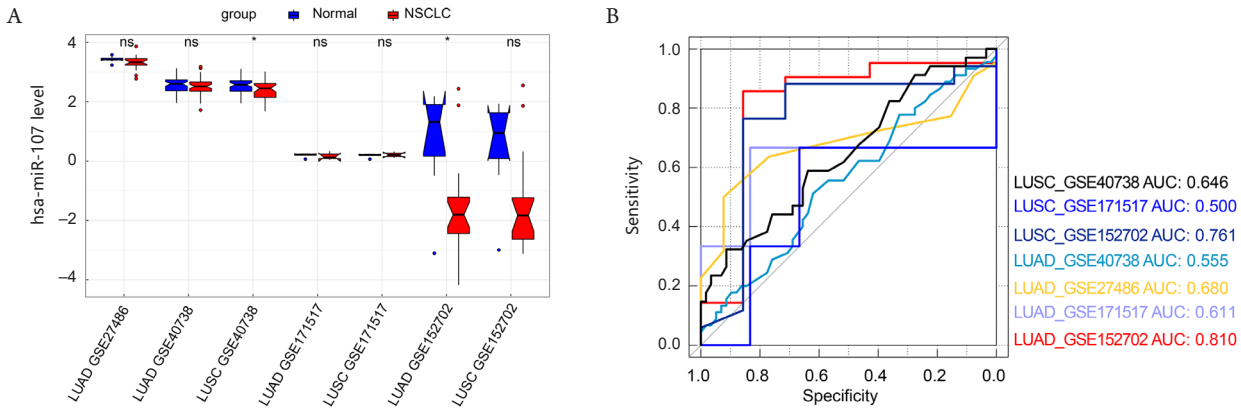


图1 肺癌患者血清hsa-miR-107表达水平及其潜在鉴别能力

Figure 1 Serum expression of hsa-miR-107 in lung cancer patients and its potential discriminatory ability

(A)Hsa-miR-107在肺癌患者血清中表达下降；(B)血清hsa-miR-107表达水平在单个数据集中用于甄别肺癌患者的效能评估。

(A) Expression of hsa-miR-107 was decreased in the serum of lung cancer patients; (B) Efficacy assessment of serum hsa-miR-107 expression levels in a single dataset for identifying lung cancer patients.

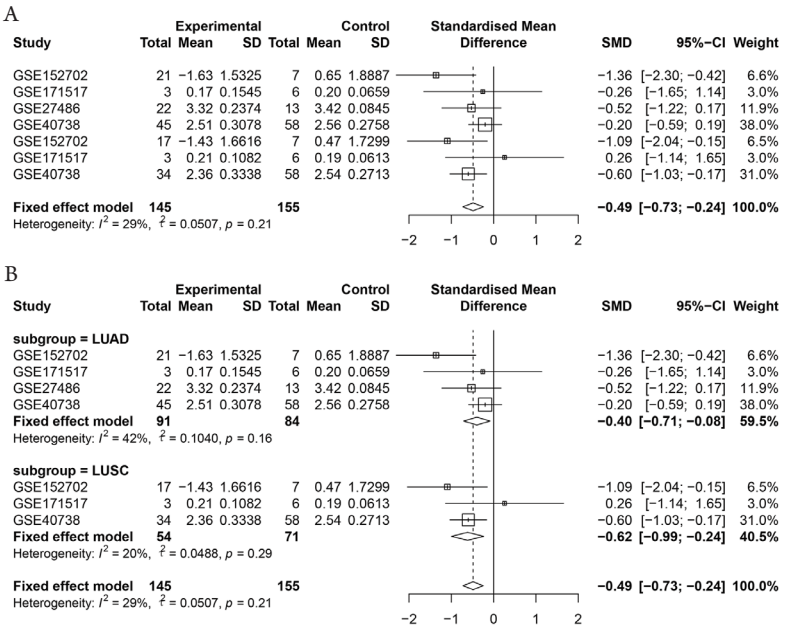


图2 Hsa-miR-107在非小细胞肺癌患者血清中的综合表达水平

Figure 2 Comprehensive expression level of hsa-miR-107 in serum of patients with non-small cell lung cancer

(A)与155例正常对照相比，hsa-miR-107在145例非小细胞肺癌患者血清中显著低表达；(B)hsa-miR-107在肺腺癌、肺鳞癌患者血清中表达水平的亚组分析。

(A) Compared with 155 normal controls, hsa-miR-107 was significantly lower in the serum of 145 NSCLC patients; (B) Subgroup analysis of the expression level of hsa-miR-107 in the serum of patients with lung adenocarcinoma and lung squamous cell carcinoma.

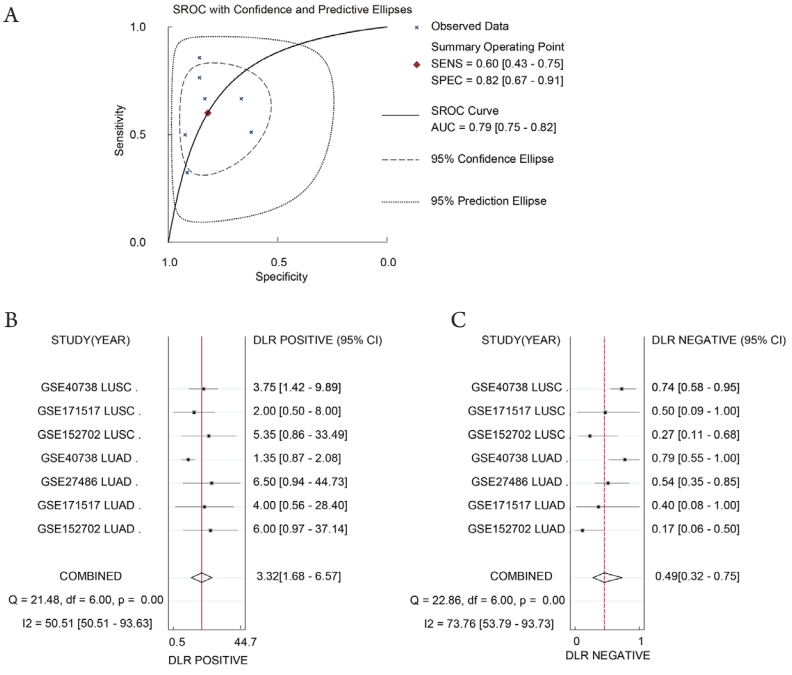


图3 血清hsa-miR-107表达水平对非小细胞肺癌患者鉴别能力的综合评估

Figure 3 Comprehensive assessment of serum hsa-miR-107 expression levels for discriminating ability of patients with non-small cell lung cancer

(A)汇总受试者操作特征曲线；(B)阳性似然比森林图；(C)阴性似然比森林图。

(A) Summary receiver operating characteristic curve; (B) Positive likelihood ratio forest plot; (C) Negative likelihood ratio forest plot.

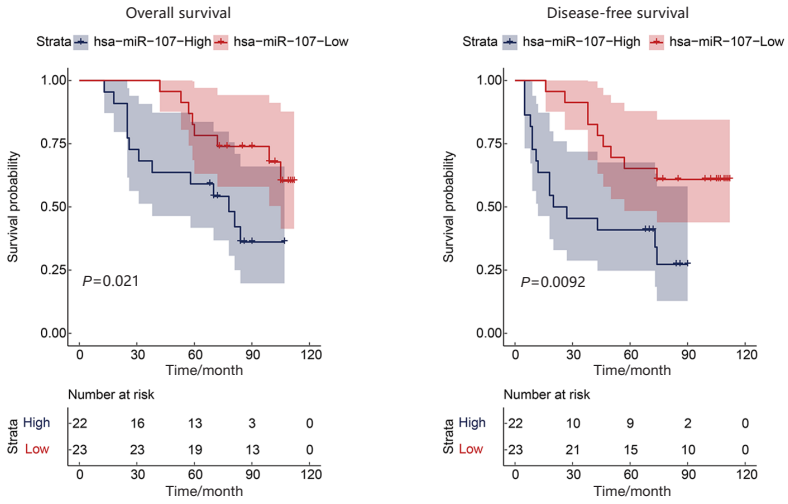


图4 肺腺癌患者血清hsa-miR-107低表达预示患者预后较好(P<0.05)

Figure 4 Low expression of serum hsa-miR-107 predicted a better prognosis in patients with lung adenocarcinoma (P<0.05)

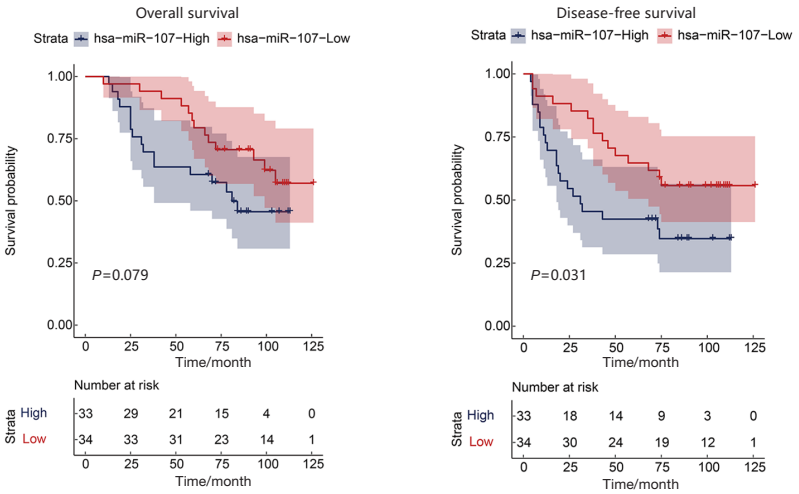


图5 NSCLC患者血清hsa-miR-107低表达预示患者预后较好(P<0.05)

Figure 5 Low expression of serum hsa-miR-107 predicted a better prognosis in patients with NSCLC (P<0.05)

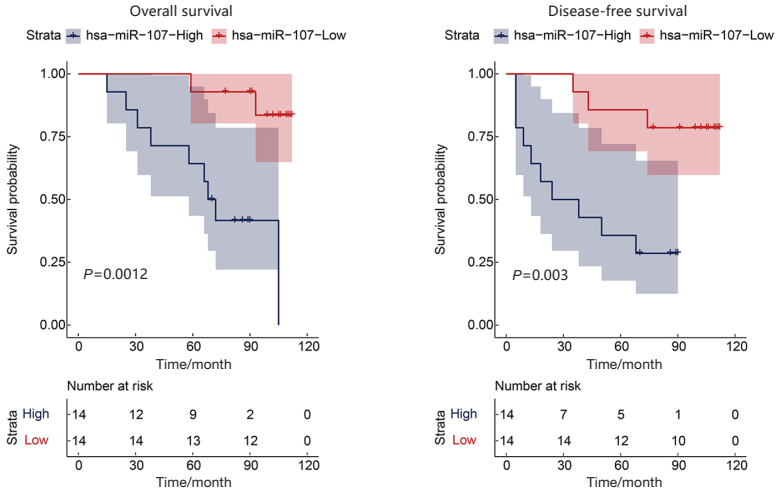


图6 I期NSCLC患者血清hsa-miR-107低表达预示患者预后较好(P<0.05)

Figure 6 Low expression of serum hsa-miR-107 predicted a better prognosis in patients with stage I NSCLC (P<0.05)

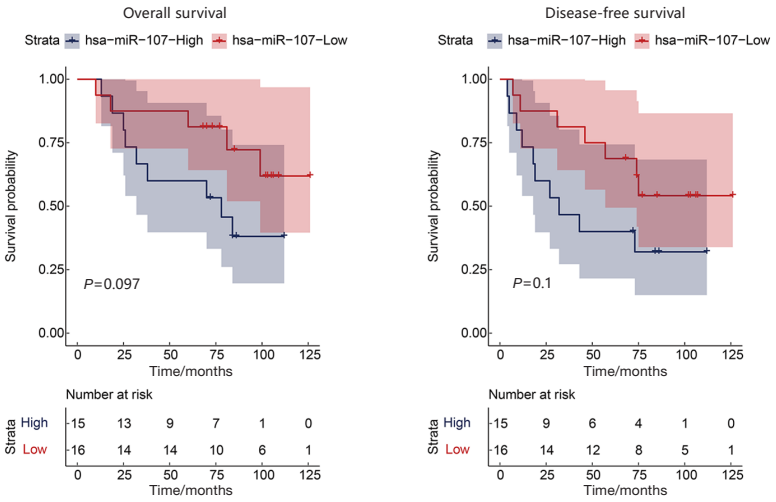


图7 II期NSCLC患者血清hsa-miR-107低表达预示患者预后较好(P>0.05)

Figure 7 Low expression of serum hsa-miR-107 predicted a better prognosis in patients with stage II NSCLC (P>0.05)

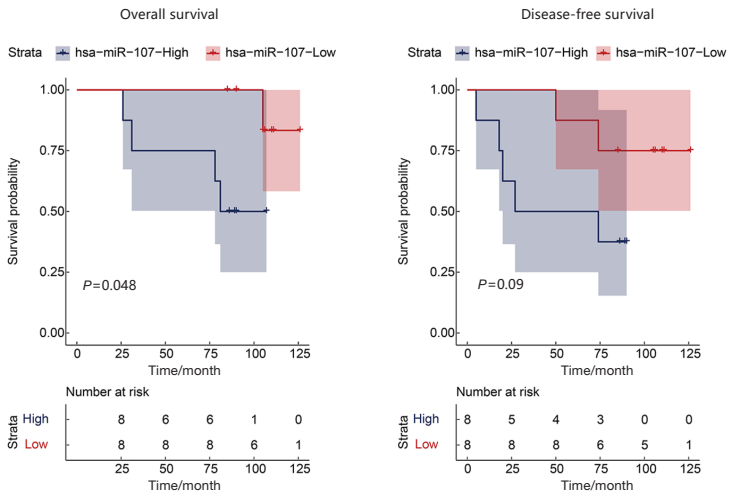


图8 NSCLC女性患者血清hsa-miR-107低表达预示患者预后较好(P<0.05)

Figure 8 Low expression of serum hsa-miR-107 predicted a better prognosis in female patients with NSCLC (P<0.05)

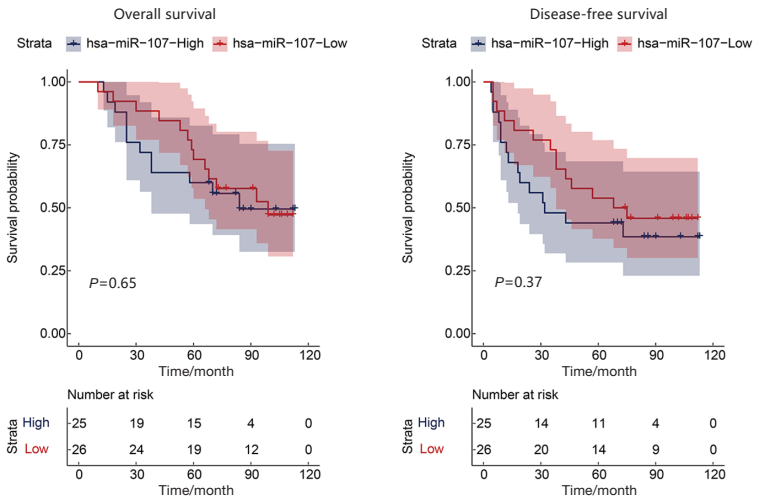


图9 NSCLC男性患者血清hsa-miR-107低表达预示患者预后较好(P>0.05)

Figure 9 Low expression of serum hsa-miR-107 predicted a better prognosis in male patients with NSCLC (P>0.05)

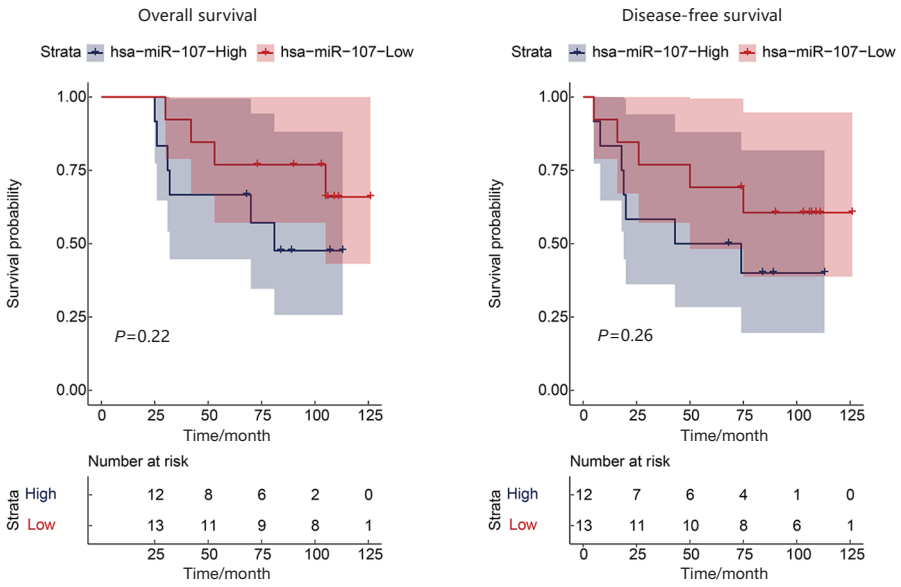


图10 年龄≤65岁NSCLC患者血清hsa-miR-107低表达预示患者预后较好(*P*>0.05)

Figure 10 Low expression of serum hsa-miR-107 predicted a better prognosis in patients with NSCLC aged ≤65 years (*P*>0.05)

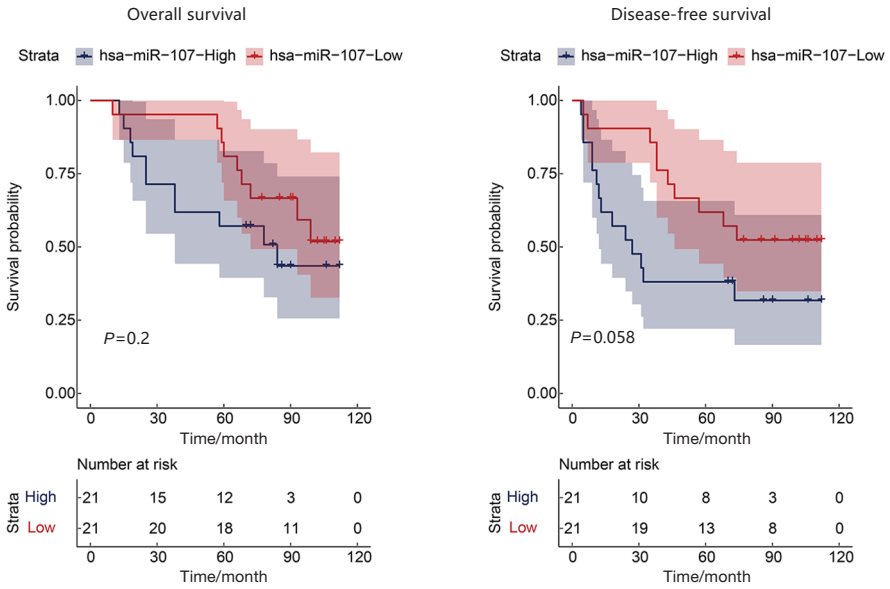


图11 年龄>65岁NSCLC患者血清hsa-miR-107低表达预示患者预后较好(*P*>0.05)

Figure 11 Low expression of serum hsa-miR-107 predicted a better prognosis in patients with NSCLC aged >65 years (*P*>0.05)

2.4 NSCLC hsa-miR-107 靶基因验证

与hsa-miR-107在NSCLC血清的低表达相反，其假定靶向枢纽基因在NSCLC组织中均为高表达(表3)，其中*CCNB1*、*CCNB2*各为LUSC、LUAD组织中表达上调水平最高的预测靶基因。*CCNE1*、*CDK1*对NSCLC组织区分性良好，且对LUSC的区分性能优于LUAD(图13A~13D)。LUSC组织中hsa-miR-107与*CCNE1*、*CDK1*表达水平呈负相关(图13E、13F)，但在LUAD组织中负相关程度则不明显($R<0$，$P>0.05$)，未予展示。最后，本研究成功预测了hsa-miR-107与*CCNE1*的匹配序列(图13G)。

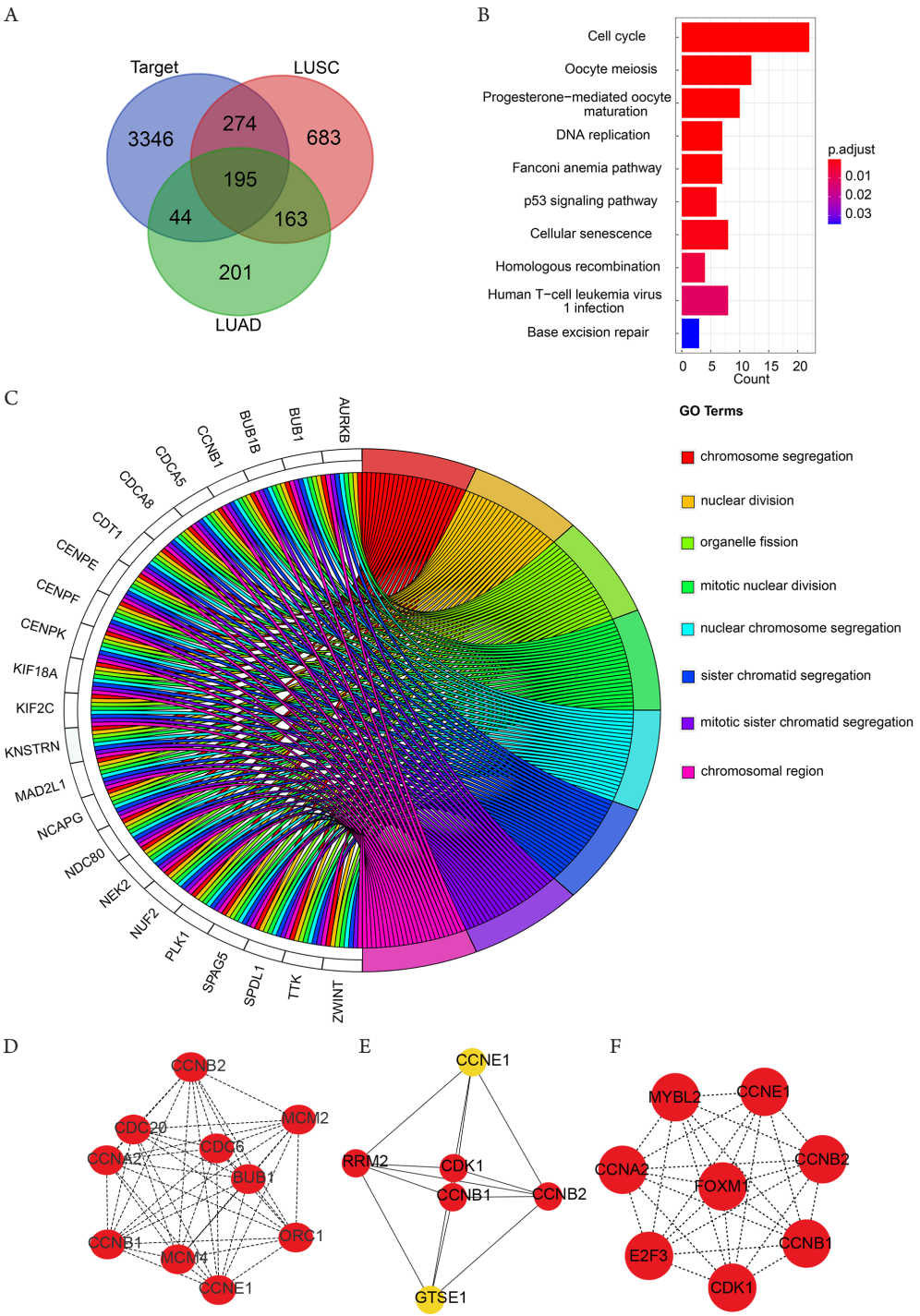


图12 NSCLC患者血清中的hsa-miR-107潜在靶向调控机制

Figure 12 Potential target regulatory mechanism of hsa-miR-107 in the serum of patients with NSCLC

(A)肺鳞癌、肺腺癌组织中hsa-miR-107靶向差异表达基因韦恩图；(B)KEGG通路富集结果；(C)GO富集结果；(D)细胞周期通路蛋白质互作网络分析；(E)p53信号通路蛋白质互作网络分析；(F)细胞衰老通路蛋白质互作网络分析。D~F：红色示通路枢纽基因。

(A) Venn diagram of differentially expressed genes targeted by hsa-miR-107 in lung squamous cell carcinoma and lung adenocarcinoma; (B) KEGG pathway enrichment results; (C) GO enrichment results; (D) Cell cycle pathway protein-protein interaction network analysis; (E) Analysis of protein-protein interaction network of p53 signaling pathway; (F) Analysis of protein-protein interaction-network in cellular senescence pathways. D–F: Pathway hub genes are shown in red.

表2 NSCLC患者血清hsa-miR-107靶基因功能注释结果

Table 2 Results of functional annotation of hsa-miR-107 target genes in serum of patients with NSCLC

术语	ID	描述	基因比率	校正*P*	*q*
GO BP	GO:0007059	Chromosome segregation	61/190	5.12E-57	4.21E-57
GO BP	GO:0000280	Nuclear division	65/190	4.93E-56	4.05E-56
GO CC	GO:0098687	Chromosomal region	50/193	1.69E-41	1.26E-41
GO CC	GO:0000775	Chromosome, centromeric region	40/193	2.17E-39	1.62E-39
GO MF	GO:0016887	ATPase activity	28/183	3.36E-13	2.93E-13
GO MF	GO:0008017	Microtubule binding	23/183	3.36E-13	2.93E-13
KEGG	hsa04110	Cell cycle	22/75	6.44E-21	5.78E-21
KEGG	hsa04114	Oocyte meiosis	12/75	1.06E-07	9.53E-08
KEGG	hsa04914	Progesterone-mediated oocyte maturation	10/75	7.45E-07	6.69E-07
KEGG	hsa03030	DNA replication	7/75	7.45E-07	6.69E-07
KEGG	hsa03460	Fanconi anemia pathway	7/75	1.11E-05	9.95E-06
KEGG	hsa04115	p53 signaling pathway	6/75	0.0009	0.0008
KEGG	hsa04218	Cellular senescence	8/75	0.0013	0.0012

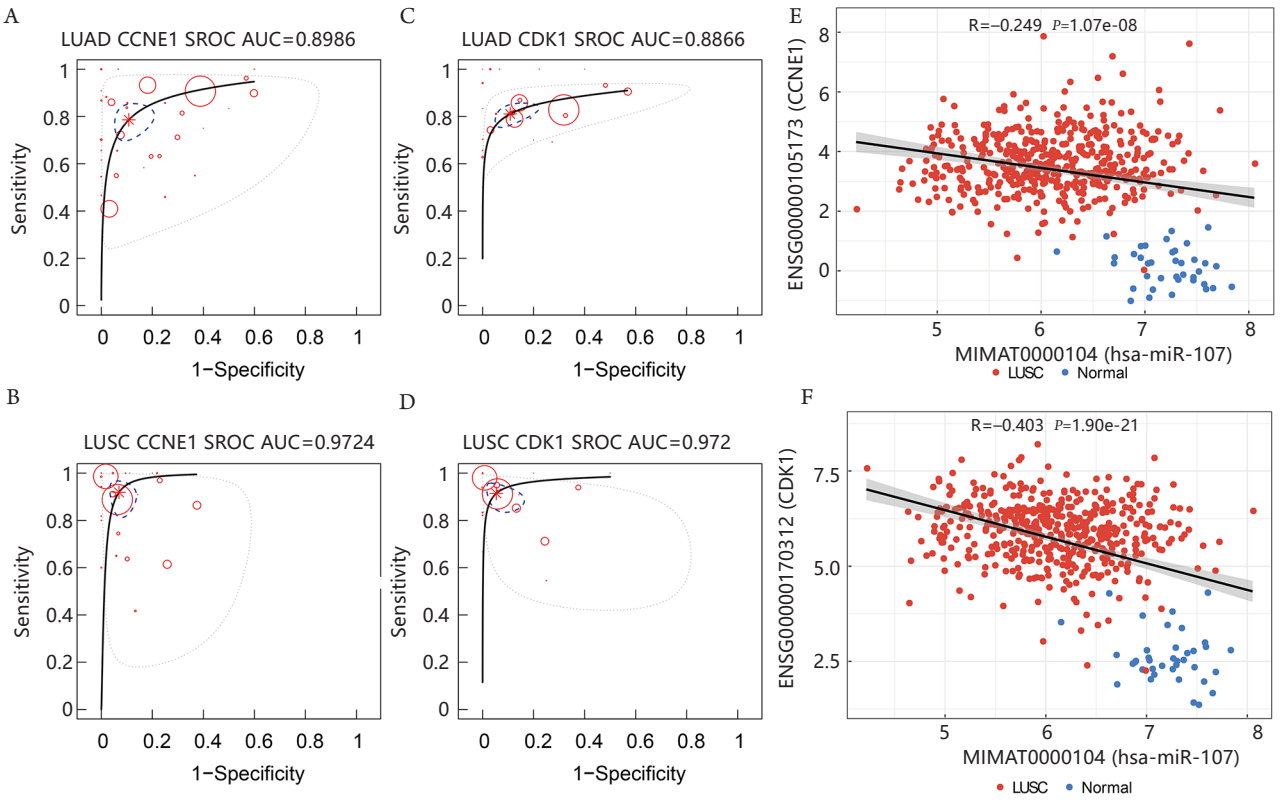
BP：生物学过程；CC：细胞成分；MF：分子功能。

BP: Biological process; CC: Cellular component; MF: Molecular function.

表3 NSCLC组织中hsa-miR-107靶向枢纽基因表达水平

Table 3 Expression levels of hsa-miR-107-targeted hub genes in NSCLC tissue samples

基因	LUAD				LUSC			
	SMD	CI下限	CI上限	I^2/%	SMD	CI下限	CI上限	I^2/%
BUB1	2.44	1.86	3.02	97.13	1.39	1.12	1.66	94.26
CCNA2	2.37	1.67	3.07	97.82	1.43	1.15	1.71	94.68
CCNB1	2.39	1.75	3.03	97.81	1.58	1.24	1.92	95.38
CCNB2	2.57	1.86	3.28	98.03	1.40	1.11	1.68	94.91
CCNE1	1.77	1.23	2.31	97.34	1.20	0.95	1.46	93.68
CDC20	2.07	1.39	2.74	98.10	1.36	1.09	1.64	94.42
CDC6	2.54	1.88	3.20	97.62	1.33	1.07	1.60	93.82
CDK1	2.06	1.36	2.77	97.81	1.42	1.19	1.65	88.28
E2F3	1.24	0.95	1.53	91.22	1.07	0.81	1.33	93.91
FOXM1	2.42	1.73	3.11	97.91	1.39	1.12	1.66	94.12
MCM2	2.23	1.64	2.83	97.49	1.20	0.94	1.47	94.27
MCM4	2.52	1.90	3.14	97.51	1.56	1.24	1.88	94.78
MYBL2	2.29	1.62	2.96	98.08	1.15	0.87	1.43	94.52
ORC1	1.85	1.09	2.61	98.27	1.11	0.79	1.44	94.80
RRM2	2.45	1.84	3.07	97.51	1.40	1.18	1.63	90.31



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BindingSite	Type	Alignment
chr19:29824023-29824029[+]	7mer-m8	Target: 5' CAGUGCGUGCUCCCGAUGCUGCU 3' :|: : |:||||||| miRNA : 3' ACUAUCGGGACAUGUUACGACGA 5'

图13 Hsa-miR-107在NSCLC中的靶向mRNA调控机制

Figure 13 Targeted mRNA regulation mechanism of hsa-miR-107 in NSCLC

(A、B)*CCNE1*对LUAD及LUSC的区分度；(C、D)*CDK1*对LUAD及LUSC的区分度；(E、F)LUSC中hsa-miR-107与*CCNE1*、*CDK1*表达水平呈负相关。Hsa-miR-107与LUAD中的*CCNE1*、*CDK1*表达水平负相关程度不明显(R<0，P>0.05)，未予展示；(G)Hsa-miR-107与*CCNE1*的匹配序列。Hsa-miR-107与靶基因的匹配序列经RNA相互作用百科全书预测。

(A, B) Discrimination of LUAD and LUSC by *CCNE1*; (C, D) Discrimination of LUAD and LUSC by *CDK1*; (E, F) Hsa-miR-107 was significantly negatively correlated with the expression levels of *CCNE1* and *CDK1* in LUSC. The negative correlation between hsa-miR-107 and the expression levels of *CCNE1* and *CDK1* in LUAD was insignificant (R<0, P>0.05), which was not shown; (G) Matching sequence of hsa-miR-107 to *CCNE1*. Matching sequences of hsa-miR-107 to target genes were predicted by the Encyclopedia of RNA Interactomes.

3 讨论

NSCLC占肺癌病例的近85%，且多数患者被发现时已出现局部进展和转移，其预后不佳[22]。目前，化疗是晚期NSCLC患者的保守治疗方案；尽管免疫检查点抑制剂[23]、小干扰RNA疗法[24]等治疗方式也如雨后春笋般涌现，但其面临的挑战依旧十分巨大。因此，寻找有效的NSCLC早期诊断生物学标志物、为晚期患者提供更多治疗干预靶点任重道远[25-26]。本研究通过检测血清miRNA表达水平，hsa-miR-107或可用于甄别部分NSCLC患者。事实上，利用血清miRNA检测技术对肺癌进行早期诊断已经早有研究[27-29]，但可能限于其诊断效能较低、成本较高等原因，目前还难以推广。寻找更多高效、敏感、简便的早期检测指标对于NSCLC早期诊断至关重要。本研究采集了来自巴西、美国和中国3个国家总共145例NSCLC样本及155例正常人血清对照样本，充分印证了hsa-miR-107在NSCLC患者血清内显著的低表达水平[20]。不仅如此，血清hsa-miR-107对NSCLC

患者及正常人还具有中等区分度。因此，在未来有望通过检测血清hsa-miR-107水平识别出部分NSCLC患者；对于NSCLC确诊患者，还有可能通过检测其血清hsa-miR-107水平预测预后状况。

本研究不仅印证了hsa-miR-107在NSCLC血清的低表达趋势，还首次对其靶向调控机制展开挖掘。前人对NSCLC中hsa-miR-107的研究较为局限，对其机制知之甚少。研究[30]发现：m6A去甲基化酶ALKBH5可通过降低YTHDFs介导的YAP表达和抑制NSCLC中hsa-miR-107/LATS2介导的YAP活性来抑制肿瘤生长和转移。还有研究[31]指出：hsa-miR-107同FOXC2-AC1相互调控，从而共同影响肺癌恶性进展。Hsa-miR-107通过与其靶基因相互作用，从而在NSCLC进展中饰演多种角色，譬如，长链非编码RNA H19可直接通过竞争性内源RNA机制下调hsa-miR-107从而促进NSCLC的细胞周期进程[32-33]。类似地，长链非编码RNA FGD5-AS1可通过吸附hsa-miR-107并上调FGFRL1从而促进NSCLC细胞增殖[17]。与之相反，circHIPK3敲除可介导hsa-miR-107上调继而抑制NSCLC细胞迁移和增殖能力[18]。还有研究[34-35]表明：诱导hsa-miR-107过表达使得A549 NSCLC细胞针对铂类药物以及抗增殖类药物小白菊内酯敏感度增加。上调的hsa-miR-107还可显著抑制A549和H1299 NSCLC细胞进展，并降低抑癌基因Rb磷酸化水平[18]。这些研究均表明hsa-miR-107可参与促进NSCLC的恶化。然而，关于hsa-miR-107在NSCLC进展中的靶向调控机制却罕有报道。在本研究中，作者立足于经实验验证的hsa-miR-107靶基因、NSCLC组织上调编码基因，对NSCLC中hsa-miR-107参与的细胞周期、p35信号通路、细胞衰老等通路基因的负性调控进行了首次探究。众所周知，细胞周期进程异常激活与癌症细胞无限增殖密切相关[36]。Hsa-miR-107与细胞周期的联系在膀胱癌[37]、卵巢癌[38]、尤文肉瘤[39]等多种癌症中已见报道，且大部分研究[40-41]提示hsa-miR-107本身可能介导细胞周期阻滞。在NSCLC细胞中，hsa-miR-107不仅呈时间和剂量依赖性抑制癌细胞增殖能力，还可将细胞周期阻滞在G_0和G_1相[42]。本研究揭示了NSCLC组织中*CCNE1*与hsa-miR-107的表达呈负相关及其碱基互补配对序列，表明*CCNE1*有可能作为hsa-miR-107的调控靶标。无独有偶，在NSCLC A549细胞系中，研究人员[42-43]通过双荧光素酶报告基因实验及实时荧光定量PCR检测，同样发现hsa-miR-107对*CCNE1*的靶向调控关系。不仅如此，利用小干扰RNA削弱A549细胞*CCNE1*的表达后，A549细胞增殖能力受到明显抑制[42]；这提示hsa-miR-107有可能介导*CCNE1*下调并削弱NSCLC细胞增殖能力。此外，也有研究报道了hsa-miR-107在前列腺癌[44]、卵巢癌[38]中靶向*CCNE1*介导的细胞周期、细胞增殖阻滞作用。因此，NSCLC中低表达的hsa-miR-107极有可能因其细胞周期阻滞作用遭到削弱，进而诱发NSCLC异常的细胞复制和增殖，由此促进NSCLC快速进展。此外，hsa-miR-107与p53抑癌信号通路也有联系。譬如，p53可通过提高hsa-miR-143/hsa-miR-107水平降低Musashi RNA结合蛋白2的表达；而利用天然抗生素Mithramycin A治疗宫颈癌细胞可增加p53和hsa-miR-143/hsa-miR-107表达并降低Musashi RNA结合蛋白2表达，导致宫颈癌细胞增殖、侵袭和球体形成受抑[45]。由此表明hsa-miR-107不仅有望成为NSCLC的早期诊断指标，还有可能作为治疗干预靶点。

总而言之，hsa-miR-107在NSCLC患者血清中低表达且预示患者不良预后。低表达hsa-miR-107可能通过调控细胞周期通路基因促进NSCLC进展。

然而，本研究尚有不足之处。譬如，作者所鉴定的NSCLC hsa-miR-107靶基因是基于前人的实验结果，虽经过了NSCLC SMD表达水平验证，但未得到临床标本检测等进一步证实。未来还需要开展体内、体外实验对此展开深入探讨。

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