体积越大,脱落率越高。Gunes 等[4]随访 12 个月,发现曼月乐在子宫肌瘤患者中的脱落率为 9.5%,脱器的原因可能是与曼月乐型号单一,与子宫的大小不符合有关。本研究脱器率为 5%,处于较低水平,这和本研究不选择有黏膜下肌瘤的患者和宫腔深度 > 9cm 的患者有关,建议在选择病例时要慎重,同时要研发多种型号的曼月乐以减少脱器率。

本研究结果表明,曼月乐在子宫肌瘤患者中的使用可以明显缓解临床症状,既减少子宫肌瘤手术率,避免了手术带来的相关风险,又保留了生育功能,提高了生活质量,是广大子宫肌瘤患者更愿意接受的一种保守治疗方案,值得临床推广,但是其对内分泌的影响尚待进一步研究。

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食管鳞状细胞癌中 miRNA – 375 的差异表达

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摘 要 目的 分析 microRNA(miRNA)表达谱在食管鳞状细胞癌及正常食管黏膜上皮组织中的差异。**方法** 8 例食管鳞状细胞癌组织及相应正常食管黏膜上皮经 microRNA 芯片技术检测分析差异 miRNA,并扩大样本量通过反转录 PCR 和荧光实时定量 PCR 技术验证差异表达的 miRNA。**结果** 芯片检测结果显示在食管鳞状细胞癌组织内明显下调的 miRNA 有 27 个,上调的 miRNA 有 16 个,并且下调差异倍数最大的为 miR – 375,通过荧光实时定量 PCR 技术验证 miR – 375 在食管鳞状细胞癌中明显下调($P < 0.005$)。**结论** miR – 375 在食管鳞状细胞癌的发生发展中发挥着关键的作用,为进一步研究食管鳞状细胞癌发生的分子机制提供依据。

关键词 食管鳞状细胞癌 miRNA 荧光实时定量 PCR

Differentiated Dysregulation of miRNA – 375 in Esophageal Squamous Cell Carcinoma. *Yao Li, Li Yanhong, Gong Li, Zhu Shaojun, Han Xiujuan, Lan Miao, Zhang Wei. Department of Pathology, Tangdu Hospital, The Fourth Military Medical University, Shaanxi 710038, China*

Abstract Objective To determine the involvement of microRNA (miRNA) in the development and progression of esophageal squamous cell carcinoma (ESCC). **Methods** The expression profiles of miRNA in ESCC tissues and corresponding normal esophageal tissues were analyzed by miRNA microarray. The selected candidate miRNAs were validated in 64 pairs of primary ESCC samples using

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SYBR – green quantitative PCR. **Results** Forty – three differential miRNAs including 27 down – regulated and 16 up – regulated miRNAs were found in ESCCs. Among them, the expression level of miR – 375 was significantly decreased ($P < 0.005$). **Conclusion** The down-regulation of miR – 375 was a frequent event in ESCC. These findings suggest that miR – 375 play important roles in the development and progression of ESCC.

Key words Esophageal squamous cell carcinoma; miRNA; qRT – PCR

食管癌是人类常见的恶性肿瘤之一,在全球范围内,食管癌在恶性肿瘤中的发生率和病死率分别为第8位和第6位[1]。食管癌的复发率很高并且预后差,5年生存率仅为14%[2]。食管癌约有90%的组织学类型为腺癌和鳞状细胞癌。在我国食管癌主要以鳞状细胞癌为主,尤其太行山南端3省交界地区更成为全球发病率最高的区域之一[3,4]。microRNA(miRNA)分子是一种单链、非编码的长约17~25nt小RNA分子,在多种肿瘤发生中发挥着关键性的作用,被认为是一组新的致癌基因或抑癌基因[5]。本研究应用microRNA芯片技术检测食管鳞状细胞癌组织和相对应正常食管黏膜上皮miRNA差异表达。以期为阐明食管鳞状细胞癌发生的分子机制提供新的依据。

材料与方法

1. 标本:食管鳞状细胞癌标本取自第四军医大学唐都医院2011~2012年手术标本。正常食管黏膜上皮标本取自同例手术标本最远端切缘。标本经10%甲醛固定,常规石蜡包埋,切片,HE染色后,显微镜下观察确定癌组织含量最多组织蜡块和完全正常食管黏膜上皮组织蜡块。

2. 芯片制备:提取样本总RNA后,使用miRNA Complete Labeling and Hyb试剂盒,对其中的miRNA进行标记。100ng标志产物同Agilent Human miRNA基因芯片(Version 16.0)杂交,随后进行洗涤,Agilent Microarray扫描仪进行扫描,数据转换。

3. 总RNA提取:5μm新鲜石蜡切片,8片,经二甲苯,100%乙醇脱蜡处理后,加240μl Buffer PKD,10μl蛋白酶K,56℃,孵育15min,80℃,15min,冰上放置3min。取上清液,加25μl DNase Booster Buffer,10μl DNase Ⅰ,上下翻转混匀,室温放置15min。加500μl Buffer RBC,1750μl 100%乙醇,混匀。加入RNeasy MinElute spin,10000r/min离心15s。经Buffer RPE洗涤后,加20μl去RNase水,洗脱RNA。

4. 反转录PCR和实时定量PCR:反转录20μl体系,2μg模版RNA,4μl miScript Hispec RT Buffer,2μl Nucleics Mix,2μl Miscript RT Mix,8μl RNase – free water。反应条件为37℃60min、90℃5min。产物cDNA进行荧光定量PCR扩增,反应体系为20μl,反应条件为预热95℃15min,进入PCR循环,循环参数为94℃15s、55℃30s、70℃35s,循环40次,管家基因U6作为内参基因,应用$2^{-\Delta\Delta Ct}$法计算差异倍数。

5. 统计学方法:数据分析采用SPSS统计软件,应用配对t检验,以$P < 0.05$为差异有统计学意义,倍数差异>2.0为显著表达差异。

结　　果

1. miRNA基因芯片结果分析:8对食管鳞状细胞癌及相同患者的正常食管黏膜上皮作为对照,通过miRNA基因芯片检测并统计分析后结果显示:表达差异大于2倍的miRNA分子共43个,包括27个在癌组织里低表达miRNA和16个在癌组织中高表达miRNA(图1)。表达差异倍数大于4倍的miRNA分子共24个,9个在癌组织中表达上调和15个在癌组织中表达下调(表1)。聚类分析结果显示miRNA表达谱在食管鳞状细胞癌组织同正常食管黏膜上皮两组内分类明显(图2)。

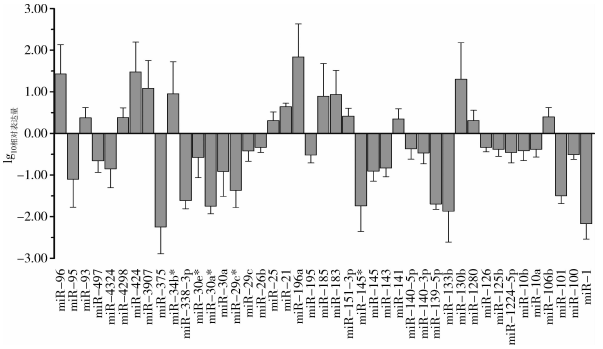

图1　食管鳞状细胞癌同正常食管黏膜上皮的miRNA芯片结果分析

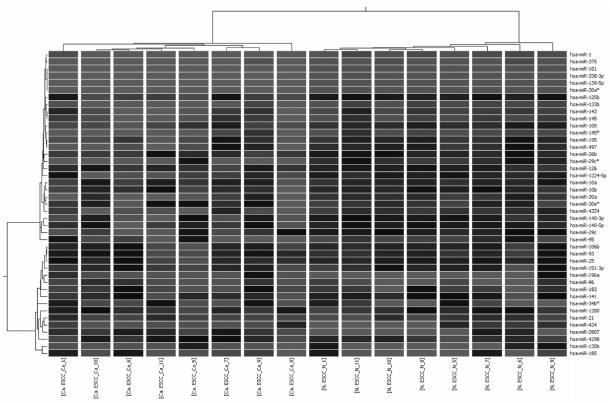

图2　miRNA基因芯片聚类分析结果

表 1 表达差异大于 4 倍的 miRNA 分子

miRNA	差异倍数
下调 miRNA	
miR－375	175.8322
miR－1	147.16624
miR－133b	73.55917
miR－30a*	55.60582
miR－145*	55.024837
miR－139－5p	49.534527
miR－338－3p	40.948067
miR－101	31.244616
miR－29c*	23.400047
miR－95	12.73129
miR－30a	8.1511965
miR－145	8.024183
miR－4324	7.0982347
miR－143	6.7646494
miR－497	4.562447
上调 miRNA	
miR－196a	69.18334
miR－424	30.001791
miR－96	26.955471
miR－130b	20.232624
miR－3907	12.135655
miR－34b*	9.071809
miR－183	8.742586
miR－185	7.89331
miR－21	4.416791

2. 实时定量 PCR 法验证：选取在癌组织中表达下调倍数最高的 miR－375，在 64 对食管鳞状细胞癌标本及配对的正常食管黏膜上皮应用 $2^{-\Delta\Delta Ct}$ 法，U6 基因作为内参基因，通过实时定量 PCR 法验证基因芯片结果。结果显示同 miRNA 基因芯片检测结果一致，miR－375 在食管鳞状细胞癌中明显下调，差异倍数为 173 倍（图 3）。

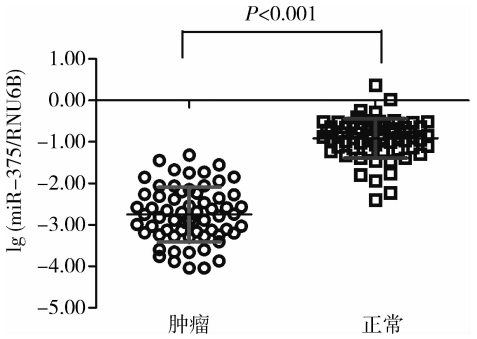

图 3 实时荧光定量 PCR 法检测 miR－375 表达差异

讨 论

食管癌约有 90% 的组织学类型为腺癌和鳞状细胞癌[2]。鳞状细胞癌主要分布在社会经济水平低的亚洲和非洲地区，危险因素包括吸烟、饮酒、食用被致癌物或霉菌污染的食物、热饮、膳食失衡、遗传易感背景和某些慢性消化系统疾病等[6]。microRNA 分子是一种单链、非编码的长约 17～25nt 小 RNA 分子，在转录后水平对 mRNA 进行调控。单链 microRNA 与 RISC 结合形成复合体后，通过与靶基因的 3′－UTR 区互补配对后对 mRNA 进行切割或者翻译抑制[7]。研究表明，microRNA 分子在多种肿瘤发生中发挥着关键性的作用，被认为是一组新的致癌基因或抑癌基因。在本实验中对食管鳞状细胞癌 miRNA 基因表达谱芯片分析发现，与正常食管黏膜上皮相比，差异表达最明显的 miRNA 是 miR－375。进一步通过荧光定量 PCR 技术验证，结果显示在食管鳞状细胞癌组织中 miR－375 确实存在较大程度的下调。其他肿瘤相关研究显示 miR－375 在胃癌、肝癌和头颈部癌中表达下调，在 Barrett's 食管中也观察到 miR－375 明显低表达[8～11]。本研究结果显示 miR－375 在食管鳞状细胞癌中表达下调，提示 miR－375 可能与食管鳞状细胞癌的发病机制有关。为食管鳞状细胞癌的预防、诊断和治疗提供新的思路和靶点。

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在母胎界面滋养细胞通过调节 Th1/Th2 及 Th17 免疫参与妊娠免疫耐受机制的研究

刘凤娟　王志华　仝进毅　董旻岳

摘　要　目的　本研究旨在探讨滋养细胞对 T 淋巴细胞细胞因子产生和平衡的影响,以及滋养细胞对 T 淋巴细胞特异性转录因子的影响。**方法**　分离早孕胎盘细胞滋养细胞,收集滋养细胞培养上清液为滋养细胞条件培养基,供淋巴细胞培养用。分离健康未孕妇女的外周血单个核细胞,用人 T 细胞富集柱纯化 $CD3^+$ T 淋巴细胞。以佛波酯(PMA)刺激 T 淋巴细胞,酶联免疫吸附分析法测定淋巴细胞产生的细胞因子浓度,实时定量聚合酶链反应检测 T 淋巴细胞中 Th1、Th2 和 Th17 免疫特异性转录因子水平。**结果**　滋养细胞条件培养基显著减少 T 淋巴细胞产生 IL - 2、IFN - γ 和 TNF - α,IL - 4 和 IL - 10 的水平无统计学差异,而 Th1/Th2(IL - 2/IL - 4、IFN - γ/IL - 4、TNF - α/IL - 4、IL - 2/IL - 10、IFN - γ/IL - 10 和 TNF - α/IL - 10)显著降低;滋养细胞条件培养基显著抑制 T 淋巴细胞产生 IL - 17。滋养细胞条件培养基显著降低 T 淋巴细胞 Th1 特异性转录因子 STAT - 4 的表达,显著增强 Th2 特异性转录因子 STAT - 6 和 GATA - 3 表达;但对 Th1 特异性转录因子 T - bet、Th17 特异性转录因子 RORC 无显著性影响。**结论**　滋养细胞主要通过抑制 T 淋巴细胞 Th1 细胞因子产生而使 Th1/Th2 平衡向 Th2 漂移,并且抑制 Th17 免疫,在妊娠免疫耐受中起着重要的作用。

关键词　滋养细胞　Th1 免疫　Th2 免疫　Th17 免疫

Placental Trophoblasts Regulated the Balance of Th1/Th2 Immunity and Th17 Immunity at the Fetomaternal Interface. *Liu Fengjuan, Wang Zhihua, Tong Jinyi, Dong Minyue. The First People's Hospital of Hangzhou, Zhejiang 310006, China*

Abstract　Objective　To clarify the effect of placental trophoblasts on T lymphocyte by observing the alteration in the production of cytokines and the expression of specific transcription factors for Th1, Th2 and Th17 immunity in T lymphocyte. **Methods**　Placental trophoblasts were isolated from chorionic villi of normal pregnancy and conditioned medium was made after 72 hours culture of trophoblast. PBMC were isolated from healthy female donors and T lymphocytes were purified and cultured in the presence or absence of conditioned medium. Enzyme - linked sorbent immune assay (ELISA) was used to detect the concentration of IL - 2, TNF - γ, IFN - α, IL - 4, IL - 10 and IL - 17 in supernatants of T cell culture and real - time PCR was used to detect the expression of specific transcription factors for Th1 immunity (T - bet and STAT - 4), Th2 (GATA - 3 and STAT - 6) and Th17 (RORC) in T lymphocyte. **Results**　The level of IL - 2, IFN - γ, TNF - α and IL - 17 was significantly decreased when the T lymphocytes were cultured in conditioned medium compared with control medium, while the level of IL - 10 and IL - 4 were comparable. The presence of conditioned medium decreased the ratio of Th1/Th2 as indexed by IL - 2/IL - 4, IFN - γ/IL - 4, TNF - α/IL - 4、IL - 2/IL - 10、IFN - γ/IL - 10 and TNF - α/IL - 10 compared with control medium. The expression of GATA - 3 and STAT - 6 were significantly increased and STAT - 4 was reduced when T cells were cultured in conditioned medium, while the expression of T - bet and RORC were comparable. **Conclusion**　Placental trophoblast - induced shift of Th1/Th2 balance toward Th2 and inhibition of Th17 might be among the mechanisms involved in maternal tolerance to fetus.

Key words　Trophoblast; Th1 immunity; Th2 immunity; Th17 immunity

妊娠是一种特殊的 Th2 现象。正常妊娠时,母体的 Th2 型免疫应答占优势,Th1 型免疫受到抑制,使 Th1/Th2 平衡向 Th2 漂移。在母胎界面,Th2 型细胞

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