新疆南部维吾尔族宫颈鳞癌microRNA研究

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［**摘要**］　**背景与目的：**宫颈癌是新疆地区尤其是新疆南部维吾尔族高发肿瘤，发病机制尚不清楚。微小RNA(microRNA，miRNA)是一类具有重要调控作用的非编码小分子RNA，其表达及功能失调与肿瘤的发生、发展密切相关。本研究筛选并初步研究新疆南部维吾尔族宫颈鳞癌差异表达的miRNA，预测并分析靶基因功能。**方法：**采用miRNA微阵列芯片技术对5例人乳头瘤病毒(human papillomavirus，HPV)16阳性的新疆南部维吾尔族宫颈鳞癌进行miRNA筛选，应用实时定量RT-PCR方法对筛选出差异表达的miRNA进行初步验证，并检测及分析在83例宫颈癌中的表达结果，应用targetscan、miRwalk、miRanda及Pictar四种软件预测其靶基因。**结果：**新疆南部维吾尔族HPV16阳性宫颈鳞癌差异表达基因18个，对差异表达显著的miRNA-138和miRNA-720进行了初步验证并预测靶基因，预测两者共同的靶基因是*EZH2*；与40例正常对照相比，miRNA-138、miRNA-720在83例宫颈鳞癌组织中均表达下调($P<0.05$)；下调的miRNA-138、miRNA-720与淋巴结转移、脉管浸润有关($P<0.05$)，但与年龄、宫颈壁累及范围及HPV16感染无明显关系($P>0.05$)；miRNA-720与临床分期、肿瘤体积有关($P<0.05$)。**结论：**miRNA-138、miRNA-720在新疆南部维吾尔族宫颈鳞癌组织中均表达下调，两者共同的靶基因是*EZH2*，可能与宫颈癌的侵润和转移有关。

［**关键词**］　新疆南部维吾尔族；人乳头瘤病毒16；宫颈鳞癌；miRNA-138；miRNA-720

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Investigation of microRNA expression on Uygur cervical squamous cell carcinoma in southern Xinjiang

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［**Abstract**］　**Background and purpose:** Cervical cancer is one of the most common cancer in Xinjiang, especially for Uygur from southern Xinjiang and its pathogenesis is not clear. MicroRNA (miRNA) is a class of small non-coding RNA playing an important regulatory role. Its expression and dysfunction is closely related to the development of tumors. In this study, we screen and preliminary analyse expression of miRNA in cervical squamous cell carcinoma samples with human papillomavirus (HPV) 16 positive of Uygur patients. The target genes of miRNA were predicted. **Methods:** miRNAs were pre-screened by using miRNA microarray technology in 5 cases of HPV16 positivity Uygur patients from southern Xinjiang with cervical squamous cell carcinoma. Fifteen cases specimens were examined by qRT-PCR for preliminary verification, and 83 cases of cervical cancer were detected and analysed the expression of miRNA; Targeted genes were predicted by using four softwares of target scan, miRwalk, miRanda and Pictar. **Results:** Eighteen differentially expressed miRNAs were selected by SAM software in 5 cases of HPV16 positivity southern Xinjiang Uygur cases with cervical squamous cell carcinoma.miRNA-138 and miRNA-720 were found expressed significantly different by initial verification. Contrasted with 40 normal cases, miR-138 and miR-720 were down-regulated in 83 Uygur patients from southern Xinjiang with cervical squamous cell carcinoma ($P<0.05$),

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and correlated with lymph node matastasis and vascular invasion ($P<0.05$), no correlation with age and the range of cervical wall involvement and HPV16 ($P>0.05$). miRNA-720 was correlated with clinical stage and tumor size ($P<0.05$); And the commonly targeted gene between miRNA-138 and miRNA-720 was EZH2. **Conclusion:** miRNA-138 and miRNA-720 were downregulated in Uygur patients from southern Xinjiang with cervical squamous cell carcinoma, and the common target gene was EZH2.The expression of miR-720 and miR-138 were correlated with relevant risk factors of invasion and metastasis.

［**Key words**］ Uygur from southern Xinjiang; Human papillomavirus 16; Cervical squamous cell carcinoma; miRNA-138; miRNA-720

宫颈癌是新疆地区高发肿瘤，其中以天山以南地区(新疆南部)为多，当地维吾尔族妇女宫颈癌的患病率为527/10万，明显高于我国其他地区，宫颈癌患病率是北京市妇女的210多倍，死亡率居女性恶性肿瘤之首[1]。宫颈癌主要病因就是人乳头瘤病毒(human papillomavirus，HPV)感染。我们的前期研究显示，新疆地区维吾尔族宫颈癌患者以HPV16感染为主，感染率可达94.31%[2]。但HPV感染导致宫颈浸润癌的机制尚不明了，研究发现致瘤病毒在感染人体的过程中表达特定微小RNA(microRNA，miRNA)谱。miRNA是一类在转录后水平上通过与靶基因miRNA的3′UTR结合后调控基因表达的小分子非编码RNA，越来越多的证据表明，miRNA的表达及功能失调与肿瘤的发生、发展密切相关。在多种肿瘤中都发现有miRNA的异常表达，并且这些miRNA在调节肿瘤的发生过程中扮演着重要的角色[3]。

目前miRNA常用的检测方法包括RNA印迹分析方法、miRNA基因芯片和实时定量逆转录聚合酶链反应(Q-RT-PCR)等。本研究拟采用微阵列芯片杂交方法筛选新疆南部维吾尔族HPV16相关宫颈鳞癌差异表达的miRNA；并进行大样本临床研究及其功能分析，寻找与新疆南部地区维吾尔族宫颈鳞癌相关的miRNA。

1 资料和方法

1.1 临床资料

收集2011年8月—2013年8月在新疆医科大学附属肿瘤医院初治原发宫颈鳞癌(癌和癌旁)组织88例、其中5例用于微阵列芯片杂交方法差异miRNA筛选，83例宫颈癌进行实时定量RT-PCR检测，非宫颈病变组织40例为正常对照，均为新疆南部维吾尔族患者。宫颈癌的临床诊断以FIGO(2009)宫颈癌临床分期为标准，其中63例为广泛子宫切除+盆腔淋巴结清扫术后标本，25例为宫颈活检术后标本。以上患者均有病理证实，且不合并其他恶性肿瘤及严重的内科疾病。宫颈癌患者年龄为36~73岁，平均52.2岁，正常对照组患者年龄为39~61岁，平均53.4岁，差异无统计学意义($P>0.05$)。

1.2 实验方法

1.2.1 琼脂糖凝胶电泳检测HPV16

HPV16引物序列为HPV 16 E7-F：5’-CAAGTGTGACTCTACGCTTCGG-3’；HPV 16 E7-R：5’-GTGCCCATTAACAGGTCTTCCAA3’。

用2%琼脂糖凝胶电泳(agarose gel electrophoresis，AGE)检测PCR扩增产物，紫外凝胶成像仪下观察。电泳条带显示亮度且电泳亮带在81 bp长度左右的即为HPV16阳性。

1.2.2 微阵列芯片技术进行microRNA筛选

CapitalBio®哺乳动物miRNA芯片V4.0，购自北京博奥公司，操作过程严格按说明书进行，采用晶芯®Slide Washer™ 8芯片洗干仪对芯片进行清洗甩干，晶芯®LuxScan™ 10K/A双通道激光扫描仪对芯片进行扫描，LuxScan 3.0图像分析软件提取数据，SAM软件挑选差异基因。

1.2.3 实时定量RT-PCR检测miRNA

根据基因芯片检测结果，从中选取差异表达显著的has-miRNA-138、has-miRNA-720进行实时定量RT-PCR检测，对芯片结果进行验证。以U6作为内参。引物序列见表1。

表 1 引物序列

Tab. 1 Primer sequences

Gene	Primer Sequence 5'→3'
miRNA Primer	GTGCAGGGTCCGAGGT
U6-F	CTCGCTTCGGCAGCACA
U6-R	AACGCTTCACGAATTTGCGT
Hsa-miR-720	UCUCGCUGGGGCCUCCA
Hsa-miR-720-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGGAGG
Hsa-miR-720-AS	GCTCTCGCTGGGGCCT
Hsa-miR-138	AGCUGGUGUUGUGAAUCAGGCCG
Hsa-miR-138-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGGCCT
Hsa-miR-138-AS	GCGAGCTGGTGTTGTGAATCA

1.2.4 miRNA的生物学信息预测

目前多个研究小组开发出多个miRNA靶基因预测软件为miRNA相关实验提供理论指导。我们利用目前常用miRNA靶基因预测网站(targetscan网址：http://www.targetscan.org；miRwalk网址：http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/；miRanda网址：http://www.microrna.org/microrna/home.do；Pictar网址：http://pictar.mdc-berlin.de/)进行操作。

1.3 统计学处理

采用SPSS 16.0软件分析，统计方法采用配对*t*检验、独立样本*t*检验及方差分析，实验数据以$\bar{x}\pm s$表示，$P<0.05$为差异有统计学意义。

2 结果

2.1 组织总RNA质量检测

使用M200Pro酶标仪检测样品总RNA纯度，A260/A280在1.8~2.0之间，说明总RNA纯度较高；1.2%甲醛变性凝胶电泳检测样品总RNA完整性，28S条带亮度约为18S的2倍，5S条带亮度最淡(图1)，说明提取的总RNA完整性没有降解。

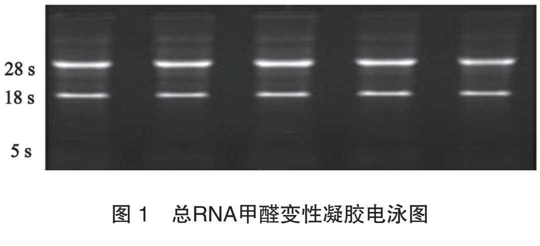

图 1 总RNA甲醛变性凝胶电泳图

Fig. 1 The picture of formaldehyde degeneration electrophoresis gel about total RNA

2.2 HPV16检测

88例宫颈鳞癌组织进行HPV16检测，67例HPV16阳性(其中5例标本用于芯片检测，15例收集到完整癌及癌旁组织的标本用于芯片验证)，21例HPV16检测结果阴性，HPV16阳性的表达率为76.1%。琼脂糖凝胶电泳检测HPV16结果见图2。电泳条带显示亮度且电泳亮带在81 bp长度左右，提示此电泳亮带为目的条带，HPV16检测结果为阳性；无电泳亮带及电泳亮带不在81 bp长度左右，提示此电泳杂带非目的条带，可能为引物二聚体或cDNA模板本身所致，HPV16检测结果为阴性。

2.3 芯片检测

5例新疆南部维吾尔族HPV16阳性宫颈鳞状细胞癌组织及癌旁组织(患者年龄为：48~58岁，临床分期为：Ⅰb1~Ⅱa1期)miRNA芯片扫描结果见图3。使用SAM软件挑选差异miRNA，18个miRNAs在癌组织中表达发生变化，其中10个miRNAs表达上调，8个miRNAs表达下调(图4、表2)。

2.4 实时定量PCR检测

2.4.1 实时定量RT-PCR检测情况

实时定量RT-PCR以U6为内参，对miRNA芯片中差异表达的miRNA，从中随机抽取2个差异表达的miRNA：miRNA-138和miRNA-720，进行实时定量RT-PCR检测，均可见典型的PCR扩增曲线，说明PCR产物量足够，引物敏感度高，目的基因和U6内参的溶解曲线均为单峰，说明产物单一，无其他非特异性产物(图5)。

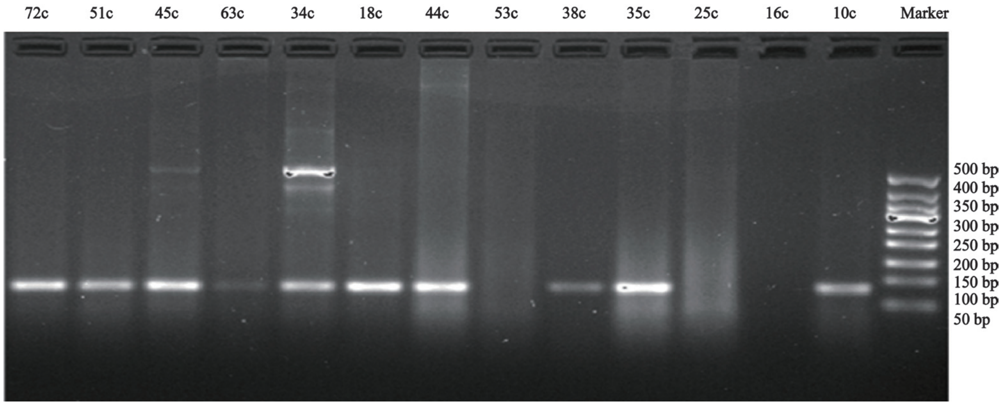

图 2　2%琼脂糖凝胶电泳检测HPV16结果

Fig. 2　The result about detection in HPV16 by 2% sepharose gel

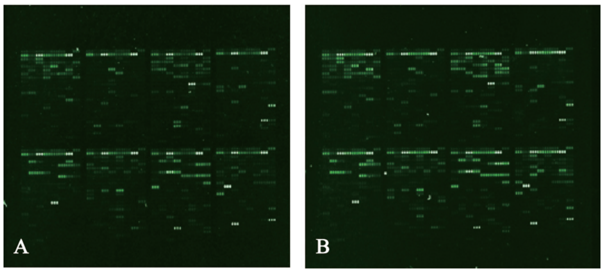

图 3　miRNA芯片检测结果

Fig. 3　miRNA microarray results

A:Cervical cancer tissue; B: Cervical cancer adjacent tissue.

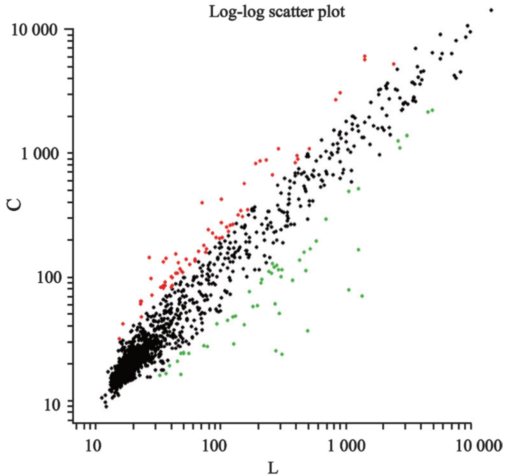

图 4　宫颈鳞癌(C)及癌旁(L)比较分析散点图

Fig. 4　The cervical squamous cell carcinoma (C) and their adjacent tissue (L) comparative analysis scatterplot

Hybridization signals for each data point represents a miRNA chip point. The data points marked in red and green were differentially expressed miRNA (fold change⩾2 or fold change⩽0.5), almost no difference in the expression of black marks belong (0.5<fold change<2).

表 2　宫颈鳞癌中差异表达的miRNAs

Tab. 2　The miRNAs differentially expressed in cervical squamous cell carcinoma

Gene	Score/d	Fold change	Q-value/%
Hsa-miR-31	12.291	2.341	0.000
Hsa-miR-142-5p	6.551	2.414	0.000
Hsa-miR-1234	5.869	1.711	0.000
Hsa-miR-1273	4.161	1.665	0.000
Hsa-miR-720	4.997	3.714	0.000
Hsa-miR-21	2.941	1.864	0.000
Hsa-miR-1285	2.349	1.814	0.000
Hsa-miR-138	3.732	3.024	0.000
Hsa-miR-1181	1.836	1.902	4.614
Hsa-miR-224	4.793	4.607	0.000
Hsa-miR-26a	-6.088	0.592	0.000
Hsa-miR-125b	-5.155	0.433	0.000
Hsa-miR-99a	-4.094	0.616	0.000
Hsa-miR-134	-6.347	0.307	0.000
Hsa-miR-195	-3.622	0.604	0.000
Hsa-miR-379	-5.031	0.206	0.000
Hsa-miR-100	-2.491	0.324	0.000
Hsa-miR-625	-4.391	0.287	1.969

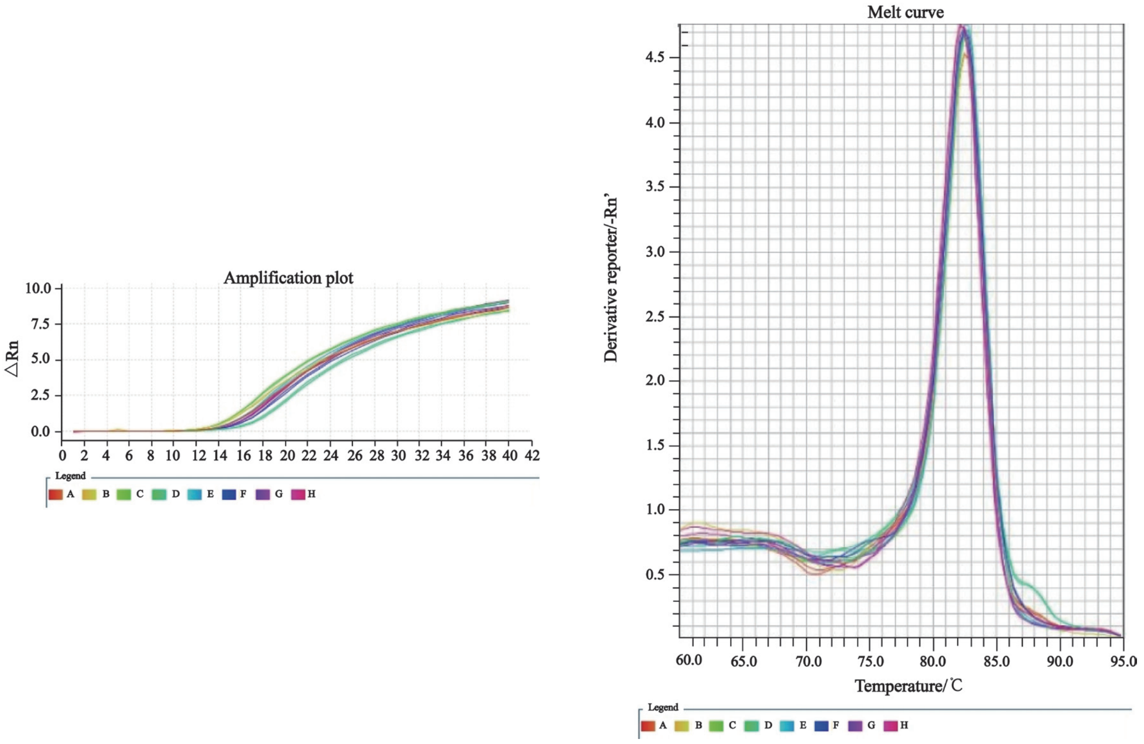

图 5 PCR扩增曲线和溶解曲线

Fig. 5 Amplification plot and melt curve of PCR

2.4.2 实时定量RT-PCR验证结果

对已用miRNA微阵列芯片技术筛选出差异表达显著的2个miRNAs：miRNA-138和miRNA-720进行实时定量RT-PCR验证。选取83例中15例Ⅰb~Ⅱa期HPV16检测阳性的新疆南部维吾尔族宫颈鳞状细胞癌组织、癌旁组织进行检测。miRNA-138和miRNA-720在宫颈鳞状细胞癌组、癌旁组织中的表达率均为100%，对宫颈鳞状细胞癌组、癌旁组的目的基因数据分别进行正态性检验，符合正态分布，采用配对*t*检验。miRNA-138、miRNA-720在癌组织与癌旁组织中表达差异均有统计学意义($P<0.05$)，癌组织中的表达量均低于癌旁组织；实时定量RT-PCR检测miRNA-138、miRNA-720在宫颈鳞状细胞癌组织中表达差异均有统计学意义，与芯片检测结果一致，说明miRNA芯片检测结果可靠(表3)。

2.5 miRNA-138及miRNA-720在宫颈癌中的表达及其与临床病理的关系

2.5.1 miRNA-138在宫颈癌中的表达

miRNA-138在宫颈组织中的表达率为100%。与正常对照组相比，miRNA-138在83例(Ⅰb~Ⅳ期)新疆维吾尔族宫颈鳞癌组中表达显著下调，宫颈癌组miRNA-138的表达平均是对照组的0.492倍，差异有统计学意义($P<0.05$)；miRNA-720在宫颈宫颈组表达率亦均为100%。与正常对照组相比，miRNA-720表达也显著下调，宫颈癌组miRNA-720的表达平均是对照组的0.294倍，差异有统计学意义($P<0.05$，表4)。

表 3 miRNA-138、miRNA-720在宫颈鳞癌及癌旁组织中的表达

Tab. 3 The expression of miRNA-138 and miRNA-720 in cervical squamous cell carcinoma tissue (C) and para-carcinoma tissue (L)

miRNA	△Ct C(n=15)	△Ct L(n=15)	△△Ct	$2^{-\triangle\triangle Ct}$	T value	P value
miR-138	14.423±1.616	13.126±2.493	1.298±2.319	0.407	2.167	0.048
miR-720	5.973±3.302	0.610±2.823	5.602±4.007	0.026	4.987	0.000

表 4　miR-138、miR-720在宫颈癌组及对照组中的表达

Tab. 4　The expression of miRNA-138 and miRNA-720 in cervical squamous cell carcinoma (C) and normal control groups (N)

miRNA	△Ct C(n=83)	△Ct N(n=40)	$2^{-\triangle\triangle Ct}$	t value	P value
miR-138	13.867±2.545	12.844±2.455	0.492	1.258	0.037
miR-720	6.279±3.935	4.514±4.471	0.294	0.461	0.031

2.5.2　miRNA-138、miRNA-720与新疆维吾尔族宫颈鳞癌临床病理因素的关系

miRNA-138与临床病理因素的相关分析显示，83例新疆维吾尔族宫颈鳞癌患者中，年龄、临床分期、肿瘤直径、浸润深度与miRNA-138的表达无明显相关，差异无统计学意义($P>0.05$)；有淋巴结转移组比无淋巴结转移的宫颈鳞癌组miRNA-138表达量低，且淋巴结转移组miRNA-138的表达水平均为无淋巴结转移组的0.334倍，差异有统计学意义($P<0.05$)；有脉管浸润组比无脉管浸润组miRNA-138表达量低，且有脉管浸润组miRNA-138的表达水平均为无脉管浸润组的0.272倍，差异有统计学意义($P<0.05$)；miRNA-720与临床病理因素的相关分析：83例新疆维吾尔族宫颈鳞癌患者中，年龄、肿瘤浸润深度与miRNA-720的表达无明显相关，差异无统计学意义($P>0.05$)；而临床分期、肿瘤直径、淋巴结转移、脉管浸润与miRNA-720的表达有关，差异有统计学意义($P<0.05$)；临床Ⅱa期miRNA-720的表达最低，差异有统计学意义($P<0.05$)。肿瘤直径>4 cm的癌组织中miRNA-720的表达水平均为肿瘤直径≤4 cm的0.304倍，差异有统计学意义($P<0.05$)；有淋巴结转移miRNA-720表达水平均为无淋巴结转移的0.105倍，差异有统计学意义($P<0.05$)；有脉管浸润miRNA-720表达水平均为无脉管浸润的0.084倍，差异有统计学意义($P<0.05$，表5)。

2.5.3　miRNA-138、miRNA-720与新疆维吾尔族宫颈鳞癌HPV16感染的关系

83例新疆维吾尔族宫颈鳞状细胞癌组织中(HPV16阳性62例、HPV16阴性21例)miRNA-138

表 5　miRNA-138、miRNA-720与维吾尔族宫颈癌临床病理因素的关系

Tab. 5　The relationship between miRNA-138, miRNA-720 and clinicopathological factors of cervical squamous cell carcinoma

Clinicopathological feature	n	miRNA-138			miRNA-720		
		△Ct(miRNA-138)	$2^{-\triangle\triangle Ct}$	P value	△Ct(miRNA-720)	$2^{-\triangle\triangle Ct}$	P value
Age/year				0.144			0.262
<40	8	13.992±1.671			5.129±1.324		
40-50	24	14.333±1.939			6.389±3.859		
50-60	31	13.046±2.243			5.608±3.661		
≥60	20	14.529±3.546			7.646±4.870		
Stage				0.118			0.027
Ⅰb	18	12.975±2.646			6.733±4.959		
Ⅱa	32	14.637±2.715			7.597±4.352		
Ⅱb	20	13.389±1.357			4.966±2.213	6.194	
Ⅲ-Ⅳ	13	13.947±3.040			4.425±1.651	9.014	
Diameter/cm				0.940			0.046
≤4 cm	44	13.847±2.303			5.471±2.878		
＞4cm	39	13.889±2.824			7.190±4.736	0.304	
Depth				0.980			0.285
＞1/2	37	13.955±2.557			7.520±4.606		
≤1/2	21	13.973±2.739			6.223±3.993		
Lymphatic metastasis				0.046			0.015
Negative	44	13.580±2.408			6.265±3.812		
Positive	14	15.162±2.900	0.334		9.517±5.319	0.105	
Vascular invasion				0.030			0.014
Negative	47	13.606±2.342			6.371±3.693		
Positive	11	15.483±3.187	0.272		9.951±6.028	0.084	

和miRNA-720的表达率为100%。miRNA-138在HPV16阳性宫颈鳞状细胞癌组织中表达水平为13.949±2.063，在HPV16阴性宫颈鳞状细胞癌组织中表达水平为13.338±3.716，差异无统计学意义($P>0.05$)；miRNA-720在HPV16阳性宫颈鳞状细胞癌组织中表达水平为6.363±3.774，在HPV16阴性宫颈鳞状细胞癌组织中表达水平为5.877±4.477，差异也无统计学意义($P>0.05$，表6)。

2.6　miRNA-138、miRNA-720生物信息学预测

2.6.1　miRNA-138靶基因预测分析

针对性地应用Targetscan、miRwalk、miRanda、Pictar 4个预测软件寻找miR-138的靶基因系列。通过数据信息交叉比对，获取miRNAs分子与3’-UTR的可能结合靶点，发现共有12个基因被一致预测为miR-138的靶基因(表7)。

表 6　miRNA-138、miRNA-720在HPV16阳/阴性宫颈鳞癌组织中的表达

Tab. 6　The expression of miRNA-138 and miRNA-720 in cervical squamous cell carcinoma of HPV16 positive/negative

miRNA	△Ct HPV16(+) (*n*=62)	△CtHPV16(-) (*n*=21)	*t* value	*P* value
miR-138	13.949±2.063	13.338±3.716	0.941	0.350
miR-720	6.363±3.774	5.877±4.477	0.486	0.628

表 7　miRNA-138靶基因预测结果及其功能

Tab. 7　miRNA-138 predicted target genes and their function

miRNA	Gene	Function
miR-138	*EZH2*	This gene encodes a member of the Polycomb-group (PcG) family. PcG family members form multimeric protein complexes, which are involved in maintaining the transcriptional repressive state of genes over successive cell generations
miR-138	*LYPLA1*	Has depalmitoylating activity toward KCNMA1 and low lysophospholipase activity.
miR-138	*ARHGEF3*	It has been identified that genetic variation in this gene plays a role in the determination of bone mineral density (BMD), indicating the implication of this gene in postmenopausal osteoporosis.
miR-138	*CLNS1A*	Performs diverse functions including regulation of small nuclear ribonucleoprotein biosynthesis, platelet activation and cytoskeletal organization.
miR-138	*EIF4EBP1*	This protein is phosphorylated in response to various signals including UV irradiation and insulin signaling, resulting in its dissociation from eIF4E and activation of mRNA translation.
miR-138	*GNAI2*	The encoded protein contains the guanine nucleotide binding site and is involved in the hormonal regulation of adenylate cyclase
miR-138	*LIMK1*	This protein is phosphorylated in response to various signals including UV irradiation and insulin signaling, resulting in its dissociation from eIF4E and activation of mRNA translation.
miR-138	*RHOC*	Overexpression of this gene is associated with tumor cell proliferation and metastasis.
miR-138	*ROCK2*	The protein encoded by this gene is a serine/threonine kinase that regulates cytokinesis, smooth muscle contraction, the formation of actin stress fibers and focal adhesions, and the activation of the c-fos serum response element. This protein, which is an isozyme of ROCK1 is a target for the small GTPase Rho.
miR-138	*SLC20A1*	The protein encoded by this gene is a sodium-phosphate symporter that absorbs phosphate from interstitial fluid for use in cellular functions such as metabolism, signal transduction, and nucleic acid and lipid synthesis.
miR-138	*TERT*	Telomerase reverse transcriptase
miR-138	*H2AFX*	This gene encodes a member of the histone H2A family, and generates two transcripts through the use of the conserved stem-loop termination motif, and the polyA addition motif.

2.6.2　miRNA-720靶基因预测分析

应用上述4个miRNA靶基因预测网站寻找miRNA-720靶基因，4个软件预测靶基因结果无完全重合者，targetscan网址和miRanda网址预测靶基因交叉重合者与miRwalk网址和miRanda网址预测靶基因结果交叉重合者列入本结果。基因预测结果显示，*EZH2*为miRNA-720与miRNA-138共同调控靶基因(表8)。

表 8 miRNA-720靶基因预测结果及其功能

Tab. 8 miRNA-720 predicted target genes and their function

miRNA	Gene	Function
miR-720	*AGAP2*	The protein mediates anti-apoptotic effects of nerve growth factor by activating nuclear phosphoinositide 3-kinase. It is overexpressed in cancer cells, and promotes cancer cell invasion.
miR-720	*SPOCK2*	The protein contains thyroglobulin type-1, follistatin-like, and calcium-binding domains, and has glycosaminoglycan attachment sites in the acidic C-terminal region. Three alternatively spliced transcript variants that encode different protein isoforms have been described for this gene.
miR-720	*FGF14*	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family, and possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion.
miR-720	*HNRNPA2B1*	These proteins are associated with pre-mRNAs in the nucleus and appear to influence pre-mRNA processing and other aspects of mRNA metabolism and transport.
miR-720	*QKI*	The protein encoded by this gene is an RNA-binding protein that regulates pre-mRNA splicing, export of mRNAs from the nucleus, protein translation, and mRNA stability. The encoded protein is involved in myelinization and oligodendrocyte differentiation and may play a role in schizophrenia
miR-720	*FOXG1*	The encoded protein, which functions as a repressor, may play a role in brain development.
miR-720	*ACVR1B*	This protein is a type I receptor which is essential for signaling. Mutations in this gene are associated with pituitary tumors.
miR-720	*DNMT3A*	This gene encodes a DNA methyltransferase that is thought to function in de novo methylation, rather than maintenance methylation.
miR-720	*EPHB2*	Ephrin receptors and their ligands, the ephrins, mediate numerous developmental processes, particularly in the nervous system.
miR-720	*LATS2*	This gene encodes a serine/threonine protein kinase belonging to the LATS tumor suppressor family.
miR-720	*KRAS*	This gene, a Kirsten ras oncogene homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase superfamily. The transforming protein that results is implicated in various malignancies.
miR-720	*CCND2*	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through the cell cycle.
miR-720	*NBN*	The protein is a member of the MRE11/RAD50 double-strand break repair complex which consists of 5 proteins. This gene product is thought to be involved in DNA double-strand break repair and DNA damage-induced checkpoint activation.
miR-720	*ENAM*	The protein is involved in the mineralization and structural organization of enamel. Defects in this gene result in amelogenesis imperfect type 1C.
miR-720	*EZH2*	This gene encodes a member of the Polycomb-group (PcG) family. PcG family members form multimeric protein complexes, which are involved in maintaining the transcriptional repressive state of genes over successive cell generations.
miR-720	*AMLX*	Mutations in this gene cause X-linked amelogenesis imperfecta. Alternative splicing results in multiple transcript variants encoding different isoforms.
miR-720	*PRNP*	This gene have been associated with Creutzfeldt-Jakob disease, fatal familial insomnia, Gerstmann-Straussler disease, Huntington disease-like 1, and kuru.
miR-720	*CALB1*	Its is a calcium-binding protein belonging to the troponin C superfamily.

3 讨 论

miRNA能通过与其靶mRNA的特异性碱基配对引起靶mRNA的降解、抑制或活化，从而对基因进行转录或转录后的调控。检测和分析miRNA的表达水平是研究miRNA功能的首要环节。研究发现在宫颈癌中有多种miRNA表达，如miRNA-205在宫颈癌组织中高表达[4]，hsa-cer-miRNA1、hsa-cer-miRNA2和hsa-cer-miRNA4在宫颈癌组织中表达上调[5]；而HR-HPV E7癌蛋白可以通过下游关键分子调控miRNA-15/miRNA-16的表达水平[6]，细胞分化过程中调控miRNA-203的活化程度[7]，影响细胞的增殖和细胞周期。本研究采用微阵列芯片技术，筛选出新疆南部维吾尔族宫颈鳞癌组织差异表达的miRNA，并采用实时定量RT-PCR法检测和分析了miRNA-138、miRNA-720在宫颈鳞癌中的表达。发现miRNA-138、miRNA-720在新疆南部维吾尔族宫颈鳞癌中呈表达下调，但未显示出与HPV16感染具有相关性。

肿瘤的复发和转移是目前肿瘤预后差的主要原因之一，miRNA在参与细胞增殖、凋亡发生、远处转移等方面均发挥着重要作用，具有评估肿瘤预后的潜在价值。本研究结果显示miRNA-138、miRNA-720在Ⅰb~Ⅳ期新疆南

部维吾尔族宫颈鳞癌组织中的表达与对照组相比均明显下调，并且在有淋巴结转移及脉管浸润者中表达下调，而宫颈癌的转移途径主要有3种：①直接蔓延(最常见)；②淋巴转移(主要转移途径)；③血行转移。提示miRNA-138、miRNA-720的表达下调与新疆南部维吾尔族宫颈癌的侵袭与转移有关，并且宫颈癌患者中这两者的表达量越低，肿瘤的侵袭转移能力越强，患者预后越差。除此之外，研究还显示miRNA-720在肿瘤体积较大的局部晚期宫颈鳞癌组织中表达量降低，提示与肿瘤预后不良因素有关。miRNA-720在Ⅱa期表达最低，未表现出与临床分期呈正相关，可能与组间病例结构有关，需进一步扩大样本研究。

miRNA-138在多种肿瘤中表达下调，与其作为抑癌基因有关[8-10]。在鼻咽鳞状细胞癌的细胞株中，miRNA-138抑制上皮-间质转化，下调的miRNA-138增强细胞迁移和侵袭，是一种多功能分子调节器[11]。在人胃癌SGC-7901细胞， 通过miR-138转染，上调细胞内miR-138表达丰度之后，能够明显抑制SGC-7901细胞的增殖[12]。Yeh等[13]的研究认为，低表达的miRNA-138与卵巢癌细胞的高侵袭性以及卵巢癌的淋巴结转移有关。Takeshi等[14]的研究发现，miRNA-138作为抑癌基因通过调控波形蛋白。抑制肾癌的转移，mirRNA介导的致癌通路是肾癌的发生和转移的潜在机制。

目前国内外有关miRNA-720与宫颈癌以外其他肿瘤的相关性报道也逐渐增多，Miguel等[15]的研究发现，miRNA-720、miRNA-21、miRNA-221和miRNA-431在前庭神经鞘瘤中均表达上调，并位于14q32染色体上。Shinozuka等[16]的研究发现，*SnoN/SKIL*基因通过下调hsa-miRNA-720控制食管癌细胞的增殖。而miRNA-720通过靶向调节*TWIST1*基因抑制乳腺癌患者肿瘤的侵袭和转移[17]，与本研究结果相符。

已证实miRNA通过各自不同的靶基因来调控肿瘤细胞的生物学行为，从而参与肿瘤的发生过程。我们通过应用软件预测miRNA-138、miRNA-720的靶基因，结果发现，*EZH2*是miRNA-138和miRNA-720共同的靶基因，*EZH2*是果蝇zeste基因增强子［E(z)］的人类同源物。该基因定位于人7q35染色体上，基因长度约40 kb。*EZH2*基因编码的蛋白属于多梳家族(PcG)成员。PcG家族成员相互结合形成了多聚蛋白复合物，*EZH2*主要通过将3个甲基基团加到组蛋白3的27号赖氨酸(H3K27)上行使基因沉默者的角色，H3K27是维持染色质凝聚的一种重要修饰，参与染色体结构的形成，调节基因的表达，控制细胞的生长，因而在细胞记忆、生长发育及X染色体灭活等方面均有重要作用。EZH2β的功能特征揭示，*EZH2*基因亚型增加了哺乳动物基因表达调控的复杂性[18]。*EZH2*过多表达意味着组蛋白甲基化抑癌基因将导致其表达沉默，就可能导致癌症形成，所以一种靶向*EZH2*的临床药物前模型显示，其能够抑制脑癌及前列腺癌的进展[19-20]。在宫颈癌组织中*EZH2*的高表达与肿瘤细胞分化、临床分期、浸润深度及淋巴结转移密切相关[21]。

总之，对miRNA-138、miRNA-720在新疆维吾尔族宫颈癌中的表达研究，及预测靶基因*EZH2*功能分析均显示，miRNA-138、miRNA-720与宫颈癌的转移，侵袭及不良预后有关。本课题下一步将进行双荧光素酶报告基因检测、以验证靶基因，继续了解相关预测靶基因的功能，进而应用蛋白质印迹(Western blot)法提取蛋白，从而得到miRNA-138、miRNA-720调控的靶基因及其所编码蛋白。

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