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·临床研究·

Claudin-2蛋白在食管鳞癌组织中的表达及其对KYSE-450细胞恶性生物学行为的影响

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[摘 要] **目的**:分析Claudin-2蛋白在食管鳞癌(esophageal squamous cell carcinomas,ESCC)组织中的表达及其与患者临床病理特征、5年生存率的关系,探索其对ESCC细胞KYSE450的增殖、迁移和侵袭的影响。**方法**:选取河南省肿瘤医院2010至2013年间初治的ESCC患者手术切除肿瘤组织52例及其中20例对应的癌旁组织标本,采用免疫组化和WB法检测Claudin-2的表达并分析其与患者临床病理特征和5年生存率的关系。WB法检测ESCC细胞(KYSE450、KYSE150、KYSE510、KYSE140)和人永生化食管上皮细胞SHEE中Claudin-2的表达,构建Claudin-2 shRNA慢病毒载体并转染KYSE450细胞构建敲低Claudin-2表达的细胞系,进一步通过克隆形成实验、细胞划痕实验及Transwell实验检测敲低Claudin-2对KYSE450细胞增殖、迁移和侵袭的影响。**结果**:ESCC组织中Claudin-2阳性率显著高于癌旁组织(P<0.05),ESCC组织中Claudin-2的表达与淋巴结转移有关(P<0.05)。Claudin-2表达阳性患者5年生存率显著低于阴性者(P<0.05)。成功构建敲低Claudin-2表达的KYSE450细胞系。sh-Claudin-2组细胞的克隆形成数、伤口愈合率和侵袭细胞数均显著低于sh-NT组和对照组(P<0.05)。**结论**:ESCC组织中Claudin-2的表达高于癌旁组织,且与患者5年生存率呈负相关,Claudin-2能够增强KYSE450细胞的增殖、迁移和侵袭能力。

[关键词] 食管鳞癌;Claudin-2;KYSE450细胞;增殖;侵袭
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Expression of Claudin-2 in human esophageal squamous cell carcinoma tissues and its effect on the malignant biological behaviors of KYSE-450 cells

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[Abstract] Objective: To analyze the expression of Claudin-2 in human esophageal squamous cell carcinoma (ESCC) tissues and its relationship with clinicopathological characteristics and 5-year survival rate of ESCC patients, and to explore its effect on proliferation, migration and invasion of ESCC KYSE-450 cells. **Methods:** A total of 52 cases of tumor tissues and 20 cases of corresponding para-cancerous tissues that surgically removed from ESCC patients who were primarily treated in Henan Cancer Hospital from 2010 to 2013 were collected for this study. Claudin-2 expression was analyzed by Immunohistochemistry and WB assay, and its relationship with clinicopathological characteristics and 5-year survival rate of ESCC patients was further investigated. The expression of Claudin-2 in ESCC cells (KYSE450, KYSE150, KYSE510 and KYSE140) and human immortalized esophageal epithelial SHEE cells was detected by WB assay. The Claudin-2 shRNA lentiviral vector was established and transfected into KYSE450 cells to establish a Claudin-2 knockdown cell line. Furthermore, the effects of knockdown of Claudin-2 on the proliferation, migration and invasion of KYSE450 cells were tested by clone formation experiment, cell scratch experiment and Transwell experiment. **Results:** The positive rate of Claudin-2 in ESCC tissues was significantly higher than that in adjacent tissues (P<0.05). The expression of Claudin-2 in ESCC tissues was related to lymph node metastasis (P<0.05). The 5-year survival rate of Claudin-2 positive patients was significantly lower than that of negative patients (P<0.05). The KYSE450 cell line with Claudin-2 knockdown was successfully constructed. The number of clone formation, wound healing rate and number of invaded cells in the sh-Claudin-2 group were significantly lower than those in the sh-NT group and the control group (all P<0.05). **Conclusion:** The expression level of Claudin-2 in ESCC tissues is higher than that of the tumor-adjacent tissues, and is negatively related with 5-year survival rate of ESCC patients. Claudin-2 can promote the proliferation,

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migration and invasion of ESCC cells.

[Key words] esophageal squamous cell carcinoma (ESCC); Claudin-2; KYSE450 cell; proliferation; invasion

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在中国，食管癌发病率位列常见癌症的第六位，5年总生存率很低（2012-2015年为30.3%）[1]，病死率较高（2015年为13.68/10万）[2]。食管鳞癌（esophageal squamous cell carcinoma，ESCC）是食管癌最常见的分型，主要发生于亚洲及非洲的发展中国家[3]，明确ESCC发生发展的相关机制，寻找其在发生、侵袭、转移过程中的分子标志物，对于ESCC的早期诊断、改善患者预后非常重要。Claudin蛋白是一种细胞间黏附分子，位于上皮细胞间紧密连接（tight junction，TJ）处，是TJ发挥细胞旁屏障及细胞旁通道功能的组成分子之一[4]。Claudin和TJ其他蛋白丢失会引起细胞黏附丧失并导致细胞屏障功能受损，这被认为是癌症的发生发展及转移过程中的重要一步[5]。已发现Claudins与肿瘤的发生、淋巴结转移、预后都有着密切的关系[6-9]。Claudin家族由27个成员组成，这些成员均为具有4个穿膜结构域的蛋白，但其细胞外区域各不相同，在TJ发挥细胞旁屏障及细胞旁通道功能中的作用各异[4]。Claudin-2参与形成细胞旁的水通道，介导上皮细胞旁水转运[9]，其表达于正常肾、肝、胰腺、小肠等[11]，在结直肠癌[12]、肺腺癌[13]中表达增加，而在骨肉瘤[14]、前列腺癌[15]中表达减少。ABU-FARSAKH等[16]发现，Claudin-2高表达于食管腺癌和鳞癌，在食管黏膜化生、异型增生和癌的发生和发展中有作用，未发现食管腺癌和鳞癌的Claudin-2表达与患者年龄、性别、分级、分期和患者生存时间相关。本研究分析ESCC组织中Claudin-2表达及其与临床病理特征的关系，并探讨Claudin-2表达对ESCC细胞增殖、迁移和侵袭的影响。

1 资料与方法

1.1 样本、细胞及试剂

1.1.1 样本资料 选取河南省肿瘤医院2010至2013年间初治的ESCC患者手术切除肿瘤组织52例及其中20例对应的癌旁组织标本。入组病例术前均未接受过放化疗，病理学诊断均经过两名病理科医生诊断后确诊，患者年龄中位数为65岁。通过复诊和电话等方式随访至2018年9月，中位随访时间为63个月。研究方案经河南省肿瘤医院伦理委员会批准和许可。本研究已获得所有患者及家属知情同意，并签署知情同意书。

1.1.2 细胞及试剂 人ESCC细胞KYSE-510、KYSE-140、KYSE-150、KYSE-450，人永生化食管上皮细胞SHEE，293T细胞、慢病毒载体pshRNA-EF1-EGFP-P2A-Puro均由中美（荷美尔）肿瘤研究院保存。细胞用含10%FBS的DMEM（KYSE-150、KYSE-510、KYSE-140）或RPMI 1640（KYSE-450、293T）或M199（SHEE）培养基于5% CO_2、37 ℃恒温加湿培养箱中培养。1640培养基、DMEM培养基均购自以色列BI公司，M199购自Gibco公司，Lipofectamine 2000转染试剂、Transwell小室均购自Sigma-Aldrich公司，Matrigel基质胶购自美国Corning公司，胰蛋白酶、蛋白裂解液、BCA蛋白定量试剂盒均购自北京索莱宝科技有限公司，ECL发光液购自上海碧云天生物技术有限公司，兔抗Claudin-2抗体和HRP标记二抗均购自英国Abcam公司。

1.2 免疫组化染色检测Claudin-2在ESCC组织中的表达

ESCC及癌旁组织标本用4%多聚甲醛于室温下固定25 min，经常规石蜡包埋、切片。用2%牛血清白蛋白封闭30 min，加入兔抗Claudin-2抗体（1∶500稀释）室温孵育2 h，PBS洗涤，加入HRP标记二抗（1∶1 000）室温孵育1.5 h，加入二氨基联苯胺显色。

Claudin-2表达情况用染色指数评估，归纳为阴性（－）、弱阳性（+）、强阳性（++）三类，染色指数依次为0、1~2、3~4。染色指数=染色强度评分+染色细胞评分。染色强度评分：0分（阴性），1或2分（中等染色），3或4分（强染色）；染色细胞评分：0分（无染色细胞），1分（10~60%阳性细胞），2分（超过60%阳性细胞）。染色强度及染色细胞评分由两名病理医师双盲独立评估。

1.3 构建Claudin-2 shRNA慢病毒载体

设计并选定Claudin-2 shRNA的靶序列GCAGTGATAAAGGAGGCATTT，插入慢病毒载体pshRNA-EF1-EGFP-P2A-Puro的寡核苷酸由上海生工合成。寡核苷酸序列：正义链5'-CCGGCCAGAGAAATCGCTCCAACTACTCGAGTAGTTGGAGCGATTTCTCTGGTTTTTG-3'，反义链5'-AATTCAAAAACCAGAGAAATCGCTCCAACTACTCGAGTAGTTGGAGCGATTTCTCTGG-3'。用*Age*Ⅰ-HF和*Eco*RⅠ消化pshRNA-EF1-EGFP-P2A-Puro获取的凝胶纯化消化载体，和合成的寡核苷酸连接，经酶切和测序证实Claudin-2 shRNA慢病毒载体pshRNA-EF1-EGFP-P2A-Puro构建成功。

1.4 Claudin-2 shRNA慢病毒载体感染KYSE-450细胞

以Lipofectamine 2000分别瞬时转染Claudin-2 shRNA慢病毒载体或空载体（sh-NT）于293T细胞

48 h后，用0.22 μm孔径滤器过滤，获取培养基含慢病毒颗粒的上清液。上清液分别加入KYSE-450细胞培养基中，设为sh-Claudin-2组和sh-NT组，24 h后，用含1 μg/ml嘌呤霉素的正常培养基筛选72 h，荧光显微镜下能够发出绿色荧光的细胞即转染成功的细胞。

1.5　WB法检测Claudin-2蛋白在ESCC组织和细胞中的表达水平

分别抽提5例ESCC患者的癌及癌旁组织、人ESCC细胞（KYSE-150、KYSE-510、KYSE-140、KYSE-450）、人永生化食管上皮细胞SHEE、sh-Claudin-2组和sh-NT组细胞的总蛋白，用BCA法制作标准曲线测定浓度。行10%SDS-PAGE，转膜，用5%脱脂奶粉室温封闭1 h，加入兔抗Claudin-2抗体（1∶500），4 ℃过夜孵育，加入HRP标记二抗（1∶5 000）室温孵育1 h，ECL发光液曝光显影。

1.6　克隆形成实验、划痕实验和Transwell侵袭实验检测敲低Claudin-2表达对KYSE-450细胞的增殖、迁移及侵袭能力的影响

取培养至对数期的KYSE-450、sh-Claudin-2组和sh-NT组细胞，以KYSE450为对照组，进行克隆实验、划痕实验和Transwell侵袭实验测定细胞的增殖、迁移及侵袭。克隆实验：将细胞以200个/孔接种于6孔板中培养1周，3.7%甲醛固定，吉姆萨染液染色，计细胞克隆数。划痕实验：在长满细胞的培养板上用200 μl枪头按照标记位置进行划痕，显微镜下观察培养0、24、48 h时划痕闭合情况。Transwell实验：收取细胞悬浮于无FBS的1640培养液成悬液（2×10^5个/ml），加入提前铺好Matrigel基质胶的小室，下室加入含10%FBS的1640培养液，培养24 h，PBS洗涤，95%乙醇固定，吉姆萨染液染色，在显微镜下计数膜下室侧细胞。

1.7　统计学处理

采用SPSS 25.0软件分析。符合正态分布的计量资料用$\bar{x}\pm s$表示，计数资料用率或百分比表示。采用χ^2检验或Fisher确切概率法对Claudin-2表达水平与临床病理特征的关系进行分析，用Kaplan-Meier方法及Log-Rank检验分析比较Claudin-2表达与ESCC患者5年生存之间的关系，COX比例风险回归单因素和多因素分析影响ESCC预后的危险因素，采用单因素方差分析比较人ESCC细胞和人永生化食管上皮细胞SHEE的Claudin-2表达以及sh-Claudin-2组、sh-NT组与空白对照组细胞之间增殖、迁移和侵袭能力的差异。以$P<0.05$表示差异具有统计学意义。

2　结　果

2.1　Claudin-2在ESCC组织中呈高表达

免疫组化染色结果（图1）显示，癌旁组织和ESCC组织中均有Claudin-2表达，ESCC组织中Claudin-2阳性表达率（71.2%）显著高于癌旁正常组织（40%，$P<0.05$）（表1，$\chi^2=8.405$，$P=0.015$）。WB检测结果（图2）显示，Claudin-2在ESCC组织表达高于癌旁组织。

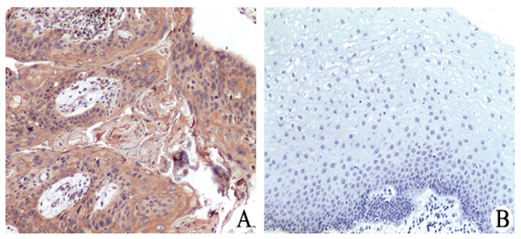


图1　ESCC组织（A）和癌旁组织（B）Claudin-2表达的免疫组化检测（×200）

Fig.1　Immunohistochemical staining of Claudin-2 in ESCC tissues (A) and adjacent tissues (B) (×200)

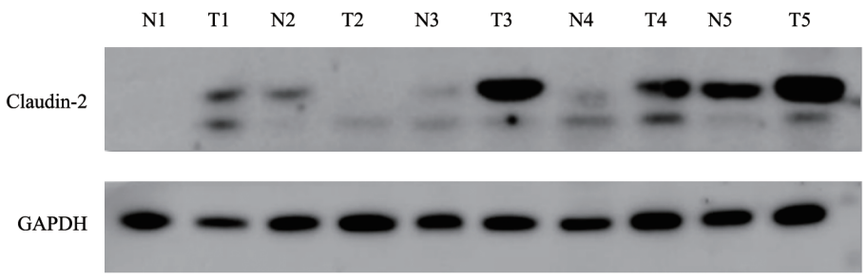
2.2　ESCC组织中Claudin-2的表达水平与患者淋巴结转移相关

52例患者的临床病理特征不同，ESCC组织Claudin-2表达也有差别。χ^2检验和Fisher精确检验结果（表2）显示，ESCC组织Claudin-2表达情况与淋巴结转移相关（$P<0.05$），与性别、年龄、吸烟史、分化程度、肿瘤位置、TNM分期未显现统计学相关性（$P>0.05$）。

表1　Claudin-2在ESCC组织及癌旁组织表达情况

Tab.1　The expression of claudin2 in ESCC tissues and adjacent tissues

Claudin-2	Negativity (*n*)	Positivity		Total	Positivity rate
		+	++		
Normal tissue (N)	12	8		20	40.00%
Tumor tissue (T)	15	28	9	52	71.20%



N: Adjacent tissue; T: Tumor tissue

图2 WB法检测Claudin-2在ESCC组织和癌旁组织中的表达（*n*=5）

Fig.2 Expression of Claudin-2 in ESCC tissues and adjacent tissues detected by WB assay (*n*=5)

表2 52例ESCC患者临床特征与Claudin-2表达的关系（*N*=52）

Tab.2 The correlation between the Claudin-2 expression and clinical characteristics (*N*=52)

Characteristic	Case [*n* (%)]		Claudin-2 expression	
	Negativity	Positivity	χ^2	*P*
Gender			0.514	0.474
Male	12 (80.0)	26 (70.3)		
Female	3 (20.0)	11 (29.7)		
Age (*t*/a)*			0.335	0.563
≤50	2 (13.3)	3 (8.1)		
>50	13 (86.7)	34 (91.9)		
Sites[a]			1.656	0.482
Higher	11 (73.4)	20 (54.1)		
Middle	2 (13.3)	9 (24.3)		
Lower	2 (13.3)	8 (21.6)		
Differentiation[b]			2.508	0.291
High	4 (26.7)	4 (10.9)		
Middle	7 (46.6)	17 (45.9)		
Low	4 (26.7)	16 (43.2)		
Tumor size (*d*/cm)			3.036	0.081
≤5	3 (20.0)	17 (45.9)		
>5	12 (80.0)	20 (54.1)		
TNM			0.435	0.509
I-II	10 (66.7)	21 (56.8)		
III-IV	5 (33.3)	16 (43.2)		
LYM			7.241	0.044
N0	11 (73.3)	22 (59.5)		
N1	3 (20.0)	9 (24.3)		
N2	0	6 (16.2)		
N3	1 (6.7)	0		
Smoking			1.201	0.273
Yes	6 (40.0)	21 (56.8)		
No	9 (60.0)	16 (43.2)		
Drinking			0.001	0.979
Yes	4 (26.7)	10 (27.0)		
No	11 (73.3)	27 (73.0)		

* Fisher exact test; a: Locations were separated as follows: upper, including cervical esophagus and upper thoracic esophagus; middle, middle thoracic esophagus; lower, lower thoracic esophagus including abdominal esophagus. b: The degree of differentiation is defined as follows: High: The differentiation of cancer cells in tumors is closer to normal cells; Low: Tumor cells in tumors are poorly differentiated, extremely immature, or apparently abnormal to normal cells, but still retain traces of some source tissues; Middle: The boundary between high and low differentiated

2.3 ESCC患者5年生存率与Claudin-2的表达呈负相关

所有患者的中位生存时间为23个月，5年生存病例为23例，5年生存率为44.2%。ESCC组织Claudin-2阴性表达组15例，中位生存时间为62.5个月，5年生存病例为11例，5年生存率73.3%。Claudin-2阳性表达组37例，中位生存时间为23个月，5年生存病例为12例，5年生存率为32.4%。Kaplan-Meier生存分析及log-rank检验结果（图3）显示，Claudin-2表达阳性组患者5年生存率显著低于Claudin-2表达阴性组（χ^2=6.513，*P*=0.011）。

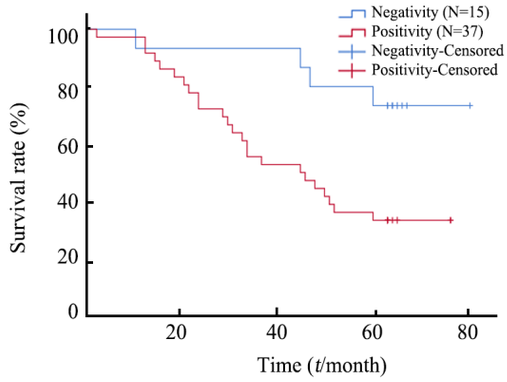


图3 Kaplan-Meier分析Claudin-2表达与ESCC患者5年生存率的关系

Fig.3 Kaplan-Meier analysis of Claudin-2 expression and 5-year survival rate of ESCC patients

COX比例风险回归单因素分析结果（表3）显示，年龄、肿瘤大小、吸烟史、饮酒史、TNM分期与患者的5年生存无显著相关性（均*P*>0.05），而性别、淋巴结转移、分化程度、肿瘤位置、Claudin-2是影响ESCC患者5年生存的危险因素（均*P*<0.05）。排除混杂因素，COX比例风险回归多因素分析结果（表4）显示，分化程度、Claudin-2表达为ESCC患者5年生存独立的危险因素（均*P*<0.05）。

表3 52例ESCC患者5年生存单因素方差分析

Tab.3 Univariate analysis of variance for 5-year survival in 52 ESCC patients

Characteristic	B	HR	Wald	95% CI	*P*
Gender	-1.326	0.613	4.675	0.08-0.883	0.031
Age(*t*/a)	0.5	0.734	0.465	0.391-6.951	0.496
Site	0.6	0.216	7.741	1.194-2.781	0.005
Differentiation	1.034	0.318	10.562	1.507-5.243	0.001
Tumor size (*d*/cm)	0.27	0.382	0.498	0.619-2.77	0.481
TNM	0.363	0.34	1.141	0.738-2.8	0.89
LYM	0.42	0.202	4.33	1.025-2.26	0.037
Smoking	0.067	0.382	0.03	0.505-2.26	0.862
Drinking	0.386	0.405	0.908	0.665-3.256	0.341
Claudin-2	-1.288	0.542	5.643	0.095-0.798	0.018

表4 Claudin-2与5年生存多因素方差分析

Tab.4 Multivariate analysis of variance for 5-year survival and Claudin-2

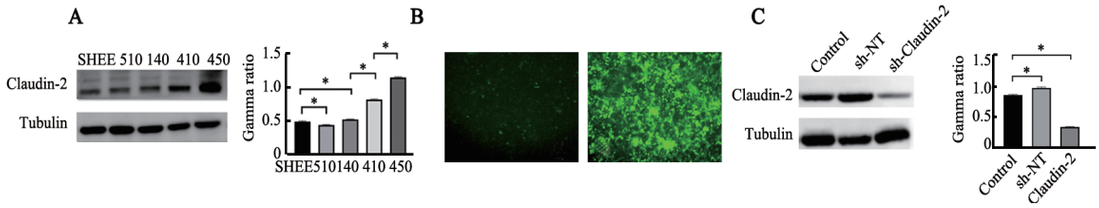
Characteristic	B	HR	Wald	95% CI	*P*
Gender	-1.082	0.342	2.614	0.091-1.258	0.106
Site	-0.382	1.696	0.411	0.213-2.193	0.522
Differentiation	0.926	2.605	5.631	1.175-5.425	0.018
LYM	-0.049	0.929	0.034	0.567-1.601	0.853
Claudin-2	1.301	3.729	5.234	1.205-11.193	0.022

2.4　成功构建敲降Claudin-2表达的ESCC细胞sh-claudin2 KYSE-450

2.4.1　KYSE450细胞高表达Claudin-2　WB检测ESCC细胞（KYSE450、KYSE150、KYSE510、KYSE140）和人永生化食管上皮细胞SHEE中Claudin-2的表达水平，结果（图4A）显示，KYSE450细胞Claudin-2的表达水平显著高于其他细胞（*P*<0.05）。

2.4.2　Claudin-2 shRNA慢病毒载体成功感染KYSE-450细胞　经293T细胞包装后的Claudin-2 shRNA慢病毒感染KYSE450细胞3 d后，荧光显微镜观察发现，sh-claudin2组和sh-NT组细胞均有绿色荧光，经含浓度为1 μg/ml嘌呤霉素培养基筛选终止时，有绿色荧光的细胞占至80%以上（图4B）。

2.4.3　sh-Claudin-2 KYSE-450细胞的Claudin-2表达降低　WB检测结果（图4C）显示，sh-Claudin-2组细胞的Claudin-2表达显著低于sh-NT组和对照组（均*P*<0.05），提示成功敲降sh-Claudin-2 KYSE-450细胞中的Claudin-2表达。



*P<0.05

A: Claudin-2 was highly expressed in ESCC cells KYSE450; B: The cells infected with lentivirus were cultured and screened successfully with 1 μg/ml purinomycin; C: Claudin-2 expression was decreased in sh-Claudin-2 KYSE-450 cells

图4 成功建立敲降Claudin-2表达的ESCC细胞si-Claudin-2 KYSE-450

Fig.4 ESCC cell line si-Claudin2 KYSE-450 with successful Claudin-2 knockdown

2.5　敲降Claudin-2抑制KYSE-450细胞的增殖、迁移和侵袭

克隆形成实验结果（图5）显示，sh-Claudin-2组KYSE-450细胞形成的克隆数显著低于sh-NT组和对照组[（44±9.54）*vs*（167.33±6.66）、（167.00±3.00）个，均*P*<0.05]。划痕实验结果（图6）显示，sh-Claudin-2

组细胞伤口愈合率显著低于sh-NT组和空白对照组[(23.33±5.77)% *vs* (51.33±8.08)%、(54.17±7.22)%，均P<0.05]。Transwell实验结果(图7)显示，sh-Claudin-2组的侵袭细胞少于sh-NT组和空白对照组[(61.67±7.64) *vs* (134.00±5.29)、(134.00±6.25)个，P<0.05]。

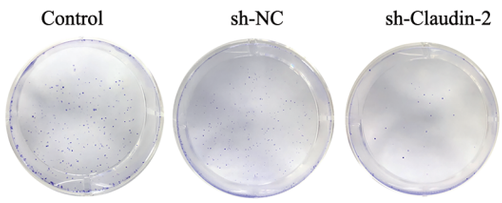


图5 敲降Claudin-2抑制细胞KYSE-450细胞的克隆形成能力

Fig.5 Knockdown of Claudin-2 inhibited the clone formation ability of KYSE-450 cells

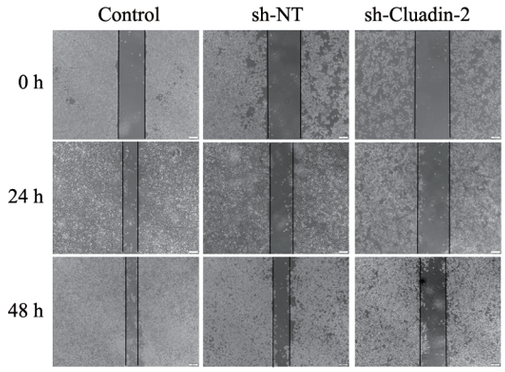


图6 敲降Claudin-2抑制细胞KYSE-450细胞的迁移能力

Fig.6 Knockdown of Claudin-2 inhibited the migration ability of KYSE-450 cells

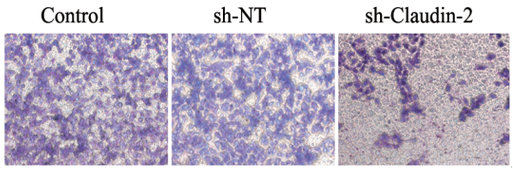


图7 敲降Claudin-2抑制细胞KYSE-450细胞的侵袭能力

Fig.7 Knockdown of Claudin-2 inhibited the invasion ability of KYSE-450 cells

3 讨 论

本研究发现Claudin-2在ESCC组织中的表达高于癌旁组织，与以往报道的Claudin-2在结直肠癌[11]、肺腺癌[12]、食管腺癌和ESCC[15]等组织中呈高表达的结果相一致。本研究发现Claudin-2表达与淋巴结转移相关，与ESCC患者五年生存率呈负相关，提示Claudin-2高表达与ESCC的预后不良相关。

本研究进一步探讨了Claudin-2可能参与的ESCC预后不良相关生物学行为。发现Claudin-2在KYSE450中高表达。敲降KYSE450细胞中Claudin-2的表达后发现，细胞的增殖、迁移和侵袭受到抑制。由此推测，Claudin-2在ESCC的发生发展过程中起重要作用，提示Claudin-2可能作为ESCC的治疗靶点及肿瘤标志物，也有作为预后标志物的潜力，这与Claudin-2可作为乳腺癌预后标志物[17]的报道一致。

目前已有研究建立了Claudin敲除或过表达的小鼠模型。比如将敲低Claudin-1的胃癌细胞通过尾静脉注入小鼠体内，发现敲低Claudin-1的表达能够显著抑制胃癌肺转移瘤的形成[18]。而在肺腺癌中，Claudin-1过表达能够抑制小鼠肺内转移结节的形成[19]。在卵巢中，沉默Claudin-3的表达能够使小鼠卵巢肿瘤荷载和腹水生成量的显着降低[20]，Claudin-3或Claudin-4表达的增加能促进小鼠体内肿瘤的生长[21]。

Claudin家族参与肿瘤生物学作用的分子机制已有一些报道。Claudin-1作为RRP1B-DNMT-Claudin-1通路分子，是胞质分裂作用因子(dedicator of cytokinesis 1，DOCK1)的靶点，可抑制三阴乳腺癌的增殖和转移[5]；Claudin-6至少部分通过激活YAP1及其下游转录靶点的转录来促进胃癌细胞的增殖、迁移和侵袭[22]；Claudin-7激活EMT促进结直肠癌的侵袭和转移[23]，但是Claudin-7可通过增加细胞间黏附抑制ESCC细胞的侵袭[24]。Claudin-2激活Afadin信号通路促进乳腺癌肝转移[25]，也可抑制Afadin/ERK信号通路来抑制骨肉瘤细胞的转移[13]，食管腺癌Claudin-2的表达与胆汁酸受体表达呈正相关[15]。ABU-FARSAKH等[15]未发现Claudin-2表达与ESCC患者生存时间相关，可能与本研究样本量52例较文献的26例更多有关。

综上所述，关于Claudin-2参与ESCC细胞肿瘤生物学作用的分子机制目前研究较少，需要进一步探索Claudin-2在ESCC发生发展、侵袭和转移过程中的相关分子机制，本研究为Claudin-2作为ESCC的潜在分子靶点、标记物和预后标志物提供了实验依据。

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