基础研究 论 著

ZCCHC12对分化型甲状腺癌细胞上皮-间质转化和侵袭的作用及其机制

刘光霞[1]，陈芳[1]，卢亚敏[1]，牛丽霞[2]，侯瞻[1]，赵连春[2*]

[1]河北省人民医院核医学科，河北石家庄 050051；[2]河北以岭医院检验科，河北石家庄 050091

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[摘要] **目的** 探讨ZCCHC12对分化型甲状腺癌细胞上皮-间质转化和侵袭的作用及其机制。**方法** 选取2017年5月—2018年12月河北省人民医院收治的50例分化型甲状腺腺癌患者作为分化型甲状腺癌组，另选取该院同期50名健康体检者作为健康对照组。采用ELISA法检测血清中PKA/cAMP反应元素结合蛋白(CREB)和p21的含量。采用Western blotting检测HUM-CELL-0097、TPC-1和FTC-133细胞系中CREB和p21蛋白的相对表达量。取TPC-1细胞和FTC-133细胞，分别设置空白对照组(正常培养TPC-1或FTC-133细胞)、NC si组(转染non-specific siRNA)、ZCCHC12 si组(转染*ZCCHC12* siRNA)、ZCCHC12 si+NC pc组(转染*ZCCHC12* siRNA后，转染pcDNA.3.1)、ZCCHC12 si+CREB pc组(转染*ZCCHC12* siRNA后，转染pcDNA.3.1-CREB) 、ZCCHC12 si+NC si组(转染*ZCCHC12* siRNA后，转染non-specific siRNA)、ZCCHC12 si+p21 si组(转染*ZCCHC12* siRNA后，转染p21 siRNA)，采用Western blotting检测ZCCHC12、CREB、P21、E-cadherin和N-cadherin蛋白的相对表达量，Transwell实验检测细胞侵袭能力。**结果** 与健康对照组比较，分化型甲状腺癌组血清中CREB含量升高($P<0.05$)，p21含量降低($P<0.01$)。与HUM-CELL-0097细胞比较，TPC-1和FTC-133细胞中CREB含量升高($P<0.01$)，p21含量降低($P<0.01$)。与NC si组比较，ZCCHC12 si组E-cadherin、p21蛋白相对表达量升高，CREB、N-cadherin蛋白相对表达量及细胞迁移数降低($P<0.01$)。ZCCHC12 si+CREB pc组p21蛋白相对表达量较ZCCHC12 si+NC pc组降低($P<0.01$)，逆转了干扰*ZCCHC12*对p21蛋白表达的促进作用。与ZCCHC12 si+NC si组比较，ZCCHC12 si+p21 si组E-cadherin蛋白相对表达量明显下降，N-cadherin蛋白相对表达量及细胞迁移数明显升高($P<0.01$)，逆转了干扰*ZCCHC12*对分化型甲状腺癌细胞上皮-间质转化和侵袭能力的抑制作用。**结论** 干扰*ZCCHC12*可通过CREB/p21信号通路有效抑制分化型甲状腺癌细胞的上皮-间质转化和侵袭，为分化型甲状腺癌的治疗提供了一定的理论基础。

[关键词] ZCCHC12；p21；分化型甲状腺癌；上皮-间质转化；侵袭

Effect and mechanism of ZCCHC12 on epithelial-mesenchymal transition and invasion in differentiated thyroid cancer cells

Liu Guang-Xia[1], Chen Fang[1], Lu Ya-Min[1], Niu Li-Xia[2], Hou Zhan[1], Zhao Lian-Chun[2*]

[1]*Department of Nuclear Medicine, Hebei General Hospital, Shijiazhuang, Hebei 050051, China*

[2]*Department of Laboratory Medicine, Hebei Yiling Hospital, Shijiazhuang, Hebei 050091, China*

[*]*Corresponding author, E-mail: zhaolian_000@163.com*

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[**Abstract**] **Objective** To investigate the effect and mechanism of ZCCHC12 on epithelial-mesenchymal transition and invasion of differentiated thyroid cancer cells. **Methods** A total of 50 patients with differentiated thyroid adenocarcinoma admitted to Hebei General Hospital from May 2017 to December 2018 were selected, and set as differentiated thyroid cancer group.

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[作者简介] 刘光霞，硕士研究生，主要从事甲状腺检验诊断方面的研究

[通信作者] 赵连春，E-mail：zhaolian_000@163.com

In addition, 50 subjects for healthy examination in the hospital during the same period were selected as the healthy control group. The contents of PKA/cAMP response element binding protein (CREB) and p21 in serum was detected by ELISA. Western blotting was used to detect the relative expressions of CREB and p21 in HUM-CELL-0097, TPC-1 and FTC-133 cell lines. TPC-1 cells and FTC-133 cells were taken and set up blank control group (normally cultured), NC si group (transfected with non-specific siRNA), and ZCCHC12 si group (transfected with *ZCCHC12* siRNA), ZCCHC12 si+NC pc group (transfected with *ZCCHC12* siRNA followed by the transfection with pcDNA.3.1), ZCCHC12 si+CREB pc group (transfected with *ZCCHC12* siRNA followed by the transfection with pcDNA.3.1-CREB), ZCCHC12 si+NC si group (transfected with *ZCCHC12* siRNA followed by the transfection with non-specific siRNA), ZCCHC12 si+p21 si group (transfected with *ZCCHC12* siRNA followed by the transfection with p21 siRNA). Western blotting was performed to detect the relative expressions of ZCCHC12, CREB, P21, E-cadherin and N-cadherin proteins. Transwell method was used to detect the cell invasion ability. **Results** Compared with healthy control group, the serum content of CREB increased ($P<0.05$), and of p21 decreased ($P<0.01$) in differentiated thyroid cancer group. Compared with HUM-CELL-0097 cells, the content of CREB increased ($P<0.01$), and of p21 decreased ($P<0.01$) in TPC-1 and FTC-133 cells. Compared with NC si group, the relative expressions of E-cadherin and p21 protein increased, while the relative protein expressions of CREB and N-cadherin and the number of cell migration decreased ($P<0.01$) in ZCCHC12 si group. The expression of p21 protein in ZCCHC12 si+CREB pc group was lower than that in ZCCHC12 si+NC pc group ($P<0.01$), which reversed the promoting effect of interfering *ZCCHC12* on p21 protein expression. Compared with ZCCHC12 si+NC si group, the relative expression of E-cadherin protein significantly decreased, while the relative expression of N-cadherin protein and the number of cell migration were significantly increased ($P<0.01$) in the ZCCHC12 si+p21 si group, which reversed the inhibitory effect of *ZCCHC12* interference on the epithelial-mesenchymal transition and invasive capacity in differentiated thyroid cancer cells. **Conclusions** Interfering with *ZCCHC12* can effectively inhibit the epithelial-mesenchymal transition and invasion of differentiated thyroid cancer cells by regulating CREB and p21, providing a certain theoretical basis for the treatment of differentiated thyroid cancer.

[**Key words**] ZCCHC12; p21; differentiated thyroid cancer; epithelial-mesenchymal transition; invasion

甲状腺肿瘤为常见的头颈部肿瘤和内分泌肿瘤[1]。甲状腺癌根据组织学特征可分为分化型和未分化型，其中分化型甲状腺癌约占75%，又可分为乳头状甲状腺癌(papillary thyroid carcinoma，PTC)和滤泡状甲状腺癌(follictalar thyroid carcinoma，FTC)[2-7]。ZCCHC12(含CCHC结构域的锌指蛋白12)是骨形态生成蛋白(bone morphogenetic protein，BMP)信号通路中的转录共激活因子[8-10]。本课题组前期研究发现，ZCCHC12在甲状腺癌组织和细胞系中呈过表达，且可能通过BMP/SMAD信号通路促进甲状腺癌的发生和发展[11]。另有研究发现，ZCCHC12可激活环磷腺苷效应元件结合蛋白(cyclic AMP-response element binding protein，CREB)[12]，而CREB可促进人脐动脉平滑肌细胞中p21的表达[13]，p21上调对甲状腺癌的发生发展具有抑制作用[14]。截至目前，关于ZCCHC12调控CREB/p21信号通路对分化型甲状腺癌的影响鲜少报道。本研究探讨了ZCCHC12对分化型甲状腺癌细胞上皮-间质转化和侵袭的作用及其机制。

1 材料与方法

1.1 细胞、试剂及仪器 人甲状腺滤泡上皮细胞HUM-CELL-0097、人PTC细胞TPC-1由河北省人民医院实验室保存；人FTC细胞FTC-133购自英国HHPACC细胞库。ZCCHC12、CREB、p21、E-cadherin、N-cadherin、GAPDH兔单抗及辣根过氧化物酶标记的羊抗兔二抗购自英国Abcam公司；BCA试剂盒购自美国Pierce公司；CREB ELISA试剂盒购自美国Raybiotech公司；p21 ELISA试剂盒购自美国Proteintech公司；空质粒pcDNA.3.1购自美国Invitrogen公司。高速离心机、移液器购自德国Eppendoff公司；电泳仪购自英国Syngene公司；蛋白凝胶成像系统、PCR仪、iMark酶标仪购自美国Bio-Rad公司；超净工作台购自北京医疗设备厂；细胞培养箱购自美国Thermo Scientific公司。

1.2 研究对象 选取2017年5月—2018年12月河北省人民医院收治的50例分化型甲状腺癌者(设为分化型甲状腺癌组)，其中男29例，女21例，年龄42~68(53.5 ± 2.6)岁，PTC 30例，FTC 20例。纳入标准：(1)经病理学检查确诊为分化型甲状腺癌；(2)临床资料完整。排除标准：(1)伴有其他系统恶性肿瘤；(2)合并心脑血管、造血系统、肝、肾等疾病；(3)伴有严重感染性疾病、免疫系统疾病；(4)伴有意识障碍或精神疾病；(5)正接受其他研究。另选取该院同期50名健康体检者作为健康对照组。本研究经河北省人民医院伦理委员会批准(批准文号：K-2018-037)，所有研究对象均签署知情同意书。

1.3 ELISA法检测血清CREB、p21含量 抽取研究对象的空腹静脉血3 ml，3500 r/min离心10 min，分离血清。按照CREB、p21 ELISA试剂盒说明书步骤操作，采用iMark酶标仪检测450 nm波长处的光密

度(OD)值，计算血清CREB、p21含量。

1.4 细胞培养及pcDNA.3.1-CREB过表达载体的构建 HUM-CELL-0097、TPC-1与FTC-133细胞分别于含10%胎牛血清、100 μg/ml链霉素和青霉素的DMEM培养基中培养。采用Trizol法提取细胞总RNA，以RNA为模板反转录成cDNA，然后扩增CREB基因序列(GenBank accession number AY347527，包括酶切位点*Xho* Ⅰ和*Eco*R Ⅰ)。将扩增获得的片段酶切后与空质粒pcDNA.3.1连接并测序，将正确的质粒命名为pcDNA.3.1-CREB。

1.5 细胞转染 将TPC-1细胞和FTC-133细胞分别接种于96孔板中，置于37 ℃、5% CO_2细胞培养箱中孵育24 h，待细胞融合至70%时，按照转染试剂操作说明进行细胞转染。设置空白对照组(正常培养TPC-1或FTC-133细胞)、NC si组(转染non-specific siRNA)、ZCCHC12 si组(转染*ZCCHC12* siRNA)、ZCCHC12 si+NC si组(转染*ZCCHC12* siRNA后，转染non-specific siRNA)、ZCCHC12 si+NC pc组(转染*ZCCHC12* siRNA后，转染pcDNA.3.1)、ZCCHC12 si+CREB pc组(转染*ZCCHC12* siRNA后，转染pcDNA.3.1-CREB)、ZCCHC12 si+p21 si组(转染*ZCCHC12* siRNA后，转染p21 siRNA)。将ZCCHC12 siRNA、p21 siRNA、non-specific siRNA、pcDNA.3.1-CREB和pcDNA.3.1各0.3 μg分别与0.6 μl Turbofect混匀，加入各组细胞中，37 ℃、5% CO_2孵育24 h，采用Western blotting检测转染效率。

1.6 Western blotting检测ZCCHC12、CREB、p21、E-cadherin、N-cadherin蛋白表达量 采用Western blotting检测HUM-CELL-009、TPC-1与FTC-133细胞中CREB、p21蛋白表达量；空白对照组、NC si组(转染non-specific siRNA)、ZCCHC12 si组、ZCCHC12 si+NC si组、ZCCHC12 si+NC pc组、ZCCHC12 si+CREB pc组CREB、p21蛋白表达量；ZCCHC12 si组、ZCCHC12 si+NC si组、ZCCHC12 si+p21 si组p21、E-cadherin、N-cadherin蛋白表达量。各组加入预冷的RIPA蛋白抽提试剂，离心后取上清。利用BCA试剂盒定量蛋白。上样，行SDS-PAGE电泳，然后转至PVDF膜上，5%脱脂奶粉室温封闭2 h；加入人源ZCCHC12单抗(1:800)、CREB单抗(1:800)、p21单抗(1:600)、E-cadherin单抗(1:600)、N-cadherin单抗(1:700)和GAPDH兔单抗(1:1000)4 ℃孵育过夜；TBST清洗，加入辣根过氧化物酶标记的羊源二抗室温孵育1 h；TBST清洗，X线曝光，采用Image-ProPlus软件分析。以GAPDH为内参，目的蛋白条带与GADPH条带灰度值的比值为目的蛋白的相对表达量。

1.7 Transwell实验检测细胞侵袭能力 设置空白对照组、ZCCHC12 si组、NC si组、ZCCHC12 si+NC si组和ZCCHC12 si+p21 si组，各组处理方法同1.5，利用Transwell实验检测细胞侵袭能力。Transwell上层小室中加入细胞悬液，下层小室加入含5%胎牛血清及0.5%小牛血清的培养基，5% CO_2、37 ℃条件下培养24 h后，擦去凝胶和滤膜上表面细胞，经苏木精染色后进行细胞计数。

1.8 统计学处理 采用SPSS 22.0软件进行统计分析。所有数据以$\bar{x}\pm s$表示，两组间比较采用*t*检验，多组间比较采用单因素方差分析，进一步两两比较采用Bonferroni检验。$P<0.05$为差异有统计学意义。

2 结 果

2.1 分化型甲状腺癌患者血清及细胞系中CREB、p21含量 与健康对照组比较，分化型甲状腺癌组血清CREB含量明显升高($P<0.05$)，p21含量明显降低($P<0.01$)(图1A)。与HUM-CELL-0097细胞比较，TPC-1、FTC-133细胞中CREB蛋白相对表达量明显升高，p21蛋白相对表达量明显降低($P<0.01$，图1B)。

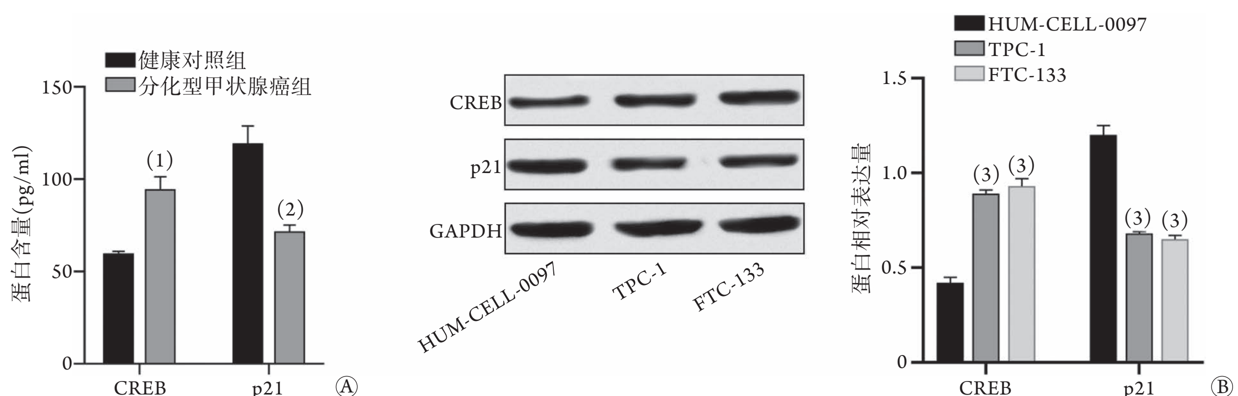

图1 分化型甲状腺癌患者血清(n=50)及细胞系(n=3)中CREB、p21含量

Fig.1 Contents of CREB and p21 in serum of patients (n=50) with differentiated thyroid cancer and cell line (n=3)

CREB. 环磷腺苷效应元件结合蛋白；A. ELISA检测患者血清CREB、p21含量；B. Western blotting检测细胞系中CREB、p21蛋白相对表达量；与健康对照组比较，(1)$P<0.05$，(2)$P<0.01$；与HUM-CELL-0097细胞比较，(3)$P<0.01$

2.2 干扰*ZCCHC12*对分化型甲状腺癌细胞上皮-间质转化及侵袭能力的影响　ZCCHC12 si组TPC-1或FTC-133细胞中ZCCHC12蛋白相对表达量较NC si组明显降低(P<0.01)，表明*ZCCHC12*干扰实验成功(图2A)。ZCCHC12 si组TPC-1或FTC-133细胞中E-cadherin蛋白相对表达量明显高于空白对照组和NC si组，N-cadherin蛋白相对表达量明显低于空白对照组和NC si组，细胞迁移数明显少于空白对照组和NC si组(P<0.01，图2)。

2.3 干扰*ZCCHC12*对分化型甲状腺癌细胞中CREB、p21蛋白表达的影响　与空白对照组比较，ZCCHC12 si组TPC-1或FTC-133细胞中CREB蛋白相对表达量明显低于空白对照组和NC si组，p21蛋白相对表达量明显高于空白对照组和NC si组(P<0.01，图3)。

2.4 干扰*ZCCHC12*且过表达CREB对分化型甲状腺癌细胞中p21蛋白表达的影响　ZCCHC12 si+CREB pc组TPC-1或FTC-133细胞中CREB蛋白相对表达量明显高于ZCCHC12 si+NC pc组和ZCCHC12 si组(P<0.01)，表明细胞转染实验成功。ZCCHC12 si+CREB pc组TPC-1或FTC-133细胞中p21蛋白相对表达量明显低于ZCCHC12 si组和ZCCHC12 si+NC pc组(P<0.01，图4)。

2.5 干扰*ZCCHC12*和*p21*对分化型甲状腺癌细胞上皮-间质转化及侵袭能力的影响　ZCCHC12 si+p21 si组TPC-1或FTC-133细胞中p21蛋白相对表达量明显低于ZCCHC12 si+NC si组和ZCCHC12 si组(P<0.01)，表明细胞转染成功(图5A)。ZCCHC12 si+p21 si组TPC-1或FTC-133细胞中E-cadherin蛋白相对表达量明显低于ZCCHC12 si组和ZCCHC12 si+NC si组，N-cadherin

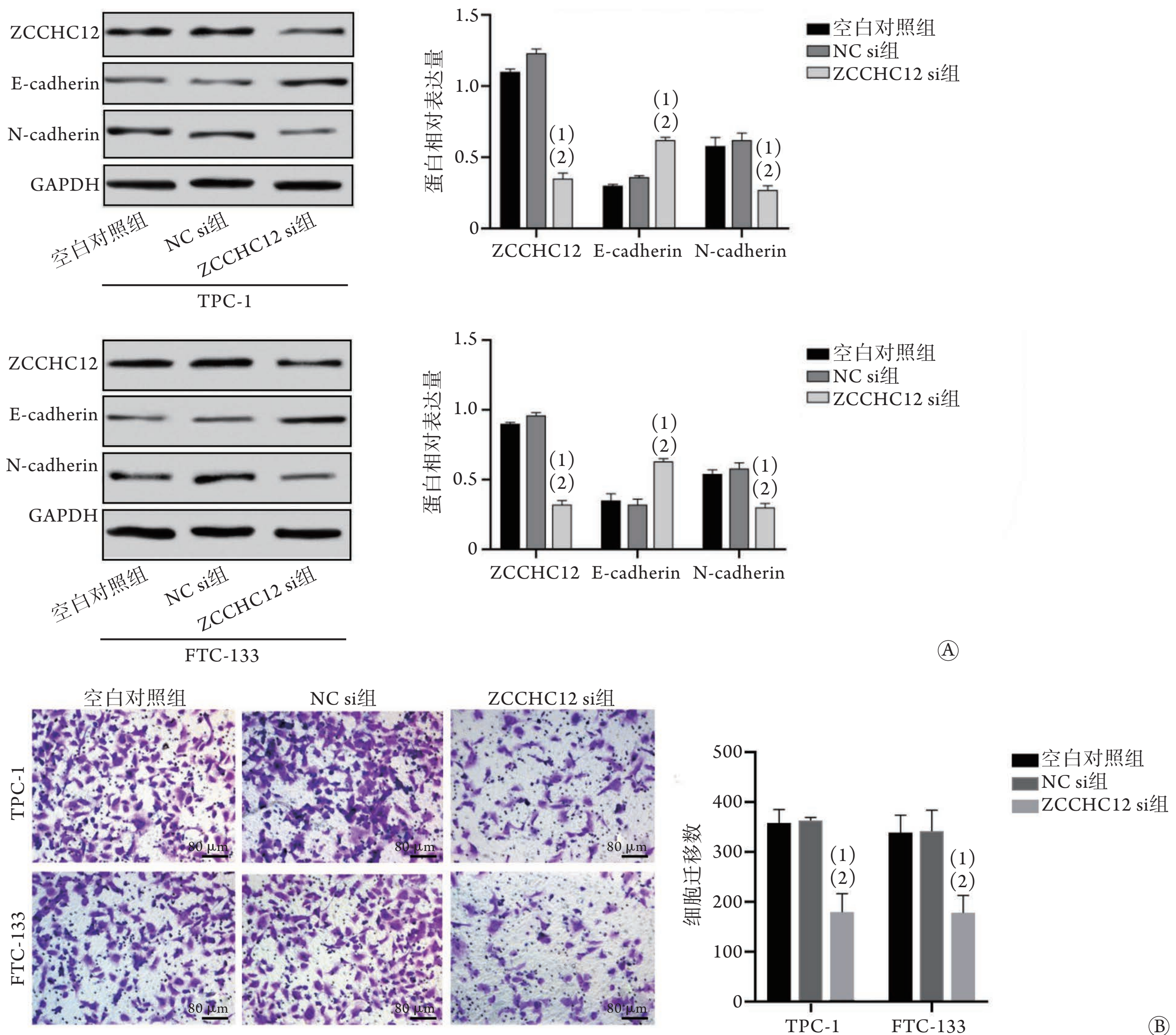

图2 干扰*ZCCHC12*对分化型甲状腺癌细胞上皮-间质转化和侵袭能力的影响

Fig.2 Influence of *ZCCHC12* interference on epithelial-mesenchymal transition and invasion of differentiated thyroid cancer cells

A. Western blotting检测上皮-间质转化相关蛋白的表达(n=3)；B. Transwell实验检测细胞侵袭能力(n=3)；与空白对照组比较，(1)P<0.01；与NC si组比较，(2)P<0.01

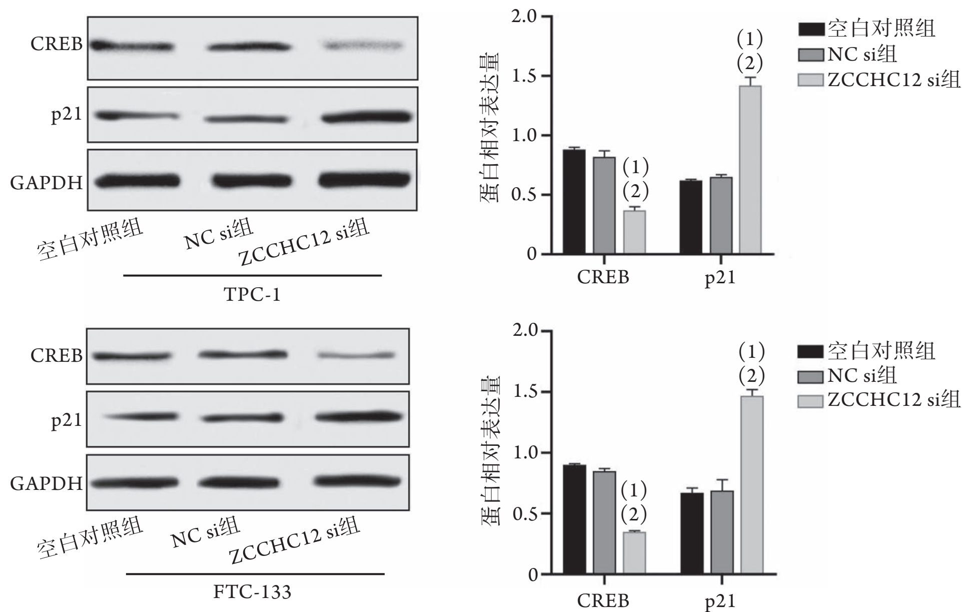

图3 干扰*ZCCHC12*对分化型甲状腺癌细胞中CREB、p21蛋白表达的影响(Western blotting, n=3)

Fig.3 Influence of *ZCCHC12* interference on the protein expressions of CREB and p21 in differentiated thyroid cancer cells (Western blotting, n=3)

CREB. 环磷腺苷效应元件结合蛋白；与空白对照组比较，(1)P<0.01；与NC si组比较，(2)P<0.01

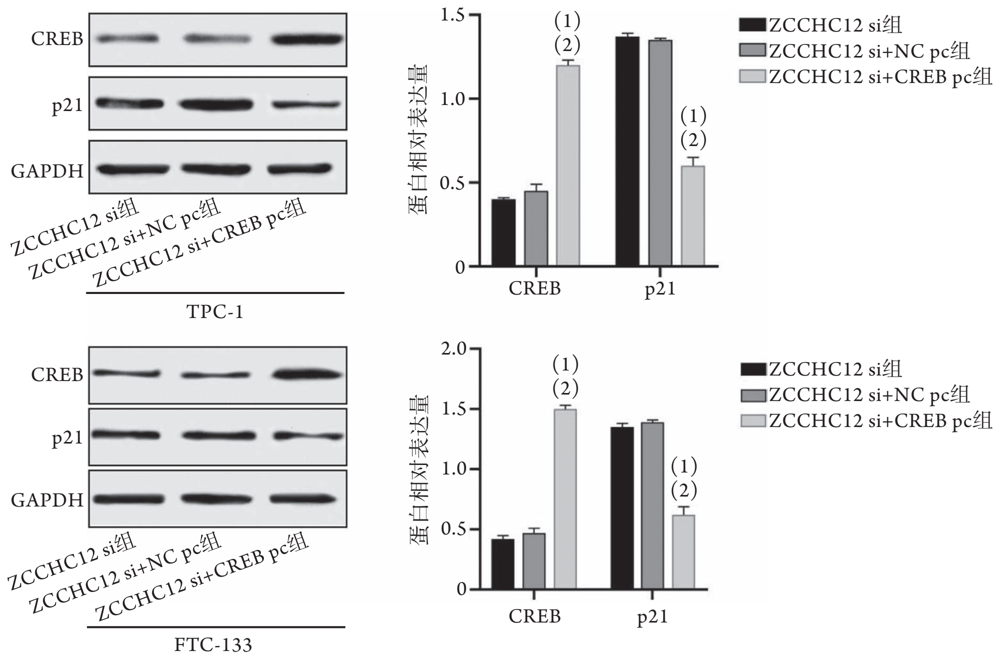

图4 干扰*ZCCHC12*且过表达*CREB*对分化型甲状腺癌细胞中p21蛋白表达的影响(Western blotting, n=3)

Fig.4 Influence of *ZCCHC12* interference and *CREB* overexpression on the protein expression of p21 in differentiated thyroid cancer cells (Western blotting, n=3)

与ZCCHC12 si组比较，(1)P<0.01；与ZCCHC12 si+NC pc组比较，(2)P<0.01

蛋白相对表达量明显高于ZCCHC12 si组和ZCCHC12 si+NC si组，细胞迁移数明显多于ZCCHC12 si组和ZCCHC12 si+NC si组(P<0.01)(图5)。

3 讨 论

既往研究发现，ZCCHC12在甲状腺癌组织中呈高表达[11]。*ZCCHC12*高表达可明显促进甲状腺癌细胞CGTH W-3和FTC-133增殖并抑制其凋亡，干扰*ZCCHC12*则可明显抑制CGTH W-3和FTC-133细胞增殖并促进其凋亡，表明ZCCHC12对甲状腺癌细胞增殖和凋亡起调控作用[11]。另有研究发现，ZCCHC12可明显促进神经细胞中CREB的表

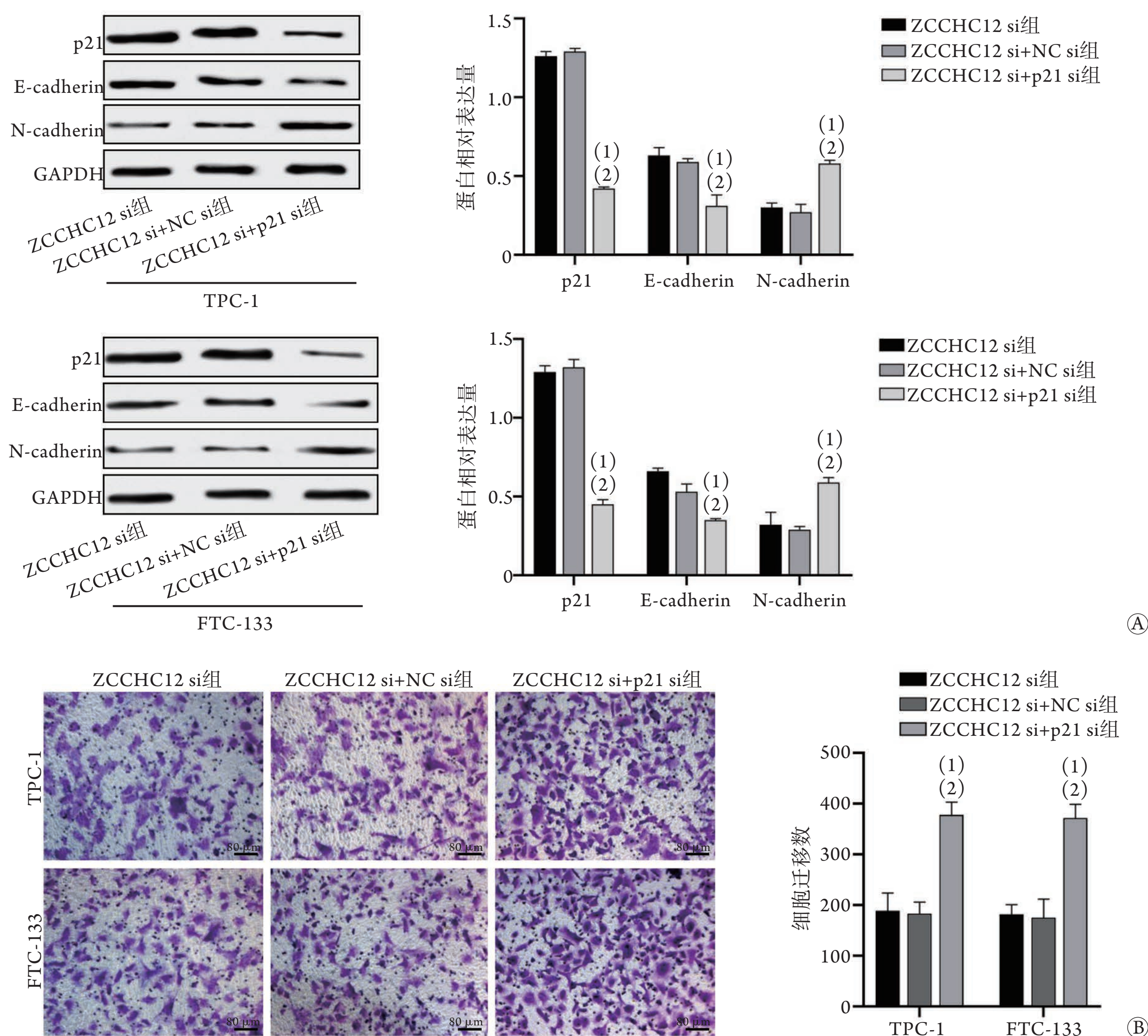

图5 干扰*ZCCHC12*和*p21*对分化型甲状腺癌细胞上皮-间质转化和侵袭能力的影响

Fig.5 Influence of interference of *ZCCHC12* and *p21* on epithelial-mesenchymal transition and invasion of differentiated thyroid cancer cells

A. Western blotting检测上皮-间质转化相关蛋白的表达(n=3)；B. Transwell实验检测细胞侵袭能力(n=3)；与ZCCHC12 si组比较，(1)P<0.01；与ZCCHC12 si+NC si组比较，(2)P<0.01

达[8]，而CREB可抑制小鼠胰岛β细胞中p21的表达[15]。Ruan等[16]发现，p21高表达可有效抑制甲状腺癌的发展。截至目前，ZCCHC12通过CREB/p21通路调控分化型甲状腺癌细胞上皮-间质转化和侵袭能力相关的研究鲜见。因此，本研究探讨了ZCCHC12、CREB和p21在分化型甲状腺癌细胞中的调控关系，以及对分化型甲状腺癌细胞上皮-间质转化和侵袭能力的影响，结果显示，干扰*ZCCHC12*可上调E-cadherin表达、下调N-cadherin表达，表明干扰*ZCCHC12*可明显抑制分化型甲状腺癌细胞的上皮-间质转化；Transwell实验结果显示，干扰*ZCCHC12*可明显抑制分化型甲状腺癌细胞的侵袭能力。

Ayroldi等[17]研究发现，CREB在甲状腺癌的发展中起着重要作用，可促进肿瘤的生长。Li等[12]发现，ZCCHC12可上调宫颈癌细胞、神经母细胞瘤细胞和脑胶质瘤细胞中CREB的表达。然而，ZCCHC12对分化型甲状腺癌细胞中CREB的调控作用尚未明确。本研究结果显示，分化型甲状腺癌患者血清中CREB含量明显升高，而抑制分化型甲状腺癌细胞中*ZCCHC12*的表达后，CREB蛋白表达量明显下降。由此可见，在分化型甲状腺癌中，ZCCHC12可促进CREB的表达，与Li等[12]报道的宫颈癌细胞、神经母细胞瘤细胞和脑胶质瘤细胞中ZCCHC12对CREB的正向调节作用一致。p21是一种细胞周期蛋白依赖性激酶抑制蛋白，作为G_1/S期限制点的调节因子，可抑制细胞从G_1期向S期转化，起到抑制细胞生长的作用[18-20]。Li等[21]研究发现，

p21过表达可有效抑制甲状腺癌细胞的增殖。Yang等[22]研究发现，p21可促进甲状腺间变性癌细胞凋亡。有研究发现，干扰lncRNA-HOTAIR可上调p21的表达，从而抑制结直肠癌细胞的侵袭能力[23]。截至目前，p21对分化型甲状腺癌细胞侵袭能力的调节作用尚不清楚。本研究结果显示，分化型甲状腺癌患者血清中p21含量明显降低。在分化型甲状腺癌细胞中，干扰*ZCCHC12*可明显上调p21蛋白的相对表达量，而过表达CREB可明显消除干扰*ZCCHC12*对p21表达的促进作用，提示在分化型甲状腺癌细胞中，干扰*ZCCHC12*可通过CREB促进p21的表达。此外，与干扰*ZCCHC12*的细胞比较，同时干扰*ZCCHC12*和*p21*可明显下调E-cadherin蛋白的表达、上调N-cadherin蛋白的表达，表明干扰*p21*可有效消除干扰*ZCCHC12*对分化型甲状腺癌细胞上皮-间质转化的影响。Transwell实验结果也表明，干扰*p21*可有效消除干扰*ZCCHC12*对分化型甲状腺癌细胞侵袭能力的影响。因此，在分化型甲状腺癌细胞中，干扰*ZCCHC12*可通过CREB增强p21的表达，进而抑制分化型甲状腺癌细胞的上皮-间质转化和侵袭。有研究发现，ZCCHC9可通过与NF-kB启动子结合抑制MAPK通路[24]，而MAPK通路可激活Notch通路，影响甲状腺乳头状癌细胞的增殖[25]。截至目前，ZCCHC12通过调控MAPK通路影响分化型甲状腺癌细胞增殖的报道较少，后期可探讨ZCCHC12/MAPK在分化型甲状腺癌细胞增殖中的作用。

综上所述，分化型甲状腺癌患者血清中CREB含量明显升高，p21含量明显降低，干扰分化型甲状腺癌细胞中的*ZCCHC12*可通过抑制CREB的表达而促进p21的表达，进而抑制分化型甲状腺癌细胞的上皮-间质转化和侵袭。但本研究未在体内探讨ZCCHC12、CREB、p21对分化型甲状腺癌发生发展的作用，后续需进一步进行大鼠体内实验探讨ZCCHC12、CREB、p21对分化型甲状腺癌发生发展的影响。

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