doi: 10.3978/j.issn.2095-6959.2019.09.003
View this article at: http://dx.doi.org/10.3978/j.issn.2095-6959.2019.09.003

CTGF缺失表达抑制动脉粥样硬化易损斑块的形成

周心涛，赵黎丙，闵新文，陈娇，张涛

(湖北医药学院附属东风医院心血管内科，湖北 十堰 442000)

[摘　要] **目的：**通过动物模型分析探讨基于调控结缔组织生长因子(connective tissue growth factor，CTGF)表达的动脉粥样硬化易损斑块形成机制。**方法：**选取8~10周龄的雌性同窝$CTGF^{+/+}ApoE^{-/-}$小鼠(观察组)与$CTGF^{-/-}ApoE^{-/-}$小鼠(对照组)，均建立动脉粥样硬化模型，给予高脂高胆固醇饲料喂养，观察小鼠状态，检测血脂指标，记录易损斑块的病理状况。**结果：**所有小鼠顺利完成实验，未观察到局部和全身性的不良反应。观察组小鼠造模后2周、4周的总胆固醇(TC)、低密度脂蛋白胆固醇(low-density lipoprotein cholesterol，LDL-C)、三酰甘油(TG)值比对照组高，高密度脂蛋白胆固醇(high-density lipoprotein cholesterol，HDL-C)值比对照组低，差异有统计学意义(P<0.05)。两组小鼠造模后2周、4周的动脉内都形成动脉粥样硬化斑块，观察组心底、主动脉根部动脉粥样硬化斑块较对照组更显著，脂质核心面积增大，泡沫细胞显著增多；观察组小鼠造模后2周、4周的易损斑块面积都显著高于对照组(P<0.05)。观察组小鼠的MMP-9相对表达水平比对照组高(P<0.05)。**结论：**CTGF缺失表达可抑制MMP-9的表达，促进血脂代谢正常，从而抑制动脉粥样硬化易损斑块的形成。

[关键词] 结缔组织生长因子；动脉粥样硬化；易损斑块；血脂

Lack of CTGF expression inhibits atherosclerotic vulnerable plaque progression

ZHOU Xintao, ZHAO Libing, MIN Xinwen, CHEN Jiao, ZHANG Tao

(Department of Cardiovascular Medicine, Dongfeng Hospital, Affiliated to Hubei University of Medicine, Shiyan Hubei 442000, China)

Abstract **Objective:** To investigate the mechanism of atherosclerotic vulnerable plaque formation based on the regulation of connective tissue growth factor (CTGF) expression by animal model analysis. **Methods:** The atherosclerosis model were established in 8-10 weeks old female littermate $CTGF^{+/+}ApoE^{-/-}$ mice (the observation group) and $CTGF^{-/-}ApoE^{-/-}$ mice (the control group). The rats were fed with high-fat and high-cholesterol diet, the state of the mice was observed, the blood lipid index was measured, and the pathological condition of the vulnerable plaques were recorded. **Results:** All mice were successfully completed there were no local or systemic adverse reactions were observed. The TC, TG and LDL-C values of the observation group were higher than those of the control group at 2 and 4 weeks after model establishment, and the HDL-C value were lower than that of the

收稿日期 (Date of reception)：2018–12–12
通信作者 (Corresponding author)：张涛，Email: zhangt8376@163.com

control group, compared the difference were statistically significant (P<0.05). The atherosclerotic plaques were formed in the arteries of the two groups at 2 and 4 weeks after modeling, the atherosclerotic plaques in the heart and aortic roots of the observation group were more significant than the control group, and the lipid core area were increased and the foam cells were increased significantly. The area of vulnerable plaques in the observation group at 2 and 4 weeks after modeling were significantly higher than that in the control group (P<0.05). The relative expression level of MMP-9 in the observation group were higher than that in the control group, and compared the difference were statistically significant (P<0.05). **Conclusion:** The lack of expression of CTGF signaling pathway can inhibit the expression of MMP-9 and promote the normal metabolism of blood lipids, thus inhibit the occurrence of atherosclerotic vulnerable plaque.

Keywords connective tissue growth factor; atherosclerosis; vulnerable plaque; blood lipid

动脉粥样硬化是一项复杂的病理过程，多种因素参与其中，巨噬细胞和T淋巴细胞等均起到了一定作用[1]。心脑血管事件发生的主要原因是粥样斑块(atheromatous plaque，AS)的破裂，其病理过程由AS稳定性决定，尤其是易损斑块破裂伴血栓形成引起是导致急性临床事件的主要原因[2-3]。易损斑块是指迅速成为罪犯病变或者易于形成血栓的斑块，其特点主要表现在胶原含量少、平滑肌细胞密度低、细胞因子合成和释放增加、淋巴细胞浸润增加等方面，伴有显著的新生血管生成或斑块内出血[4-5]。动脉粥样硬化易损斑块的形成是一个漫长复杂的过程，有多种因素参与，炎性反应、脂代谢紊乱等贯穿于易损斑块形成与破裂的全过程[6]。随着低密度脂蛋白(low-density lipoprotein，LDL)水平的升高，脂质渗透至内皮下积聚，导致血管内皮功能受损，继而趋化炎症细胞释放一系列炎症因子，使血管发生重构，导致斑块内脂质池增大，诱发稳定斑块演变为易损斑块[7-8]。结缔组织生长因子(connective tissue growth factor，CTGF)属于TGF-β1诱导纤维化的一种下游信号因子，可调节基质细胞整合素受体表达而促进细胞与基质黏附及基质沉积，在调节免疫系统平衡、维持机体内环境的稳定等方面发挥重要作用[9-10]。CTGF表达失衡将会破坏机体免疫内环境的稳定，导致炎症反应的无限放大[11]，但其是否影响易损斑块的稳定性鲜有报道。本研究拟建立动物模型，探讨基于调控CTGF表达的动脉粥样硬化易损斑块形成机制。

1 材料与方法

1.1 材料

选取8~10周龄(体重≥18 g)、状态良好的雌性同窝$CTGF^{+/+}ApoE^{-/-}$小鼠(观察组)与$CTGF^{-/-}ApoE^{-/-}$小鼠(对照组)，每组各8只小鼠。小鼠购自美国Jackson实验室，由湖北医药学院动物实验中心保种、繁育。本研究经湖北医药学院附属东风医院实验动物伦理委员会批准。高脂高胆固醇小鼠饲料由北京科澳协力饲料有限公司提供。

1.2 方法

两组小鼠均给予高脂高胆固醇饲料(含21%脂肪和0.15%胆固醇)喂养持续10周，建立动脉粥样硬化模型。腹腔注射0.08%戊巴比妥钠麻醉小鼠后，固定后从颈部正中皮肤位置剪开，暴露右侧颈总动脉，将内径为0.3 mm、长度为3 mm的硅胶管套置于颈总动脉外周并固定套管，缝合皮肤切口，苏醒后回笼。

1.3 观察指标

1)在造模后实时观察小鼠状态。2)两组分别在造模后2周、4周各取4只小鼠，经心室采血并分离血清，采用Hitachi 7180型全自动生化分析仪检测总胆固醇(TC)、三酰甘油(TG)、高密度脂蛋白胆固醇(high-density lipoprotein cholesterol，HDL-C)和低密度脂蛋白胆固醇(low-density lipoprotein cholesterol，LDL-C)的变化。3)在上述同一时间点取血后处死小鼠，取小鼠颈总动脉组织块，以4 μm厚度连续切片，行HE染色，选取每个小鼠斑块面积最大的层面行斑块形态学分析。4)小鼠斑块病变分型分为纤维性病变、AS、薄纤维帽斑块、破裂斑、斑块腐蚀，前2类被认为是稳定斑块，后3类被认定是易损斑块，记录易损斑块面积。5)将取材的组织样本进行研磨，取总蛋白20 μg的样本加样到10% SDS-PAGE电泳，转膜后加入兔抗大鼠MMP-9抗

体(1:500)，或β-actin抗体(1:1 000)一抗，4 ℃孵育过夜。加入HRP二抗(1:5 000)，于室温下孵育30 min，ECL发光后进行半定量分析，明确CTGF蛋白的相对表达水平。

1.4 统计学处理

采用SPSS 22.00软件进行数据分析，计量资料以均数±标准差($\bar{x}\pm s$)表示，数据对比采用t检验与One-Way ANOVA分析，检验水准为α=0.05。P<0.05为差异有统计学意义。

2 结果

2.1 体重与血脂变化

所有小鼠顺利完成实验，未观察到局部和全身性的不良反应。观察组小鼠造模后2周、4周的TC，LDL-C，TG值比对照组高，HDL-C值比对照组低，差异有统计学意义(P<0.05，表1)。

2.2 斑块形态学对比

两组小鼠造模后2周、4周的动脉内均形成动脉粥样硬化斑块，内膜下脂质池形成；观察组心底、主动脉根部动脉粥样硬化斑块较对照组更显著，脂质核心面积增大，泡沫细胞显著增多，纤维帽较薄，坏死显著(图1)。

2.3 易损斑块面积

观察组小鼠造模后2周、4周的易损斑块面积都显著高于对照组，差异有统计学意义(P<0.05，表2)。

表1 两组不同时间点的血脂对比(n=4)

Table 1 Comparison of blood lipids at different time points between the 2 groups (n=4)

组别	造模后2周/(mmol·L^{-1})				造模后4周/(mmol·L^{-1})			
	TC	TG	LDL-C	HDL-C	TC	TG	LDL-C	HDL-C
观察组	25.28 ± 2.49	2.76 ± 0.99	16.14 ± 3.22	0.70 ± 0.34	26.29 ± 2.55	4.19 ± 0.22	18.49 ± 1.48	0.65 ± 0.11
对照组	23.87 ± 2.91	2.62 ± 0.28	13.11 ± 2.11	0.78 ± 0.11	23.52 ± 3.33	2.97 ± 0.29	15.10 ± 2.58	0.75 ± 0.13
t	5.683	4.583	8.193	3.785	7.493	11.832	6.833	4.722
P	0.012	0.022	0.005	0.037	0.010	<0.001	0.005	0.020

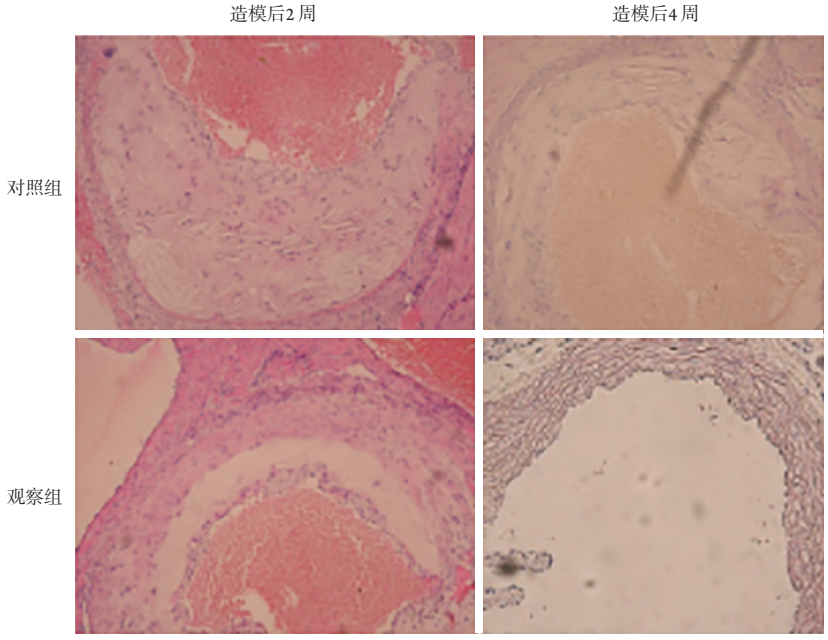

图1 两组不同时间点的斑块形态学(HE，×400)

Figure 1 Plaque morphology at two different time points (HE, ×400)

表2 两组不同时间点的动脉粥样硬化易损斑块面积对比（*n*=4）

Table 2 Comparison of atherosclerotic vulnerable plaque area at different time points between the 2 groups (*n*=4)

组别	造模后2周/μm^2	造模后4周/μm^2
观察组	86 781 ± 11 342	118 100 ± 10 847
对照组	79 872 ± 10 444	99 214 ± 11 842
t	7.482	6.444
P	0.010	0.014

2.4 MMP-9 表达情况对比

观察组小鼠的MMP-9相对表达水平比对照组高，差异有统计学意义（*P*<0.05，图2）。

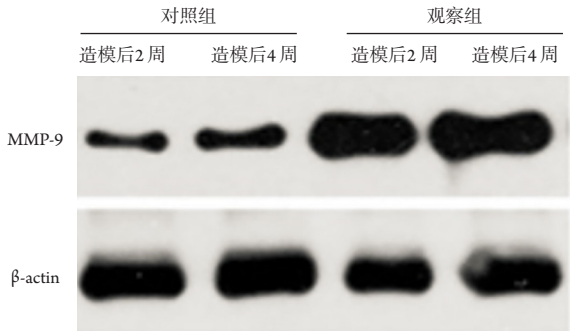

图2 两组不同时间点的MMP-9表达

Figure 2 MMP-9 expression at two different time points

3 讨论

易损斑块是指易于形成血栓或可能迅速进展为罪犯病变的斑块，部分斑块内部伴有显著的新生血管生成。动脉粥样易损斑块可使斑块破裂或表面溃疡，继而引发不同程度的血栓形成，引起动脉阻塞，导致心脑血管疾病的发生[12]。研究[13-14]显示：动脉粥样硬化病灶中不仅聚集了较多的T淋巴细胞，而且还能释放相对较多的炎症因子，促使巨噬细胞激活，导致出现局部炎症；此外，还可使凝血系统激活，加快释放出组织因子，促进斑块的易损性增加。

动脉粥样硬化是对动脉壁脂质累积产生的炎症反应的过程，起始于血浆TG，TC水平的升高，继而导致动脉内皮细胞渗透性改变，使得脂质穿过内膜进入血管壁，导致动脉粥样硬化斑块形成[15]。CTGF是属于富含半胱氨酸的一种多肽，分子量为30~40 kD(1 D=1 u)，对纤维细胞具有一定影响，可促进其产生、趋化及增殖等诸多功能[16]。研究[17]显示：CTGF可能参与了动脉粥样硬化的炎症反应，不仅心力衰竭、脑卒中患者，甚至心肌梗死患者也有CTGF表达异常情况。本研究显示：观察组小鼠造模后2周、4周的TC，LDL-C，TG值比对照组高，HDL-C值比对照组低，差异有统计学意义（*P*<0.05），表明CTGF的表达可导致机体血脂代谢紊乱。研究[18]表明：CTGF可参与调控体内多种生物学过程，如糖代谢、细胞生存与增殖、脂肪代谢等，敲除CTGF可促使血脂恢复正常。

目前较公认的易损斑块的特点是脂质核心面积巨大、纤维帽变薄、活动性炎症反应。斑块溃疡变、纤维帽破裂和斑块内出血是易损斑块发生的前奏，动脉粥样硬化是也脂质核心的必然过程[19]。CTGF在不同动脉粥样硬化发展阶段，因炎症状态不同，其发生的作用也不同。炎症活跃时，CTGF可促进动脉粥样硬化斑块向不稳定斑块转变以及斑块破裂的发生，可通过促进巨噬细胞炎症活性而发挥作用[20]。CTGF也会通过抑制平滑肌细胞增殖，减少巨噬细胞数目，减少斑块纤维帽中胶原比例，从而使斑块易损性增加[21]。本研究结果显示：两组小鼠造模后2周、4周的动脉内都形成动脉粥样硬化斑块，观察组心底、主动脉根部动脉粥样硬化斑块较对照组更显著，脂质核心面积增大，泡沫细胞显著增多；观察组小鼠造模后2周、4周的易损斑块面积都显著高于对照组（*P*<0.05）。CTGF可在疾病状态下，穿梭到细胞质中行使“非基因功能”，在巨噬细胞中，CTGF被多种促炎症因子快速诱导表达，诱导促炎症基因激活，也可增加NF-κB的活性，增加巨噬细胞对脂蛋白的摄取，诱发形成易损斑块[22]。

基质金属蛋白酶的释放是引起纤维帽损伤，导致纤维帽破裂继而形成易损斑块的一种重要物质。MMP-9激活，纤维帽变薄，胶原降解增多，斑块趋于不稳定[23]。CTGF缺失表达可改变斑块的组成成分，增加平滑肌细胞和斑块内胶原的含量，促使巨噬细胞的浸润和脂质的沉积减少，斑块转变为稳定型的表型[24]。其机制主要包括减轻或抑制机体出现局部炎症反应，降低MMP-9的释放量，分泌更多的抗炎因子，抑制易损斑块的形成[25]。本研究显示：观察组小鼠的MMP-9相对表达水平比对照组高，差异有统计学意义（*P*<0.05）。然而受限于研究时间与成本，本研究仅探讨了前期证实的CTGF通路，未能更深入地研究信号通路。

综上，CTGF缺失表达可抑制MMP-9的表达，促进血脂代谢正常，从而抑制动脉粥样硬化易损斑块的形成。

参考文献

1. 漆仲文, 李萌, 张军平. 从滋养血管成熟化探讨稳定动脉粥样硬化易损斑块的作用[J]. 中国动脉硬化杂志, 2017, 25(7): 737-740.
   QI Zhongwen, LI Meng, ZHANG Junping. Study on the role of vasa vasorum maturation in stabilizing vulnerable plaque of atherosclerosis[J]. Chinese Journal of Arteriosclerosis, 2017, 25(7): 737-740.
2. Zhang YP, Huang YT, Huang TS, et al. The mammalian target of rapamycin and DNA methyltransferase 1 axis mediates vascular endothelial dysfunction in response to disturbed flow[J]. Sci Rep, 2017, 7(1): 14996.
3. 李晶津, 陈红, 任景怡. 不稳定型心绞痛患者循环microRNA对神经生长因子通路的靶向调节作用及其功能调控网络[J]. 北京大学学报(医学版), 2014, 46(6): 868-874.
   LI Jingjin, CHEN Hong, REN Jingyi. Targeted effect of microRNA on nerve growth factor pathway and its functional network in patients with unstable angina[J]. Journal of Peking University (Health Sciences), 2014, 46(6): 868-874.
4. Yao Y, Li B, Fu C, et al. Anti-connective tissue growth factor detects and reduces plaque inflammation in early-stage carotid atherosclerotic lesions[J]. Nanomedicine, 2017, 13(8): 2385-2394.
5. 胡木, 张永生, 孔柄坛, 等. mTOR信号通路与动脉粥样硬化[J]. 中国动脉硬化杂志, 2016, 24(12): 1269-1272.
   HU Mu, ZHANG Yongsheng, KONG Bingtan, et al. mTOR Signaling Pathway and Its Relationship with Atherosclerosis[J]. Chinese Journal of Arteriosclerosis, 2016, 24(12): 1269-1272.
6. Shi H, Zhang C, Pasupuleti V, et al. CCN3 regulates macrophage foam cell formation and atherosclerosis[J]. Am J Pathol, 2017, 187(6): 1230-1237.
7. 肖亚莉, 黄鹤, 黄丹, 等. G蛋白信号调节因子5在动脉粥样硬化发展中的作用[J]. 医学研究杂志, 2016, 45(3): 178-180.
   XIAO Yali, HUANG He, HUANG Dan, et al. The role of G protein signal regulator 5 in the development of atherosclerosis[J]. Journal of Medical Research, 2016, 45(3): 178-180.
8. Wu W, Li J, Zhao M, et al. HIF-1α mediates visfatin-induced CTGF expression in vascular endothelial cells[J]. Cell Mol Biol (Noisy-le-grand), 2017, 63(4): 28-32.
9. Khuman MW, Harikumar SK, Sadam A, et al. Candesartan ameliorates arsenic-induced hypertensive vascular remodeling by regularizing angiotensin II and TGF-beta signaling in rats[J]. Toxicology, 2016, 30(374): 29-41.
10. 景怡, 蔡丹凤, 林超, 等. 慢性内质网应激诱导的凋亡在动脉粥样硬化斑块形成中的作用[J]. 中国动脉硬化杂志, 2017, 25(9): 957-962.
    JING Yi, CAI Danfeng, LIN Chao, et al. Role of chronic endoplasmic reticulum stress induced apoptosis in atherosclerotic plaque formation[J]. Chinese Journal of Arteriosclerosis, 2017, 25(9): 957-962.
11. 刘俊丽, 张沐新, 梁迪, 等. 拮抗结缔组织生长因子小分子化合物的研究进展[J]. 中国药物警戒, 2018, 15(2): 98-102.
    LIU Junli, ZHANG Muxin, LIANG Di, et al. Research progress on small-molecule compounds against connective tissue growth factor[J]. Chinese Journal of Pharmacovigilance, 2018, 15(2): 98-102.
12. 杨俊, 岳增辉, 谢涛, 等. 动脉粥样硬化易损斑块与TLR4/NFκB信号通路关系的研究进展[J]. 中西医结合心脑血管病杂志, 2014, 12(6): 747-749.
    YANG Jun, YUE Zenghui, XIE Tao, et al. Toll Receptor 4/NF kB Signaling Pathway in Atherosclerotic Vulnerable Plaque[J]. Chinese Journal of Integrative Medicine on Cardio/Cerebrovascular Disease, 2014, 12(6): 747-749.
13. Gerritsen KG, Falke LL, van Vuuren SH, et al. Plasma CTGF is independently related to an increased risk of cardiovascular events and mortality in patients with atherosclerotic disease: the SMART study[J]. Growth Factors, 2016, 34(3/4): 149-58.
14. 谢涛, 岳增辉. TLR2/4 IRF3信号通路调控胆固醇逆转运及其对动脉粥样硬化影响的研究进展1)[J]. 中西医结合心脑血管病杂志, 2014, 12(4): 493-495.
    XIE Tao, YUE Zenghui. TLR2/4 IRF3 signaling pathway regulates reverse cholesterol transport and its effect on atherosclerosis 1)[J]. Chinese Journal of Integrative Medicine on Cardio/Cerebrovascular Disease, 2014, 12(4): 493-495.
15. Wang KC, Yeh YT, Nguyen P, et al. Flow-dependent YAP/TAZ activities regulate endothelial phenotypes and atherosclerosis[J]. Proc Natl Acad Sci U S A, 2016, 113(41): 11525-11530.
16. 周明学, 康群甫, 刘卫红, 等. 动脉粥样硬化斑块稳定期和易损期小鼠主动脉基因启动子区DNA甲基化及基因表达差异[J]. 中国病理生理杂志, 2015, 28(10): 1828.
    ZHOU Mingxue, KANG Qunfu, LIU Weihong, et al. Differences in DNA methylation and gene expression in promoter region of aorta gene between stable and vulnerable mice with atherosclerotic plaque[J]. Chinese Journal of Pathophysiology, 2015, 28(10): 1828.
17. Taylor E, Huang N, Bodde J, et al. MRI of atherosclerosis and fatty liver disease in cholesterol fed rabbits[J]. J Transl Med, 2018, 16(1): 215.
18. Tu Y, Sun Y, Fan Y, et al. Multimodality molecular imaging of cardiovascular disease based on nanoprobes[J]. Cell Physiol Biochem, 2018, 48(4): 1401-1415.
19. 巩清波. 茯苓复方与有氧运动干预动脉粥样硬化模型大鼠血脂及黏附分子的变化[J]. 中国组织工程研究, 2015, 19(40):

6465-6469.
GONG Qingbo. Effect of Poria compound combined with aerobic exercise on lipid and adhesion molecules in atherosclerosis rats[J]. Journal of Clinical Rehabilitative Tissue Engineering Research, 2015, 19(40): 6465-6469.

20. West NEJ, Corrigan JP, Owen RHG, et al. Percutaneous sampling of local biomolecule gradients across coronary artery atherosclerotic plaques[J]. JACC Basic Transl Sci, 2017, 2(6): 646-654.
21. 王和峰, 翟纯刚, 庞文会, 等. PI3K/Akt/mTOR信号通路在巨噬细胞自噬及动脉粥样硬化斑块不稳定中的作用[J]. 中国病理生理杂志, 2013, 29(3): 390-397.
WANG Hefeng, ZHAI Chungang, PANG Wenhui, et al. Roles of PI3K/Akt/mTOR signaling pathway in macrophage autophagy and atherosclerotic plaque instability[J]. Chinese Journal of Pathophysiology, 2013, 29(3): 390-397.
22. Luque A, Farwati A, Krupinski J, et al. Association between low levels of serum miR-638 and atherosclerotic plaque vulnerability in patients with high-grade carotid stenosis[J]. J Neurosurg, 2018 [Epub ahead of print].
23. Frostegård J, Hellström C, Nilsson P, et al. Autoantibody profiling reveals four protein candidate autoantigens associated with systemic lupus erythematosus[J]. Lupus, 2018, 27(10): 1670-1678.
24. 付文波, 丁世芳, 李大主, 等. 急性冠脉综合征诱导单核-巨噬细胞中Notch信号受体1及炎性细胞因子的表达[J]. 心脏杂志, 2012, 24(3): 335-339.
FU Wenbo, DING Shifang, LI Dazhu, et al. Expression of Notch signal receptor 1 and inflammatory cytokines in monocytes-macrophages induced by acute coronary syndrome[J]. Chinese Heart Journal, 2012, 24(3): 335-339.
25. Ma S, Motevalli SM, Chen J, et al. Precise theranostic nanomedicines for inhibiting vulnerable atherosclerotic plaque progression through regulation of vascular smooth muscle cell phenotype switching[J]. Theranostics, 2018, 8(13): 3693-3706.

本文引用： 周心涛, 赵黎丙, 闵新文, 陈娇, 张涛. CTGF缺失表达抑制动脉粥样硬化易损斑块的形成[J]. 临床与病理杂志, 2019, 39(9): 1872-1877. doi: 10.3978/j.issn.2095-6959.2019.09.003
Cite this article as: ZHOU Xintao, ZHAO Libing, MIN Xinwen, CHEN Jiao, ZHANG Tao. Lack of CTGF expression inhibits atherosclerotic vulnerable plaque progression[J]. Journal of Clinical and Pathological Research, 2019, 39(9): 1872-1877. doi: 10.3978/j.issn.2095-6959.2019.09.003