

## 慢性应激对大鼠牙周组织 IL-1 $\beta$ 和 TNF- $\alpha$ 表达的影响

宋红丽,李斌,陈耀忠\*

东南大学附属中大医院口腔科,江苏 南京 210009

**[摘要]** 目的:探讨慢性应激促进牙周炎的可能机制。方法:选择慢性不可预知应激大鼠模型(chronic unpredictable stress, CUS)。将大鼠分为正常对照和慢性应激组(4周后根据糖水消耗实验将应激组又分为抑郁组和非抑郁组)。每周记录大鼠体重,8周后称取肾上腺重量。分别使用糖水消耗实验、酶联免疫法、蛋白质印迹法(Western blot),实时荧光定量 PCR(Real-time PCR)检测各组大鼠抑郁样行为,血浆皮质醇和胰岛素水平,牙周组织总的及磷酸化胰岛素受体水平及白细胞介素 1 $\beta$ (interleukin-1 $\beta$ , IL-1 $\beta$ )和肿瘤坏死因子 $\alpha$ (tumor necrosis factor- $\alpha$ , TNF- $\alpha$ )含量。结果:慢性应激4周后,约59%的大鼠表现出明显的快感缺失;与对照组相比,无论是否存在快感缺失,应激3周后所有大鼠均出现体重下降。与对照组相比,应激8周后大鼠肾上腺重量明显增加、血浆皮质醇水平明显升高;应激大鼠血浆胰岛素水平下降,牙周组织内磷酸化的胰岛素受体底物1表达下降;应激大鼠牙周组织内 IL-1 $\beta$ 和 TNF- $\alpha$ 的 mRNA 表达增加,上述指标在应激组内(抑郁组和非抑郁组之间)无显著性变化。结论:应激本身,而非应激引起的情绪障碍,引起了下丘脑-垂体-肾上腺(HPA)轴紊乱,从而导致胰岛素信号通路异常,牙周组织炎症因子表达明显增加。这可能是慢性应激后易患牙周炎的可能原因。

**[关键词]** 慢性应激;HPA轴;胰岛素信号通路;炎症因子

**[中图分类号]** R782.42

**[文献标志码]** A

**[文章编号]** 1007-4368(2018)06-843-05

**doi:**10.7655/NYDXBNS20180626

## The effect of chronic stress on IL-1 $\beta$ and TNF- $\alpha$ expression in periodontal tissues of rats

Song Hongli, Li Bin, Chen Yaozhong\*

Department of Stomatology, Affiliated ZhongDa Hospital, School of Medicine, Southeast University, Nanjing 210009, China

**[Abstract]** **Objective:** To explore the mechanism of the chronic stress contribution to periodontitis. **Method:** Sprague-Dawley rats (2 months) were received chronic unpredicted mild stress (CUS) for eight weeks. Then, four weeks later, they were divided into susceptible (depressive) or unsusceptible (resistant) groups according to sucrose preference test. Total body and adrenal weights of rats were weighed weekly and at 8 week, respectively. The levels of plasma corticosterone and insulin, the total insulin receptor (IRS) and activity-dependent phosphorylated IRS in the periodontal tissues, and the mRNA expression of IL-1 $\beta$  and TNF- $\alpha$  in the periodontal tissues were detected by enzyme-linked immunosorbent assay (ELISA), Western blot and Real-time PCR, respectively. **Results:** After 4 weeks of CUS, 59% of rats showed a decrease of sucrose preference indicating anhedonic or depressive-like behavior (CMSA). Compared with controls, all the stressed rats showed decreased weight after three weeks; after eight weeks, they also exhibited increased corticosterone and decreased insulin in plasma, decreased p-IRS and increased mRNA levels of IL-1 $\beta$  and TNF- $\alpha$  in the periodontal tissue. The depressive and resistant groups showed no significant differences in above measures. **Conclusion:** The stress itself, rather than stress-induced mood disorders, leads to HPA axis disorders, abnormality of insulin signal pathway, and increased expression of periodontal tissue inflammatory factor. These changes may serve as the mechanism in contributing to stress susceptible periodontal disease.

**[Key words]** chronic stress; HPA axis; insulin signaling pathway; inflammatory factor

[Acta Univ Med Nanjing, 2018, 38(06): 843-847]

**[基金项目]** 南京市卫计委项目(YKK15245)

\*通信作者(Corresponding author), E-mail: chenyz2003@126.com

牙周炎是一种多因素疾病,也是我国成年人牙齿丧失的首要原因。其特点是炎症导致牙周结构破坏<sup>[1]</sup>。越来越多的证据显示,基因和环境因素,如吸烟、糖尿病、冠心病等都与牙周炎密切相关<sup>[2]</sup>。寻找牙周炎的危险因素,并采取针对性措施将对于预防牙周疾病起到及其重要作用。

流行病学研究显示,应激可能是导致牙周炎的危险因素之一<sup>[3-4]</sup>。临床研究也证实,工作压力大、失业及生活中的重大事件均与牙周炎发病密切相关<sup>[5]</sup>。然而,慢性应激促进牙周炎发生发展的内在机制尚不清楚。本研究旨在观察慢性不可预知性应激对成年大鼠牙周组织炎症因子IL-1 $\beta$ 和TNF- $\alpha$ 表达的影响,来探讨慢性应激促进牙周炎发生发展的可能机制。

## 1 材料和方法

### 1.1 材料

雄性Sprague-Dawley(SD)大鼠(180-220 g)36只[清洁级,南京医科大学SCXK(苏)2008-00X],在安静、室温22℃(±2℃)的实验环境中饲养,昼夜节律为12 h:12 h。

按照随机数字表法分组,实验动物分组为:正常对照(Control)、和慢性应激组,慢性应激4周后根据行为学结果又分为抑郁组(chronic unpredictable stress depressive, CUSA)、非抑郁组(chronic unpredictable stress depressive resistant, CUSR)。抑郁组和非抑郁组继续给予慢性应激4周。实验总周期为8周。

### 1.2 方法

2月龄的雄性SD大鼠给予慢性温和和不可预知性应激(包括夹尾1 min、冰水游泳5 min、束缚1 h、通宵照明、禁饮禁食24 h、斜笼12 h、湿笼18 h,每天随机予以1种应激方式,1周循环1次)。

#### 1.2.1 体重和糖水消耗实验

大鼠于实验开始前和每周应激结束时称量体重并记录。糖水消耗实验是反映动物快感缺失的重要实验。对动物进行蔗糖水消耗实验前,先给予大鼠自来水和1%蔗糖水适应性喂养48 h,并于24 h调换自来水和蔗糖水的位置,训练大鼠学习饮用糖水。训练结束24 h后,每只大鼠单笼喂养,进行蔗糖水消耗实验基线值测量,同时给予每只大鼠事先称重好的两瓶水:1瓶1%蔗糖水,1瓶自来水,检测时间为24 h。中间更换蔗糖水和自来水的位置,结束后取走瓶子并称重。计算大鼠的糖水消耗比例:

糖水消耗=蔗糖水消耗量/(糖水消耗量+自来水消耗量)×100%。

#### 1.2.2 蛋白免疫印迹(Western blot)

蛋白提取:将2组大鼠(每组3只)牙周组织和匀浆液按每0.01 g牙周组织加100  $\mu$ L匀浆液进行匀浆。超声3 s×10次;4℃12 000 g离心15 min;取出上清,BCA法测蛋白浓度。加入1/5体积6×上样buffer,沸水浴10 min,进行后续检测总的及磷酸化胰岛素受体表达检测,蛋白上样量为30  $\mu$ g,蛋白质经SDS-PAGE凝胶分离后转移至PVDF(polyvinylidene fluoride, Millipore公司,美国)膜上。将PVDF膜用5% BSA/TBST封闭液于室温振荡封闭60 min,分别加入总的胰岛素受体底物1(insulin receptor substrate 1, IRS-1)(1:1 000; Cell Signaling公司,美国),磷酸化的IRS-1(Ser636/639)(1:1 000; Cell Signaling公司,美国)一抗,4℃孵育过夜,TBST缓冲液冲洗3次,滴加辣根过氧化物酶标记的二抗(1:5 000, Pierce公司,美国),37℃孵育1 h,TBST缓冲液冲洗,ECL显色。

#### 1.2.3 酶联免疫吸附实验(ELISA)

基于抗原与抗体竞争性结合来检测皮质酮水平的。能特异性结合皮质酮的多克隆抗体结合到包被在微型板驴抗羊抗体上。洗去多余的多克隆抗体后,血浆中的皮质酮与辣根过氧化物酶标记的有能够识别皮质酮的化合物与微型板上的多克隆抗体竞争性结合。然后清洗掉多余的皮质酮与辣根过氧化物酶标记的有能够识别皮质酮的化合物。显色液加入微型板孔中来显示辣根过氧化物酶的活性。加入终止液并读取450 nm的吸光度。吸光度的大小与样本中皮质酮的浓度呈反比。

#### 1.2.4 实时荧光定量PCR检测(Real-time PCR)

牙周组织中IL-1 $\beta$ 和TNF- $\alpha$  mRNA检测按照试剂盒说明进行[GoTaq<sup>®</sup> qPCR Master Mix(Promega公司,美国)]引物序列如下:

IL-1 $\beta$  上游:5'-AATCTCACAGCACATCAA-3';下游:5'-AGCCCATACTTTAGGAAGACA-3';

TNF- $\alpha$  上游:5'-TTCGAGTGACAAGCCTGTAGC-3';下游:5'-AGATTGACCTCAGCGCTGAGT-3';

GAPDH 上游:5'-GCAGTGGCAAAGTGGAGATTG-3';下游:5'-TGCAGGATGCATTGCTGACA-3'

### 1.3 统计学方法

应用SPSS 18.0统计学软件进行数据分析。计量资料均以均数±标准差( $\bar{x} \pm s$ )表示,多组平均的比较采用单因素方差分析(one-way ANOVA),两组之

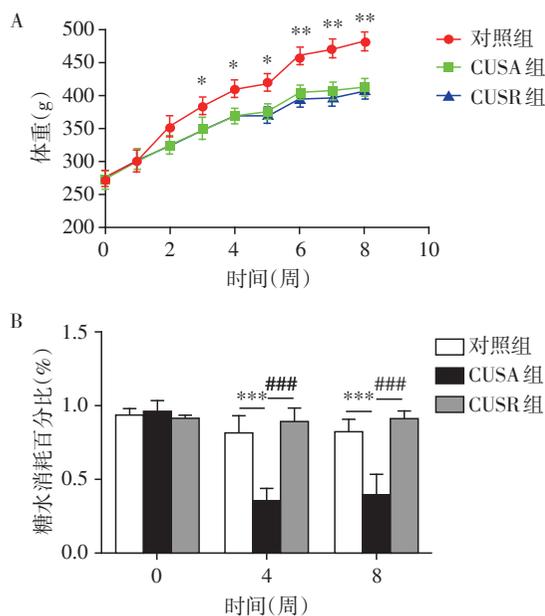
间的比较采用 Bonferroin 检验。  $P \leq 0.05$  为差异有统计学意义。

## 2 结果

### 2.1 慢性不可预知应激导致大鼠抑郁样行为改变

随着动物月龄增加,体重逐渐增加,各个组别体重差异可反映应激对动物成长的影响。从应激第3周开始,CUSA 与 CUSR 大鼠体重显著低于对照组大鼠,直至实验结束(图 1A)。

快感缺失是抑郁的一个核心症状,本研究采用糖水消耗实验检测大鼠快感缺失的情况。各组动物造模前糖水消耗水平没有差异,造模4周后,有59%的大鼠糖水消耗量低于50%,剩余41%大鼠为糖水消耗量高于50%。据此将造模组动物分为CUSA 和 CUSR。糖水消耗实验结果显示,与对照组相比,CUSA 大鼠于实验第4周及第8周糖水消耗显著下降(图 1B)。CUSR 大鼠第4、8周糖水消耗水平与对照组无明显差别。



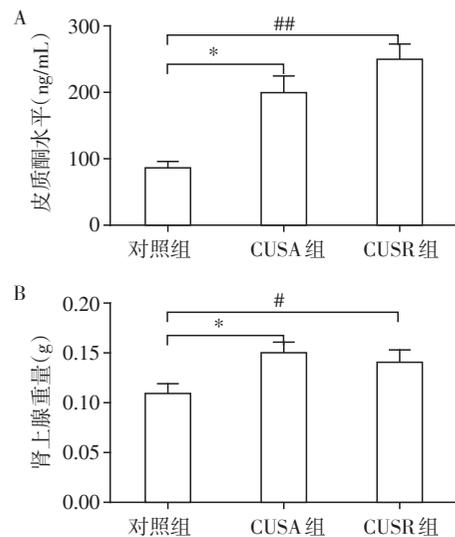
A: 体重变化; B: 糖水消耗实验。与对照组相比, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; 与抑郁组相比, ### $P < 0.001$ 。

图1 慢性不可预知应激导致大鼠抑郁样行为改变

Figure 1 Chronic unpredictable stress induced depression like behavior in rats

### 2.2 应激引起的大鼠 HPA 轴变化

与对照组相比,ELISA 检测发现应激引起的 CUSA 和 CUSR 大鼠血浆皮质酮水平均明显增高(图 2A)。而且,与正常对照组相比,CUSA 和 CUSR 的肾上腺的重量也明显增加(图 2B)。



A: 各组大鼠血浆皮质酮水平; B: 各组大鼠肾上腺重量。与对照组相比, \* $P < 0.01$ , \* $P < 0.05$ , ## $P < 0.001$ 。

图2 应激引起的大鼠 HPA 轴变化

Figure 2 Stress induced changes of HPA axis in rats

### 2.3 应激对大鼠胰岛素信号系统的影响

检测血浆胰岛素水平,结果发现,CUSA 和 CUSR 血浆的胰岛素水平均出现了明显下降(图 3A)。此外,CUSA 和 CUSR 胰岛素受体底物1(是胰岛素受体的活化形式)均明显减少(图 3B)。而总的胰岛素受体底物表达未发生明显改变(图 3B)。

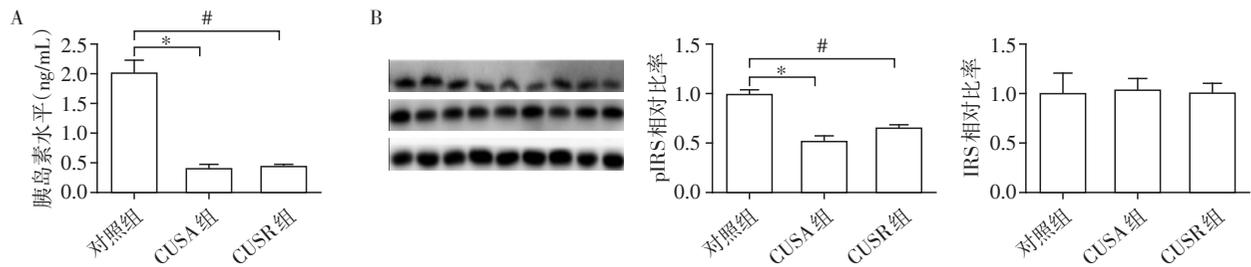
### 2.4 应激对牙周组织炎症因子表达的影响

通过 RT-PCR 方法检测牙周组织炎症因子 IL-1 $\beta$ 和 TNF- $\alpha$ 的 mRNA 表达,结果发现,与正常对照组相比,CUSA 和 CUSR 牙周组织 IL-1 $\beta$ 和 TNF- $\alpha$ 的 mRNA 表达均出现明显下降(图 4)。

## 3 讨论

慢性应激严重影响人类健康。研究显示,慢性应激可以导致焦虑、抑郁、认知功能障碍等神经精神系统疾病,也可以导致血压升高、糖尿病等系统性疾病<sup>[6-8]</sup>。近年来研究发现,慢性应激与牙周疾病也密切相关<sup>[3-4]</sup>。本文采用慢性温和和不可预知性应激对大鼠造模,将大鼠分为抑郁组及非抑郁组,研究应激及应激导致的情绪障碍对大鼠牙周组织炎症因子表达的影响,探讨应激促进牙周疾病的可能机制。研究结果显示,应激本身,而非应激引起的情绪障碍,可导致 HPA 轴紊乱,损害胰岛素信号通路,导致牙周组织炎症因子 IL-1 $\beta$ 和 TNF- $\alpha$ 表达明显增加,这可能是应激人群易患牙周炎的可能原因。

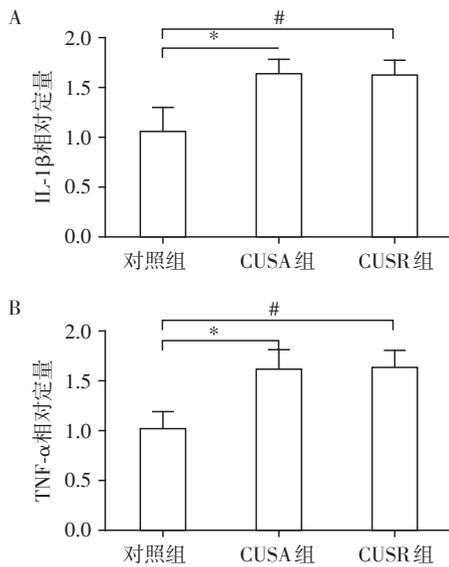
慢性应激可以导致 HPA 轴激活,表现为糖皮质



A: 各组大鼠血浆胰岛素水平; B: 总的以及磷酸化胰岛素受体底物表达。与对照组相比, \* $P < 0.01$ , # $P < 0.01$ 。

图3 应激对大鼠胰岛素信号系统的影响

Figure 3 Effects of stress on insulin signaling system in rats



A: 各组大鼠 IL-1 $\beta$  mRNA 相对定量; B: 各组大鼠 TNF- $\alpha$  mRNA 相对定量。与对照组相比, \* $P < 0.05$ , # $P < 0.05$ 。

图4 应激对牙周组织炎症因子表达的影响

Figure 4 Effects of stress on the expression of inflammatory cytokines in periodontal tissues

激素分泌增加<sup>[9]</sup>。本研究发现,慢性应激可以导致肾上腺重量明显增加、血浆糖皮质激素水平增高,说明我们慢性应激模型是成功的。而且,在抑郁组和非抑郁组都发现了血浆皮质激素水平增加,提示无论在抑郁组还是非抑郁组,均存在HPA轴激活。

上世纪90年代提出了抑郁的炎症假说,基础和临床研究均可提供大量证据来说明炎症与抑郁之间存在密切关系。慢性应激是一种经典的动物抑郁模型,既往研究发现慢性应激大鼠血及海马中,均发现了IL-6及TNF等炎症因子含量明显升高,而降低炎症因子表达后,抑郁症状明显改善<sup>[10]</sup>;研究发现抑郁患者的血及脑脊液中炎症生物标记物和前炎症因子水平明显升高<sup>[11]</sup>。上述证据均提示应激与机体慢性炎症密切相关。然而,应激对牙周组织炎症因子表达影响,目前尚未见报道。在本研究

中,根据行为学结果,将应激大鼠进一步区分为抑郁组及应激非抑郁组,发现无论是抑郁组还是非抑郁组牙周组织内IL-1 $\beta$ 和TNF- $\alpha$ 等炎症因子含量明显增加,提示慢性应激可以通过诱导炎症因子产生从而诱发牙周炎。

在研究中,尽管并没有发现血中存在高胰岛素水平,然而,发现牙周组织内胰岛素受体底物明显降低,与之相对应的血胰岛素水平下降,提示胰岛素信号通路受损。近年来许多研究发现,胰岛素抵抗人群中某些炎症标志物如IL-1 $\beta$ 、TNF- $\alpha$ 等血浆浓度明显升高<sup>[12]</sup>。说明胰岛素信号通路损害可以导致炎症因子含量增加。因此本文认为,应激导致胰岛素信号通路损害可能是导致IL-1 $\beta$ 、TNF- $\alpha$ 增高的原因。

【参考文献】

- [1] Deo V, Bhongade ML. Pathogenesis of periodontitis: role of cytokines in host response [J]. Dent Today, 2010, 29 (9):60-62
- [2] Cardoso EM, Reis C, Manzanera-Céspedes MC. Chronic periodontitis, inflammatory cytokines, and interrelationship with other chronic diseases [J]. Postgrad Med, 2018, 130(1):98-104
- [3] Jaiswal R, Shenoy N, Thomas B. Evaluation of association between psychological stress and serum cortisol levels in patients with chronic periodontitis - Estimation of relationship between psychological stress and periodontal status [J]. J Indian Soc Periodontol, 2016, 20(4):381-385
- [4] Genco RJ, Ho AW, Grossi SG, et al. Relationship of stress, distress and inadequate coping behaviors to periodontal disease [J]. J Periodontol, 1999, 70(7):711-723
- [5] Croucher R, Marcenes WS, Torres MC, et al. The relationship between life-events and periodontitis. A case-control study [J]. J Clin Periodontol, 1997, 24(1):39-43
- [6] Gonda X, Hullam G, Antal P, et al. Significance of risk polymorphisms for depression depends on stress exposure [J]. Sci Rep, 2018, 8(1):3946

- [7] Harris ML, Oldmeadow C, Hure A, et al. Stress increases the risk of type 2 diabetes onset in women: A 12-year longitudinal study using causal modelling [J]. *PLoS One*, 2017, 12(2): e0172126
- [8] Alenezi F, Brummett BH, Boyle SH, et al. Usefulness of myocardial annular velocity change during mental stress to predict cardiovascular outcome in patients with coronary artery disease (from the responses of mental stress-induced myocardial ischemia to escitalopram treatment trial) [J]. *Am J Cardiol*, 2017, 120(9): 1495-1500
- [9] Salari AA, Fatehi - Gharehlar L, Motayagheni N, et al. Fluoxetine normalizes the effects of prenatal maternal stress on depression- and anxiety-like behaviors in mouse dams and male offspring [J]. *Behav Brain Res*, 2016, 311: 354-367
- [10] Wang YL, Han QQ, Gong WQ, et al. Microglial activation mediates chronic mild stress - induced depressive - and anxiety-like behavior in adult rats [J]. *J Neuroinflammation*, 2018, 15(1): 21
- [11] Xia QR, Liang J, Cao Y, et al. Increased plasma nesfatin-1 levels may be associated with corticosterone, IL-6, and CRP levels in patients with major depressive disorder [J]. *Clin Chim Acta*, 2018, pii: S0009-8981(18)30065-2
- [12] Sosale B, Chandrashekhara S, Aravind SR, et al. Influence of cytokine status on insulin resistance and circulating endothelial progenitor cells in type 2 diabetes mellitus [J]. *Cytokine*, 2017, 99(2): 179-185
- [收稿日期] 2017-12-27

(上接第 832 页)

- perimentally induced early cerebral aneurysms [J]. *Gastrointestinal Endoscopy*, 2013, 9(2): 41
- [7] 刘思玉,谢 龙,祁 兵,等.口腔鳞状细胞癌组织中差异微小RNA/mRNA 表达谱的对接研究[J]. *华西口腔医学杂志*, 2014, 32(4): 400-403
- [8] Rautava J, Luukka M, Heikinheimo K, et al. Squamous cell carcinomas arising from different types of oral epithelia differ in their tumor and patient characteristics and survival [J]. *Oral Oncol*, 2007, 43: 911-919
- [9] Kunej T, Godnic I, Ferdin J, et al. Epigenetic regulation of microRNAs in cancer: An integrated review of literature [J]. *Mutat Res*, 2011, 717(1): 77-84
- [10] Jiang YW, Chen LA. MicroRNAs as tumor inhibitors, oncogenes, biomarkers for drug efficacy and outcome predictors in lung cancer (review) [J]. *Mol Med Rep*, 2012, 5: 890-894
- [11] Mansoori B, Mohammadi A, Shirjang S, et al. MicroRNAs in the diagnosis and treatment of cancer [J]. *Immunol Invest*, 2017, 46(8): 880-897
- [12] Li Chen, Liang Luoc, Wei Chen, et al. MicroRNA-24 increases hepatocellular carcinoma cell metastasis and invasion by targeting p53: miR-24 targeted p53 [J]. *Biomedicine & Pharmacotherapy*, 2016, 84: 1113-1118
- [13] Lynch SM, McKenna MM, Walsh CP, et al. miR-24 regulates CDKN1B/p27 expression in prostate cancer [J]. *Epub*, 2016, 76(7): 637-648
- [14] Reis PP, Tomenson M, Cervigne NK, et al. Programmed cell death 4 loss increases tumor cell invasion and is regulated by miR-21 in oral squamous cell carcinoma [J]. *Mol Cancer*, 2010, 9(1): 238
- [15] Jiang L, Liu X, Kolokythas A, et al. Downregulation of the Rho GTPase signaling pathway is involved in the microRNA-138-mediated inhibition of cell migration and invasion in tongue squamous cell carcinoma [J]. *Int J Cancer*, 2010, 127(3): 505-512
- [16] Liu X, Wang C, Chen Z, et al. MicroRNA-138 suppresses epithelial-mesenchymal transition in squamous cell carcinoma cell lines [J]. *Biochem J*, 2011, 440(1): 23-31
- [17] Xu G, Zhang Y, Jia H, et al. Cloning and identification of microRNAs in bovine alveolar macrophages [J]. *Mol Cell Biochem*, 2009, 332(1/2): 9-16
- [18] Esposito I, Kayed H, Keleg S, et al. Tumor-suppressor function of SPARC-like protein 1/Hevin in pancreatic cancer [J]. *Neoplasia*, 2007, 9(1): 8-17
- [19] Hurley PJ, Marchionni L, Simons BW, et al. Secreted protein, acidic and rich in cysteine - like 1 (SPARCL1) is downregulated in aggressive prostate cancers and is prognostic for poor clinical outcome [J]. *Proc Natl Acad Sci USA*, 2012, 109(37): 14977-14982
- [20] Li P, Qian J, Yu G, et al. Down-regulated SPARCL1 is associated with clinical significance in human gastric Cancer [J]. *Epub*, 2012, 105(1): 31-37
- [收稿日期] 2017-12-12