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兔肝 VX2 肿瘤射频消融术后残癌中基质金属蛋白酶 9 表达

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摘要 目的:探讨兔肝脏 VX2 肿瘤射频消融术(radio-frequency ablation,RFA)后残余肿瘤组织中基质金属蛋白酶 9(MMP-9)表达。**方法:**超声引导下将 VX2 肿瘤组织块接种于 23 只新西兰大白兔肝脏中,造模成功后随机分为 5 组,对照组(A 组, n=3),不行 RFA 治疗;余 20 只为实验组行 RFA 治疗,在超声引导下射频针插入肿瘤偏心位置,展开电极致损毁范围最大为肿瘤总体积的 2/3,人为造成残存肿瘤组织。根据治疗结束后不同时间点分为 4 组:0 小时组(B 组, n=5)、术后 1 周组(C 组, n=5)、术后 2 周组(D 组, n=5)、术后 4 周组(E 组, n=5),行超声检查结束后处死大白兔,取肿瘤组织采取免疫组化法观察残存肿瘤组织中及未治疗肿瘤组中 MMP-9 的表达情况。**结果:**RFA 术后 0 小时、1 周、2 周、4 周残存肿瘤组织中 MMP-9 的表达均较对照组明显降低（P< 0.05）。**结论:**RFA 治疗后残存肿瘤细胞中 MMP-9 水平表达减低。

关键词:VX2 肿瘤;射频消融;基质金属蛋白酶 9;超声检查

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The Expression of the MMP-9 in Rabbit Liver VX2 Tumor-remnant Tissues after Radio-frequency Ablation

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ABSTRACT Objective: To investigate the expression of the MMP-9 in rabbit liver VX2 tumor-remnant tissues after radio-frequency ablation treatment (RFA). **Methods:** Ultrasound-guided the VX2 tumor mass were planted into the liver of 23 New Zealand rabbits, models of rabbit VX2 liver tumor were divided into 5 groups at random, there were 3 rabbits in group A as the control group with no RFA treatment; the remaining 20 rabbits as experimental group with the RFA treatment, Ultrasound-guided the Rf needle was inserted into the location of the liver tumor eccentric, tumor-remnant tissues were remained by opening the electrode to control the maximum damage range of total tumor volume 2/3. 20 rabbits were divided into 4 groups according to their different time after treatment, that was after RFA group (B group), one week after RFA group (C group), two weeks after RFA group (D group), four weeks after RFA group(E group), 5 rats in each group. The rabbits were executed after taking ultrasonographical examination, Immunohistochemistry was used to detect the expression status of MMP-9 in rabbit liver VX2 tumor-remnant tissues after RFA treatment and the untreated tumor tissues. **Results:** The expression of MMP-9 in tumor-remnant tissues of 0 hour, 1 week, 2 weeks, 4 weeks after RFA were significantly lower than the control group (P<0.05). **Conclusion:** After RFA the expression of MMP-9 in the remnant tumor cells is decreased.

Key words: VX2 carcinoma; Radio-frequency ablation; MMP-9; Ultrasonography

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前言

目前手术切除肿瘤、经导管动脉化疗栓塞术(transcatheter arterialchemoembolization,TACE)、局部热消融治疗等已成为临床认可的、在治疗肝脏恶性肿瘤中有明确疗效的方法,但因受到肿瘤的大小、数目、部位、毗邻关系、滋养血管有无等因素影响,上述方法无法达到彻底根除治疗的目的,造成残留,影响疗效。因此残存肿瘤的生物学行为成为众多学者研究的热点。目前许多研究已证明基质金属蛋白酶 9(MMP-9)活性的增加与人类多种恶性肿瘤的侵袭转移及预后的能力是密切相关。本研究通过用 RFA 治疗兔肝 VX2 肿瘤模型,制成术后残癌,观察治疗前及治疗后残癌中不同时期 MMP-9 浓度变化并探讨其意义。

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1 材料和方法

1.1 材料

1.1.1 实验动物 新西兰大白兔 23 只，由大连医科大学动物实验中心提供,雌雄不论 5-8 月龄,体重约 1.6-3 kg,VX2 荷瘤兔 1 只,由山东省医学科学院附属医院惠赠。

1.1.2 实验设备 射频仪器为美国达隆公司生产的 RF-2000。超声诊断仪器为东芝 PV7500 型彩色多普勒诊断仪。

1.1.3 主要实验试剂 MMP-9 购于北京博奥森公司,二抗即用型 SP 免疫组化试剂盒,DAB 显色试剂盒均购于北京中杉金桥

生物技术有限公司。

1.2 方法

1.2.1 VX2 肝肿瘤模型制作 采用经皮穿刺瘤块植入法:切取VX2 荷瘤兔肿块,选取接近包膜的鱼肉样组织,剔除筋膜及坏死组织,生理盐水多次冲洗后,用眼科剪将肿瘤组织剪成 1 mm^3 大小瘤块置于生理盐水中备用。将 23 只实验兔分批接种,术前禁食 8 小时以上,用 3 %戊巴比妥钠(1 mL/kg)经耳缘静脉麻醉后固定于兔台上,备皮后碘伏局部消毒。于剑突下偏左用手术刀将皮肤纵向切开 0.3 cm 的小口, 超声引导下将 14 G 穿刺针从切口处刺入已定位的肝左叶位置,稳住针鞘,拔出针芯,用平头针芯将 2-3 块瘤块推送入肝内,确定植入成功后拔出穿刺针,缝合切口,再次消毒。术后肌肉注射青霉素 40 万单位,持续使用 3 d,防止感染。

1.2.2 实验分组 接种成功后 14-20 天行超声检查, 待肿瘤直径约为 1.5 cm 左右时,将 23 只新西兰大白兔分为 5 组:对照组(A 组, n=3),不行 RFA 治疗;余 20 只为实验组行 RFA 治疗,根据治疗结束后不同时间点分为:0 小时组(B 组, n=5)、术后 1 周组(C 组, n=5)、术后 2 周组(D 组, n=5)、术后 4 周组(E 组,n=5)。

1.2.3 射频消融术 用 3 %戊巴比妥钠麻醉实验兔, 给腹部及背部备皮,消毒铺巾。将电极板置于背部并固定。于穿刺点做 2-3 cm 的小切口,超声确定肿瘤位置后将射频针插入肿瘤偏心位置,弹开电极。设计 RFA 毁损范围(肿瘤损毁气化半径约为肿瘤总体积的 2/3),即制成残癌模型。接通电源,输出功率 20 W,阻抗 616-1528 Ω ,治疗时间为(119± 13) s,消融灶温度达到 60 ℃便可引起细胞不可逆性死亡。按分组条件,取出瘤体后处死,进行免疫组化实验。

1.2.4 免疫组化染色 采用 SP 法:常规脱蜡至水,3 % H_2O_2 室温孵育 10-15 分钟;100 ℃微波抗原修复 10 分钟后自然冷却,加动物血清封阻 10-15 分钟, 分别滴加 1:100 的 MMP-9 抗体 37 ℃孵育 2-3 小时;滴加相应的二抗;DAB 显色,苏木复染,脱水透明,树胶封片。用 PBS 液替代一抗作为阴性对照、已知阳性片作为阳性对照。

1.2.5 判定标准 用 Mattern 积分法[1]染色指数来评定染色结果阳性细胞百分率。每例标本选取 10 个含有阳性细胞的高倍视野(× 400)分别计数 100 个肿瘤细胞,取其平均值计算阳性细胞百分率。0 为无阳性细胞;1 为阳性细胞≤25 %;2 为阳性细胞 25% ~ 50 %;3 为阳性细胞 >50%。染色强度:0 为阴性;1 为浅黄色;2 为深黄色;3 为棕黄色。染色指数为阳性细胞百分率和染色强度之和,最小值 0,最大值 6。阳性表达指数 >3 定为免疫组化染色阳性;≤3 定为阴性。

1.2.6 观察指标 a: 兔肝 VX2 肿瘤的二维超声表现及多普勒血流显像情况。b:射频消融术后的二维超声表现及多普勒血流显像情况。c:射频消融术前及术后肿瘤组织中 MMP-9 的表达情况。

1.3 统计学处理

统计结果经 SPSS16.0 统计软件处理, 数据以均数± 标准差($\bar{X}$± S)表示,采用方差分析,以 P<0.05 为差异有统计学意义。

2 结果

2.1 RFA 治疗术后超声检查结果

RFA 治疗后 B 组射频区可见气体强回声区,回声不均匀,边界模糊,呈类圆形或圆形,血流信号无法检出。残癌区呈不均匀低回声,内见条状、点状高回声。C 组、D 组射频区呈高回声,部分高回声内可见不规则低回声区, 与正常肝组织分界不清,CDF、CDE 射频治疗区中央未见血流信号, 射频区内部及周边血流信号减少或消失,残癌区呈均匀低回声,边界清,未见血流信号。E 组射频区呈低回声区,内可见不均匀分布的高回声带,残癌区仍呈均匀的低回声,CDF、CDE 周边可见条状血流信号。(见图 1)。

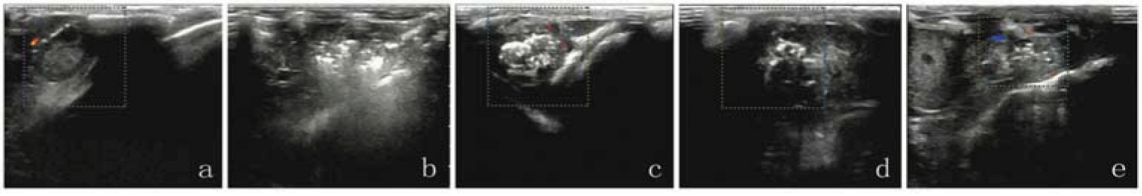

图 1 对照组与实验组肿瘤超声表现。a.A 组(未行 RFA 治疗)。b.B 组(RFA 治疗后 0 小时)。c.C 组(FRA 治疗后 1 周)。d.D 组(RFA 治疗后 2 周)。e.E 组(RFA 治疗后 4 周)

Fig.1 The control group and experimental group tumor ultrasound findings. a. Group A (with no RFA treatment). b.Group B (0 hour after RFA). c. Group C (one week after RFA). d. Group D (two weeks after RFA). e. Group E (four weeks after RFA)

表 1 RFA 治疗前后各组 MMP-9 的表达变化情况(%, $\bar{x}$± s)

Table 1 RFA therapy group before and after the meaning of the changes in the expression of MMP-9(%, $\bar{x}$± s)

Group	n	Positive expression rate of MMP-9
A group	3	70.5± 29.3
B group	5	42.2± 23.4
C group	5	37.6± 18.7
D group	5	35.0± 13.5
E group	5	31.1± 11.2

2.2 免疫组化结果

MMP-9 的阳性表达产物主要在细胞浆及细胞核上，A 组（对照组）在肿瘤组织中呈高表达；B、C、D、E 组(实验组)在残癌区表达明显低于 A 组，差别有统计学意义(P<0.05)，其四组间表达无明显差别。（具体结果见表 1 及图 2）。

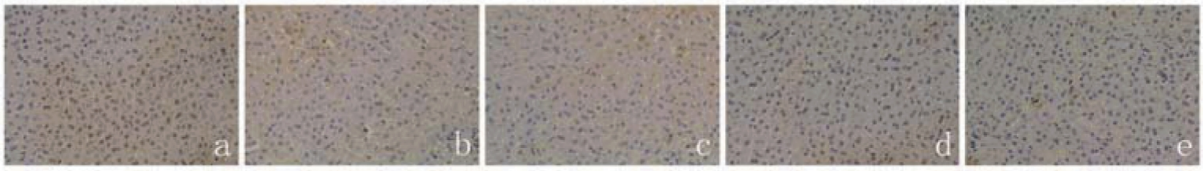

图 2 MMP-9 在对照组与实验组的表达，SP 组化染色× 200。a.A 组(未行 RFA 治疗)。b.B 组(RFA 治疗后 0 小时)。c. C 组(FRA 治疗后 1 周)。d. D 组(RFA 治疗后 2 周)。e. E 组(RFA 治疗后 4 周)

Fig.2 The expression of MMP-9 in the control group and experimental group. SP immunohistochemical× 200. a. Group A (with no RFA treatment). b. Group B(0 hour after RFA). c. Group C (one week after RFA). d. Group D (two weeks after RFA). e. Group E (four weeks after RFA)

3 讨论

射频消融治疗通过活体组织离子随高频电流变化的方向振动，电极附近组织离子因受电流的作用互相碰撞生热，使组织温度上升，当温度超过 50 ℃ 以上可使局部组织发生不可逆的蛋白凝固、细胞膜破裂溶解、凝固性坏死从而起到治疗目的[2]。在过去数十年，射频消融作为一种微创治疗手段，因其效果确切，已经在肝癌治疗中取得了重要的地位[3,4]。而对于病灶位置特殊靠近重要脏器(肠管、胆囊、膈肌、大血管、心脏等)的肝癌消融治疗时，很难直接将肿瘤完全消融，存在残留，影响疗效，因此残存肿瘤的生物学行为成为众多学者研究的热点。

本实验利用免疫组化手段评价残癌的生物学行为。研究已表明：肿瘤的生长、复发依赖于新生血管。当肿瘤直径为 1-2 cm 时，因缺乏新生血管生成，而依赖于周围组织供血，肿瘤处于血管前期，因此瘤体生长速度较为缓慢。当肿瘤继续生长，肿瘤细胞本身分泌多种促血管因子时，新生血管开始生成，肿瘤进入血管期，此时肿瘤在充足的血液供应下而快速生长并发生转移[5]。基质金属蛋白酶(matrix metalloproteinases，MMPs) 是一类蛋白水解酶，细胞外基质中大多数蛋白成分被其降解，肿瘤细胞的组织学屏障被破坏[6]。而 MMP-9 是 MMPs 家族中一个非常重要的分子，主要降解Ⅳ型胶原和其他变质的胶原(明胶)。目前发现 MMP-9 已在肝癌[7]、食管癌[8]、直肠癌[9]、膀胱癌[10]、卵巢癌[11]、乳腺癌[12]等多种肿瘤组织中表达异常，因此在肿瘤的侵袭转移中 MMP-9 起到重要的作用[13,14]。文献报道，MMP-9 与肿瘤血管生成有关，但 MMP-9 在具有血管生成拟态和不同血管生成模式的肿瘤类型中拥有重要的地位，而肝癌正是这一类的肿瘤，因此 MMP-9 在肝癌血管生成中的表达和意义与既往的肿瘤机制研究相符[15]。另有文献报凭借 MMP-9 的浓度检测也能够评估损伤或疾病的程度[16]，并可以初步估测出肝癌治疗的预后效果[17]。本研究结果提示射频消融在有效范围内对肿瘤能够完全灭活，短期内能够抑制残存肿瘤的新生血管形成，起到治疗及抑制其发展的作用。

综上所述射频消融是治疗肝肿瘤效果确切可靠的方法，可以在某种程度上改善残存肝肿瘤生物学行为，在短期内抑制残癌的生长和转移。

参 考 文 献(References)

[1] Volta M, Mattern J, Koomagi R. Angiostatin expression in non-small cell lung cancer [J]. Clin Cancer Res, 2000, 6(8):3236-3240

[2] 阚雪锋，郑传胜. 射频消融治疗肝癌的进展 [J]. 临床放射学杂志，2011, 30(8):1227-1229
Kan Xue-feng, Zheng Chuan-sheng. Advances in the treatment of liver cancer with radiofrequency ablation [J]. Journal of Clinical radiology, 2011, 30(8):1227-1229

[3] Cho YK, Kim JK, Kim MY, et al. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies [J]. Hepatology, 2009, 49 (2):453-459

[4] Kong WT, Zhang ww, Qiu YD, et al. Major complications after radiofrequency ablation for liver tumors:analysis of 255 patients [J]. World J Gastroenterol, 2009,15(21):2651-2656

[5] Yang LY, Lu WQ, Huang GW, Wang W. Correlation between CD105 expression and postoperative recurrence and metastasis of hepatocellular carcinoma[J]. BMC Cancer, 2006, 6(1):110-110

[6] Hernández-Pérez M, Mahalingam M. Matrix metalloproteinases in health and disease: insights from dermatopathology [J]. Am J Dermatopathol, 2012, 34(6):565-579

[7] Deniz Nart, Banu Yaman, Funda Y, et al. Expression of Matrix Metalloproteinase-9 In Predicting Prognosis of Hepatocellular Carcinoma After Liver Transplantation [J]. Liver Transplantation, 2010, 16(5): 621-630

[8] Groblewska M, Siewko M, Mroczko B, et al. The role of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in the development of esophageal cancer [J]. Folia Histochem Cytobiol, 2012,50(1): 12-19

[9] Svagzdys S, Lesauskaite V, Pangonyte D, et al. Matrix metalloproteinase-9 is a prognostic marker to predict survival of patients who underwent surgery due to rectal carcinoma [J]. Tohoku J Exp Med, 2011, 223(1):67-73

[10] Reis ST, Leite KR, Piovesan LF, et al. Increased expression of MMP-9 and IL-8 are correlated with poor prognosis of Bladder Cancer [J]. BMC Urol, 2012, 12(1):18-18

[11] Yan L, Lin B, Gao L, et al. Lewis (y) Antigen Overexpression Increases the Expression of MMP-2 and MMP-9 and Invasion of Human Ovarian Cancer Cells [J].Int J Mol Sci, 2010, 11 (11): 4441-4452

[12] Li Wen-tao, Wu Gang, Zhi Hui, et al. Correlation of DCC and MMP-9 expression with the ultrasonic appearance in breast cancer [J]. Chinese Journal of Practical Surgery, 2012, 32(11):942-944

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参 考 文 献(References)

[1] Ma B, Yang B, Guo H, et al. The association between tumor necrosis factor alpha promoter polymorphisms and ankylosing spondylitis: A meta-analysis[J]. Human immunology, 2013, 74(10): 1357-1362

[2] Rees JD, Bennett AN, Harris D, et al. Superior outcomes for military ankylosing spondylitis patients treated with anti-TNF [J]. J R Army Med Corps, 2014

[3] Jin DE, Zhao LD, Yan XP, et al. The re-evaluation of 140 patients diagnosed as ankylosing spondylitis and nonradiographic axial spondyloarthritis [J]. Chinese Journal of Internal Medicine, 2013, 52 (11): 920-923

[4] Braun J, Van den Berg R, Baraliakos X, et al. 2010 update of the ASAS/EULAR recommendations for the management of ankylosing spondylitis[J]. Annals of the rheumatic diseases, 2011, 70(6): 896-904

[5] Zhao LD, Zhao Y, Zhang X, et al. Nonradiographic axial spondyloarthritis: early ankylosing spondylitis[J]. Chinese Journal of Internal Medicine, 2013, 52(11):910-912

[6] Feng XH1, Jiang Q2, Liu HX2, et al. Clinical effect analysis of ankylosing spondylitis treated by Chinese medical syndrome differentiation [J]. Chinese Journal of Internal Medicine, 2013; 33 (10): 1309-1314

[7] Prevosto C, Goodall J C, Gaston J S H. Cytokine Secretion by Pathogen Recognition Receptor-stimulated Dendritic Cells in Rheumatoid Arthritis and Ankylosing Spondylitis[J]. The Journal of Rheumatology, 2012, 39(10): 1918-1928

[8] Suh YS, Kwok SK, Ju JH, et al. Safe Re-administration of Tumor Necrosis Factor-alpha (TNFα) Inhibitors in Patients with Rheumatoid Arthritis or Ankylosing Spondylitis Who Developed Active Tuberculosis on Previous Anti-TNFα Therapy[J]. J Korean Med Sci, 2014, 29(1): 38-42

[9] 李伟, 高杰, 赵育松, 等. 局部应用依那西普治疗强直性脊柱炎髋关节受累的临床疗效分析 [J]. 现代生物医学进展, 2013, 13(25): 4917-4920
Li Wei, Gao Jie, Zhao Yu-song, et al. Clinical Efficacy of Etanercept hip joint Injection in the Treatment of Patients with Ankylosing Spondylitis [J]. Progress in Modern Biomedicine, 2013,13 (25): 4917-4920

[10] 吕艳霞. 强直性脊柱炎治疗的新手段—生物制剂 [J]. 基层医学论坛, 2011, 15(002): 49-49
Lv Yan-xia. Treatment of ankylosing spondylitis new means-biologics[J]. The Medical Forum, 2011, 15(002): 49-49

[11] Poddubnyy D A, M rker-Hermann E, Kaluza-Schilling W, et al. Relation of HLA-B27, Tumor Necrosis Factor-α Promoter Gene Polymorphisms, and T Cell Cytokine Production in Ankylosing Spondylitis-A Comprehensive Genotype-Phenotype Analysis from an Observational Cohort[J]. The Journal of Rheumatology, 2011, 38(11): 2436-2441

[12] Asifa GZ1, Liaquat A1, Murtaza I1, et al. Tumor necrosis factor-alpha gene promoter region polymorphism and the risk of coronary heart disease[J]. Scientific World Journal, 2013, 5, 2013: 203492-203498

[13] 韦蓉梅, 董丽平, 吴红慧, 等. 强直性脊柱炎患者骨代谢生化指标和 RANKL-RANK-OPG 系统表达之间的相关性 [J]. 现代生物医学进展, 2013, 13(13): 2528-2531
Wei Rong-mei, Dong Li-ping, Wu Hong-hui, et al. Correlation of Bone Metabolic Indexes and Levels of RANKL-RANK-OPG System in Ankylosing Spondylitis Patients [J]. Progress in Modern Biomedicine, 2013, 13(13): 2528-2531,2546

[14] Mattey D L, Packham J C, Nixon N B, et al. Association of cytokine and matrix metalloproteinase profiles with disease activity and function in ankylosing spondylitis [J]. Arthritis Research & Therapy, 2012, 14(3): R127

[15] Bowness P, Ridley A, Shaw J, et al. Th17 cells expressing KIR3DL2+ and responsive to HLA-B27 homodimers are increased in ankylosing spondylitis[J]. The Journal of Immunology, 2011, 186(4): 2672-2680

[16] Appel H, Maier R, Bleil J, et al. In Situ Analysis of Interleukin-23-and Interleukin-12-Positive Cells in the Spine of Patients With Ankylosing Spondylitis[J]. Arthritis & Rheumatism, 2013, 65(6): 1522-1529

[17] Mei Y, Pan F, Gao J, et al. Increased serum IL-17 and IL-23 in the patient with ankylosing spondylitis [J]. Clinical rheumatology, 2011, 30(2): 269-273

[18] Zambrano-Zaragoza JF, Agraz-Cibrian JM, González-Reyes C, et al. Ankylosing spondylitis: from cells to genes [J]. Int J Inflam, 2013,2013:501653-501660

[19] Miranda-Filloy J A, López-Mejias R, Genre F, et al. Leptin and visfatin serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-α antagonist therapy[J]. Clin Exp Rheumato, 2013, 31(4):538-545

[20] Genre F, López-Mejí as R, Miranda-Filloy JA, et al.Gelsolin levels are decreased in ankylosing spondylitis patients undergoing anti-TNF-alpha therapy[J].Clin Exp Rheumatol, 2013 Dec 16

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[13] Raithatha SA, Muzik H, Muik H, et al. Localization of gelatinase-A and gelatinase-B mRNA and protein in human gliomas [J]. Neuro-oncol, 2000, 2(3):145-150

[14] Deryugina EI, Iuo GX, Reisfeld RA, et al. Tumor cell invasion through matrigel is regulated by activated matrix metalloproteinase-2 [J]. Anficaneer Res, 1997, 17(5A):3201-3210

[15] Sun B, Zhang S, Zhang D, et al. Vasculogenic mimicry is associated with high tumor grade, invasion and metastasis,and short survival in patients with hepatocellular carcinoma [J]. Oncol Rep, 2006, 16(4): 693-698

[16] Kotyza J, Pesek M, Puzman P, et al. Progelatinase B/matrix metalloproteinase-9 proenzyme as a marker of Neural inflammation [J]. ExpLung Res, 2004, 30(4):297-309

[17] Watelet JB, Claeys C, Van Cauwenberge P, et al. Predictive and monitoring value of matrix metalloproteinase-9 for heahng quality after sinus surgery [J]. Wound Repair Regen, 2004, 12(4):412-418