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基质金属蛋白酶 MMP-2/9 分子探针对临床结肠癌组织的检测

余祖红[1], 栾富娟[1], 冷霞[1], 史玉雪[1], 王洁[1], 尹玲[2,3], 何蕾[2,3], 史海斌[2,3*], 陈卫昌[1*]

1. 苏州大学附属第一医院消化内科, 苏州 215006;
2. 苏州大学医学部放射医学与防护学院, 苏州 215123;
3. 苏州大学放射医学及交叉学科研究院(RAD-X), 苏州 215123

* 联系人, E-mail: hbshi@suda.edu.cn; weichangchen@126.com

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摘要 研发基质金属蛋白酶MMP-2/9特异性识别的荧光分子探针并用于临床结肠癌组织的快速、准确性检测意义重大, 但仍然具有一定的挑战性. 本文基于基质金属蛋白酶MMP-2和MMP-9 (MMP-2/9)特异性识别的多肽底物, 利用一价铜离子催化的"点击"化学和酰胺化反应, 分别将荧光素(FITC-N_3)和荧光淬灭基团Dabcyl-NHS偶联于多肽底物两端, 制备得到MMP-2/9特异性识别的荧光能量共振转移(FRET)荧光分子探针Dab-GPLGVRGY-FITC. 通过将重组基质金属蛋白酶MMP-2/9与探针作用, 跟踪检测溶液在520 nm处荧光信号变化的情况, 对探针的特异性和检测灵敏度进行评估. 此外, 利用人源大肠癌细胞LoVo和30例临床结肠癌组织样本开展了结肠癌快速体外检测初步研究. 体外重组酶检测实验表明, 探针对MMP-2/9具有较强的特异性和选择性, 对MMP-2的最低检测限可达14 ng/mL. 共聚焦显微成像实验显示, 相比于正常人胃黏膜上皮细胞GES-1, 肠癌细胞LoVo表现出较强的绿色荧光信号, 如预先加入抑制剂, 细胞的荧光信号则很弱. 另外, 15例活性MMP-2高表达的临床结直肠癌组织裂解液与探针作用后荧光信号较胃炎组织增强5~9倍, 而15例癌旁组织裂解液荧光则增强约0.9~3.0倍. 值得注意的是, 如果将探针喷洒到结肠癌组织和癌旁组织表面, 相比于胃炎组织, 荧光信号明显增强. 总之, 该探针对基质金属蛋白酶MMP-2/9过表达肠癌细胞和结肠癌组织表现出较好的特异性和检测灵敏度, 具有应用于临床结肠癌早期诊断的极大潜力.

关键词 荧光探针, 荧光能量共振转移(FRET), 基质金属蛋白酶 MMP-2/9, 结肠癌

结肠癌是全球常见恶性肿瘤之一, 每年约有140万新发病例[1]. 在我国, 近20年结肠癌发病率亦逐渐增加[2]. 结肠癌由于诊治时期的不同, 其5年生存率具有明显差异, 早期结肠癌手术后5年存活率可高达90%左右, 而晚期仅为17%[3]. 因此, 结肠癌的早期诊断与治疗对于结肠癌的治愈非常重要. 目前, 临床中纤维结肠镜仍然是结肠癌早期诊断的金标准[4], 但受操作医师技能的限制及结肠生理弯曲等因素, 普通结肠镜检查往往存在一定的漏诊率[5].

基质金属蛋白酶(MMPs)是一类蛋白水解酶, 广泛参与细胞外基质(ECM)的降解和肿瘤血管生成. 大量研究表明, 基质金属蛋白酶与肿瘤的发生、发

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展、侵袭和转移关系极为密切，尤其是MMP-2和MMP-9 (MMP-2/9)，目前已经成为临床肿瘤诊断与治疗的一类重要的生物标志物[6~13]. 因此，提高MMP-2/9检测的特异性和灵敏度是实现癌症早期诊断与有效治疗的一个重要的途径.

近年来，随着材料科学与医学影像技术的飞速发展，基于不同材料，如小分子多肽[14~17]、聚合物[18~21]、硅纳米颗粒[22,23]、无机金属纳米材料[24~27]等，大量MMP-2/9特异性识别的分子探针被制备和报道. 探针的信号检测手段从早期的光学技术逐渐发展为磁共振、光声、核医学等先进的影像技术，大大地提高了MMP-2/9检测的灵敏度，为临床肿瘤的早期诊断、治疗及预后评估提供了重要的技术参考. 因此，构建基质金属蛋白酶MMP-2/9特异性分子探针，开展临床肿瘤样本检测对于癌症的早期诊断与治疗具有重要的临床意义. 目前，已报道的MMPs特异性识别的分子探针大多集中在体外或小动物肿瘤模型检测研究，而用于临床肿瘤样本检测研究仍然很少. 本研究通过将绿色荧光素(FITC)和荧光淬灭基团(Dabcyl)策略性偶联于MMP-2/9特异性识别多肽底物GPLGVRGY[28]两端，构建基于荧光能量共振转移(FRET)机理的荧光分子探针，利用结肠癌细胞中高表达的MMP-2/9在多肽特异性位点剪切释放荧光信号的特性，初步开展临床结肠癌快速、准确诊断的相关基础研究(图1).

1 材料和方法

(ⅰ) 材料与仪器. 异硫氰酸荧光素(fluorescein isothiocyanate, FITC)购于上海阿拉丁公司；GPLGVRGY-Pra多肽定制于上海吉尔生化有限公司；琥珀酰亚胺(DABCYL acid, SE)购于北京欧凯纳斯公司；重组基质金属蛋白酶-2/9(recombinant matrix metalloproteinase, MMP-2/9)购于美国R&D Systems公司；RPMI 1640培养基、胎牛血清购于美国Gibco公司；RIPA裂解液、噻唑蓝均购于上海碧云天公司；大肠癌细胞LoVo、正常胃黏膜细胞GES-1购自中国科学院上海生命科学研究院细胞库.

(ⅱ) 探针Dab-GPLGVRGY-FITC的合成、纯化与表征. 首先，将3-azidopropan-1-amine (7 mg, 0.05 mmol/L)与异硫氰酸荧光素(FITC-NCS, 14 mg, 0.04 mmol/L)溶于DMF溶剂，室温下搅拌6 h后，经真空浓缩和柱层析纯化后制备得到异硫氰酸荧光素-叠氮(FITC-N_3). 然后，将末端炔修饰多肽GPLGVRGY-Pra (27 mg, 0.03 mmol/L)与FITC-N_3 (17 mg, 0.04 mmol/L)在抗坏血酸钠(Na Asb)和硫酸铜($CuSO_4$)的催化下通过“点击”化学反应合成得到NH_2-GPLGVRGY-FITC-Pra，并经高效液相色谱仪HPLC和质谱进行纯化和表征. 多肽NH_2-GPLGVRGY-FITC-Pra (42 mg, 0.03 mmol/L)与Dabcyl酸琥珀酰亚胺脂(13 mg, 0.04 mmol/L)溶于DMF溶剂中，于室温下避光搅拌6 h后真空浓缩并经HPLC纯化得到探针Dab-GPLGVRGY-FITC，溶于二甲基亚砜备用. 探针经过高分辨质谱确认.

(ⅲ) 体外研究探针对MMP-2检测的特异性和灵敏度. 为了研究探针对MMP-2检测的灵敏度，探针(2 μmol/L)在37℃下与不同浓度的MMP-2 (0, 0.7, 1.4, 2.8, 5.6, 8.5, 11.3 nmol/L)在PBS溶液中孵育6 h，然后利用荧光光谱仪检测溶液的荧光信号变化. 为进一步研究探针的特异性，在有无MMP-2抑制剂存在的条件下，探针(2 μmol/L)和8.5 nmol/L MMP-2在37℃下分别孵育6 h. 同时，在相同条件下，探针还分别与Furin, Dansa, Ransa, BSA, MMP-2, MMP-9等其他生物酶在37℃下孵育6 h，溶液的荧光强度最终经荧光光谱仪测定.

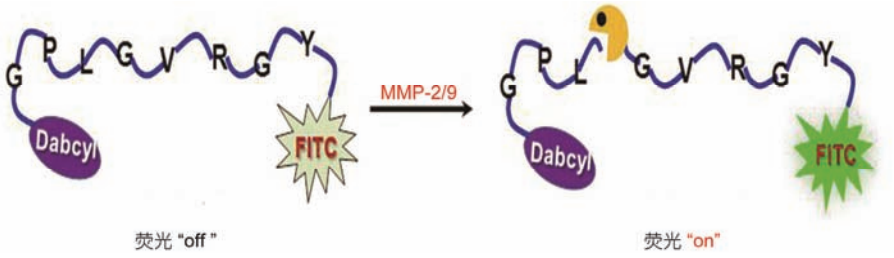


图 1 (网络版彩色) 探针Dab-GPLGVRGY-FITC对MMP-2/9 特异性检测
Figure 1 (Color online) Specific detection of MMP-2/9 using probe Dab-GPLGVRGY-FITC

(ⅳ) 细胞成像. 大肠癌细胞LoVo与不同浓度的探针(0, 0.1, 1, 10, 50, 100 μmol/L)在5% CO_2和37℃下孵育24 h后, 加入0.5 mg/mL噻唑蓝, 用MTT实验对探针的细胞毒性进行评估后, 大肠癌细胞LoVo和正常胃黏膜上皮细胞GES-1分别与2 μmol/L探针于5% CO_2, 37℃下孵育8 h, 荧光共聚焦显微镜下记录细胞荧光成像情况. 此外, 大肠癌细胞LoVo中分别加入探针(2 μmol/L)和MMP-2抑制剂(25 μmol/L)开展竞争实验研究.

(ⅴ) 临床组织样本检测分析. 30例临床结肠癌肿瘤组织样品(含15例结肠癌组织与15例癌旁组织, 均来自于苏州大学附属第一医院普外科肿瘤切除术后2 h内标本)和1例胃炎黏膜组织(来自于苏州大学附属第一医院内镜室)经RIPA裂解液裂解、定量. 探针(2 μmol/L)和组织样本(1.0 mg/mL)在37℃孵育2 h. 同时, 添加MMP-2抑制剂(25 μmol/L)作为阴性对照实验, 利用荧光光谱仪记录溶液的荧光强度变化. 为进一步研究探针对临床组织显像的效果, 将结肠癌、癌旁及胃炎组织分别切割成小块置于探针(2 μmol/L)的PBS溶液中孵育4 h后(MMP-2抑制剂添加为阴性对照), 通过小动物活体光学成像系统扫描记录组织的荧光成像情况. 最后, 将组织冰冻切片, 荧光共聚焦显微镜下观测其荧光信号.

2 结果与讨论

2.1 探针的设计、纯化与表征

末端炔修饰MMP-2/9多肽底物(GPLGVRGY-Pra)分别经过Cu(I)催化"点击"化学反应和酰胺化反应与叠氮异硫氰酸荧光素(FITC-N_3)和Dabcyl琥珀酰亚胺脂(DABCYL acid, SE)偶联, 制备得到探针Dab-PLGVRGY-FITC (图2), 其理论分子量为1652.8300 D. 图3(a)和(b)分别为Dab-GPLGVRGY-FITC的HPLC图谱和高分辨质谱. 探针纯度在95%以上, 并经高分辨质谱确证.

2.2 探针对MMP-2/9的特异性和灵敏度

首先, 考察了探针对MMP-2/9的特异性和灵敏度. 如图4(a), 当探针与MMP-2作用后, 荧光光谱仪扫描显示, 520 nm波长处溶液的荧光强度较探针自身

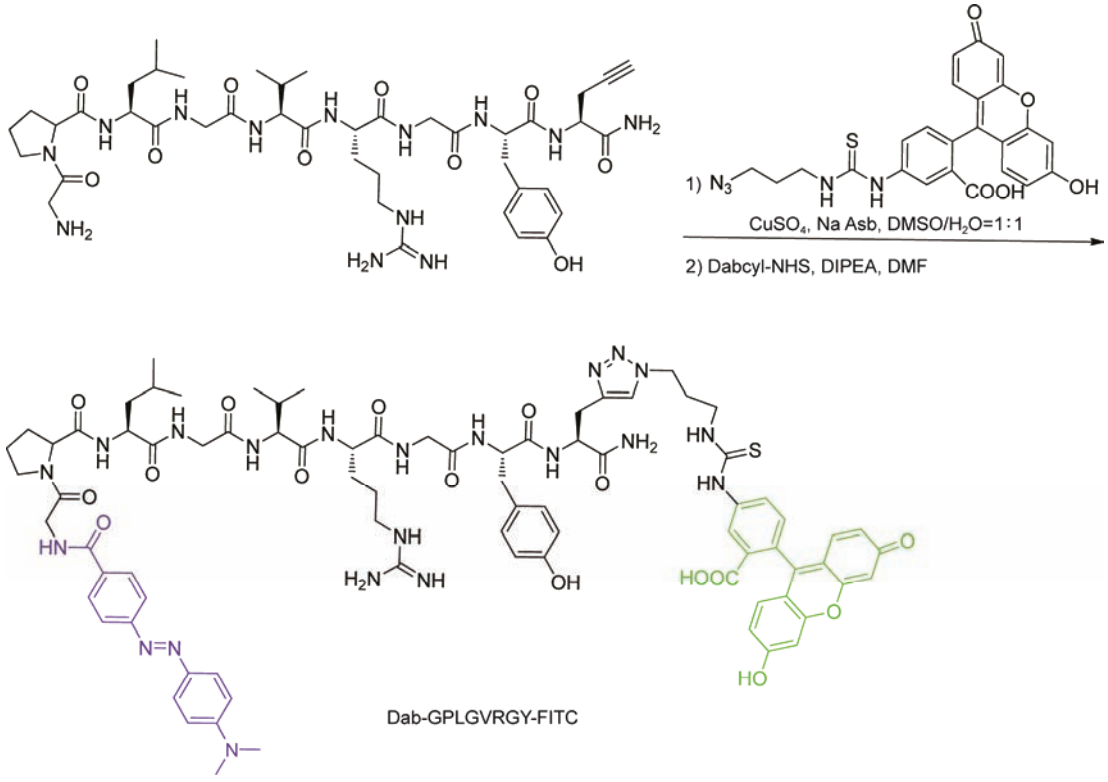


图 2 探针的合成路线

Figure 2 The synthetic route of probe

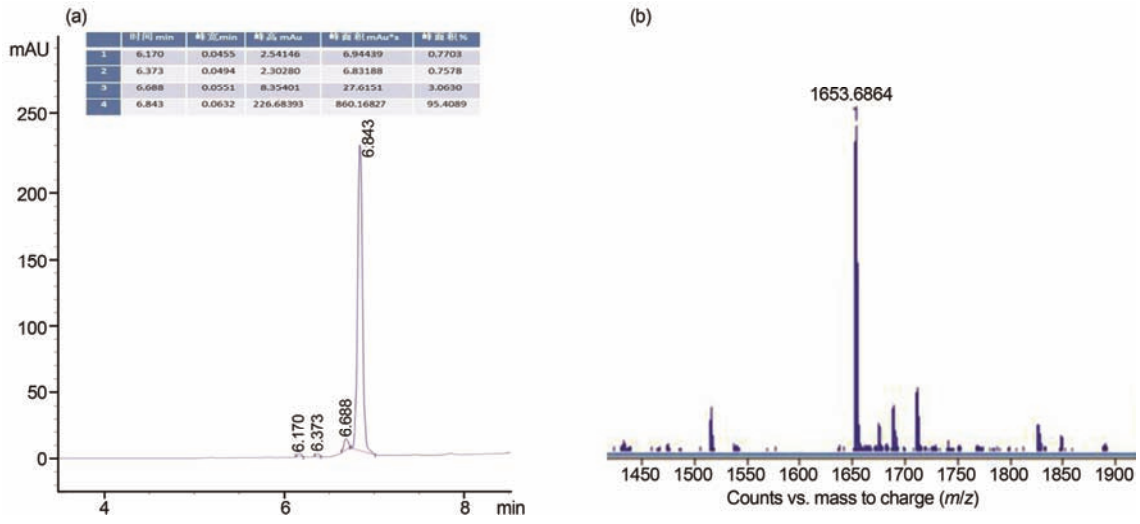


图 3 探针的纯度和高分辨质谱结果. (a) 探针的HPLC图谱; (b) 探针的高分辨质谱图

Figure 3 The purity and high-resolution mass spectra results of probe. (a) HPLC profile of probe; (b) high-resolution mass spectrum of probe

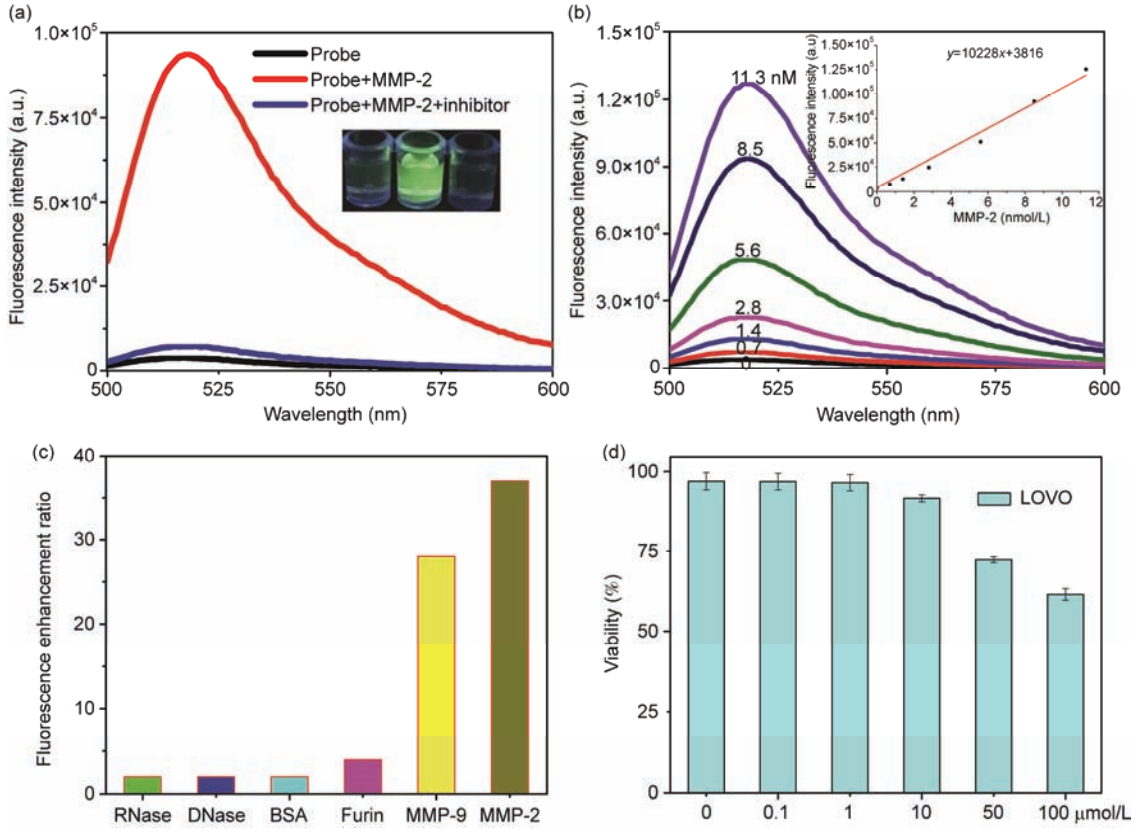


图 4 (网络版彩色)探针对MMP-2/9 的特异性和检测灵敏度研究. (a) 探针自身、探针与MMP-2 及抑制剂竞争实验的荧光强度变化; (b) 探针在不同量MMP-2 的作用下的荧光强度; (c)探针对MMP-2/9 的特异性和选择性研究; (d) 探针对LoVo细胞的毒性研究. [probe]=2 μmol/L, [MMP-2]=8.5 nmol/L, [抑制剂]=25 μmol/L

Figure 4 (Color online) Study of the specificity and sensitivity of probe for MMP-2/9 detection. (a) Fluorescent intensity of indicated amount of probes after incubation with MMP-2 and inhibitor, respectively; (b) fluorescence of probes after incubation with different concentrations of MMP-2; (c) the fluorescence enhancement ratio of probes after incubation with different proteins; (d) cytotoxicity of probe to LoVo cells. [probe]=2 μmol/L, [MMP-2]=8.5 nmol/L, [inhibitor]=25 μmol/L

荧光有明显增强，达40倍，同时，在紫外灯照射下，溶液的颜色由无色变为绿色. 而预先添加了抑制剂的溶液则无明显荧光增强和颜色变化. 此外，如图4(b)显示溶液的荧光强度随着MMP-2用量的增加呈线性增强，最低检测限可达14 ng/mL. 为进一步研究探针的选择性，图4(c)为探针与不同种类的生物酶(如RNase, DNase, BSA, Furin, MMP-9和MMP-2)作用后溶液荧光信号变化情况. 仅MMP-2和MMP-9在520 nm波长处有较大的荧光增强，增强达30~40倍，而其他酶荧光增强微小. 上述实验结果表明，探针对MMP-2/9检测具有较强的特异性和灵敏度，能潜在应用于MMP-2/9过表达肿瘤的检测研究.

2.3 对大肠癌细胞的成像

为了考察探针是否能用于活性MMP-2/9过表达肿瘤细胞成像，首先利用MTT实验对探针的细胞毒性进行了评估. 图4(d)显示，大肠癌细胞LoVo即使与100 μmol/L探针孵育24 h后，仍然保持70%的存活率，表明探针对细胞具有较低的毒性. 然后，进一步开展了探针对肿瘤细胞的成像研究. 将大肠癌细胞LoVo和正常细胞GES-1分别与探针孵育8 h后，通过荧光共聚焦显微镜对细胞进行成像，图5结果显示，LoVo细胞表现出较强的绿色荧光，且荧光信号能够被MMP-2抑制剂所抑制，而正常细胞GES-1则几乎无荧光信号检测得到，表明探针对活性MMP-2具有较好的特异性，能够选择性对肠癌细胞成像.

2.4 临床结肠癌组织样本检测研究

为了进一步考察探针用于临床结肠癌组织检测的可行性. 临床结肠癌、癌旁及炎症组织分别经RIPA裂解，与探针作用后，其结果如图6(a)所示，结肠癌组织溶液的荧光信号明显增强，较探针自身背景荧

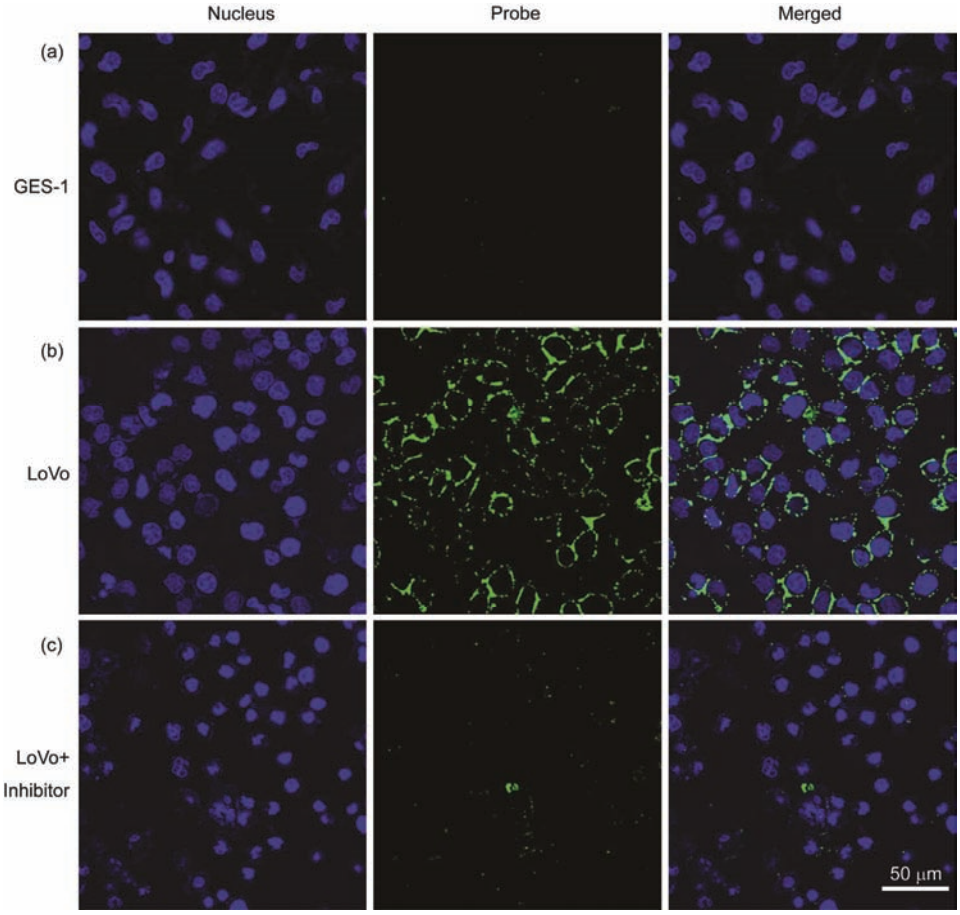


图 5 (网络版彩色)细胞成像研究. (a) GES-1+探针; (b) LoVo+探针; (c) LoVo+抑制剂+探针. [probe]=2 μmol/L, [inhibitor]=25 μmol/L

Figure 5 (Color online) Cell imaging study. (a) GES-1+probe; (b) LoVo+probe; (c) LoVo+inhibitor+probe. [probe]=2 μmol/L, [inhibitor]=25 μmol/L

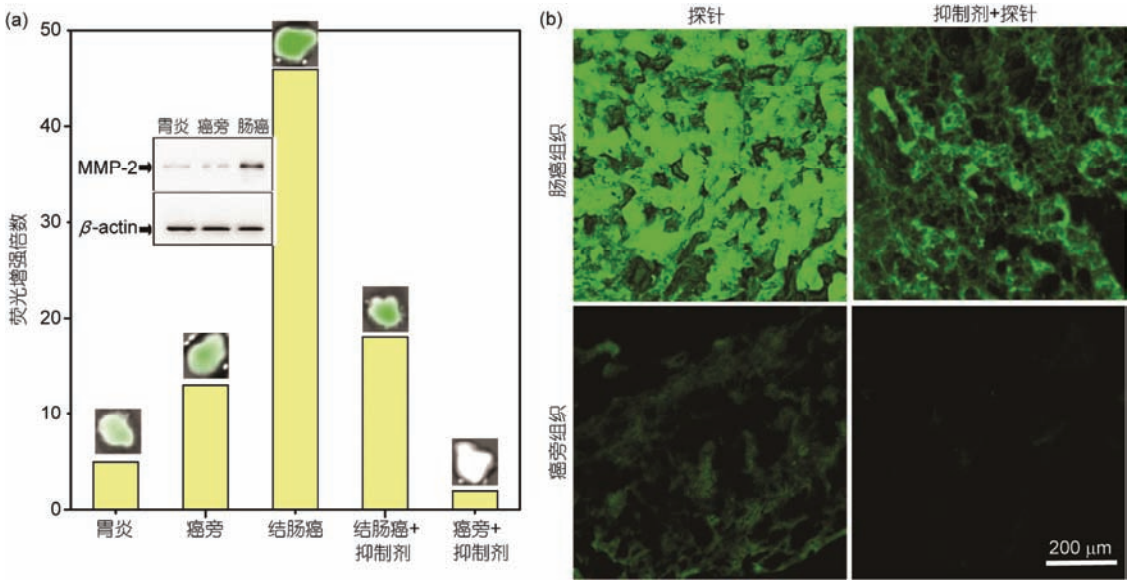


图 6 (网络版彩色) 探针对临床组织样本的检测. (a) 结肠癌、癌旁及胃炎组织和裂解液分别在探针或抑制剂的作用下荧光变化情况及Western blotting 检测组织内活性MMP-2 的表达情况; (b) 临床组织样本切片在荧光共聚焦显微镜下的成像. [探针]=2 μmol/L, [组织裂解液]=1.0 mg/mL, [抑制剂]=25 μmol/L

Figure 6 (Color online) Detection of clinical tissue samples using probe. (a) Fluorescence enhancements of probes after incubation with different clinical samples with and without inhibitor; (b) fluorescence confocal microscopy imaging of clinical tissues. [probe]=2 μmol/L, [tissue lysate]=1.0 mg/mL, [inhibitor]=25 μmol/L

光增强达46倍; 癌旁组织则表现出约12倍的荧光增强, 而胃炎组织荧光信号增强微弱. 抑制剂竞争实验结果显示, MMP-2抑制剂的加入, 结肠癌及癌旁组织的荧光信号将被大大地抑制. 利用MMP-2用量与溶液荧光强度线性关系, 可推断出该结肠癌组织样品中活性MMP-2含量约为1.33 ng/mg, 而结肠癌旁组织中活性MMP-2含量约为0.25 ng/mg. 这与利用Western blotting所测得的肠癌和肠癌旁组织内活性MMP-2的表达量(2.90和1.12 ng/mg)很接近, 因此具有一定的参考价值. 此外, 荧光共聚焦显微镜下, 结肠癌组织相比于癌旁组织表现出较强的绿色荧光. 同样, 在抑制剂的作用下, 荧光信号会大大减弱, 结果如图6(b)所示. 这些实验结果表明, 利用该探针能够对结肠癌、癌旁和炎症组织进行成像和区分.

最后, 以1例胃炎组织作为阴性对照, 利用探针对15例临床结肠癌组织样本和15例癌旁组织进行检测, 实验结果统计如表1, 15例结肠癌组织较胃炎组织荧光强度高达5.2~9.2倍, 而癌旁组织的荧光强度则约是胃炎组织的0.9~3.0倍. 若以荧光强度增强3倍以上为阳性统计, 肿瘤阳性检测率高达95%以上. 因此, 该探针具有应用于临床辅助快速诊断结肠癌的极大潜力. 本研究中由于临床样本数量有限, 探针对临床结肠癌组织检测的阳性率和准确性还需进一步深入研究. 此外, 本实验仅对肿瘤探针的特异性和灵敏度进行了体外评估, 能否进行体内肿瘤的早期、准确检测还需进一步研究.

3 结论

本研究针对与结肠癌细胞发生、发展和转移密切相关的MMP-2和MMP-9[29~31], 基于基质金属蛋白酶MMP-2/9特异性识别多肽底物设计和构建了FRET荧光分子探针, 将其成功应用于重组基质金属蛋白酶MMP-2/9的体外检测研究, 并充分证明了探针对MMP-2/9检测具有较强的特异性和灵敏度. 细胞成像结果表明, 探针能够对MMP-2/9过表达人源大肠癌细胞LoVo特异性成像. 此外, 临床确诊的结直肠癌组织样本检测结果进一步表明, 探针对临床结直肠癌组织的检测率高达95%以上. 总之, 本研究成功构建了一种基质金属蛋白酶MMP-2/9特异性荧光分子探针, 其能够对体外结直肠癌组织与细胞进行灵敏性检测, 这为我们下一步开展分子显像早期诊断结直肠癌提供了重要依据.

表 1 临床组织来源的患者年龄、性别、肿瘤TNM分期及与探针作用后的荧光强度变化

Table 1 The corresponding patient's age, sex, tumor TNM staging for clinical tissues and the fluorescence intensities of the clinical samples after treated with probe

病例	性别	年龄(岁)	TNM分期	荧光强度(520 nm处)				
				胃炎组织	癌旁组织	癌旁/胃炎	肠癌组织	肠癌/胃炎
胃炎者	男	37		2.91E+4				
1	男	67	ⅢB		5.36×10^4	1.84	2.59×10^5	8.90
2	男	61	ⅡA		6.63×10^4	2.28	2.40×10^5	8.25
3	女	73	ⅢB		6.12×10^4	2.1	2.04×10^5	7.01
4	女	60	ⅢB		6.52×10^4	2.24	2.30×10^5	7.9
5	男	68	ⅢB		8.68×10^4	2.98	2.32×10^5	7.97
6	男	80	ⅡA		6.60×10^4	2.27	2.33×10^5	8.0
7	女	75	ⅢB		5.94×10^4	2.04	2.12×10^5	7.29
8	男	89	ⅡA		6.83×10^4	2.35	2.19×10^5	7.53
9	女	86	ⅡA		6.60×10^4	2.27	2.09×10^5	7.18
10	男	47	ⅡA		6.52×10^4	2.24	2.24×10^5	7.7
11	男	80	ⅢB		8.11×10^4	2.79	2.67×10^5	9.18
12	男	61	Ⅳ		2.50×10^4	0.86	1.67×10^5	5.74
13	男	63	ⅡA		7.21×10^4	2.48	2.21×10^5	7.59
14	男	78	ⅡA		6.35×10^4	2.18	1.51×10^5	5.19
15	男	72	ⅠB		6.12×10^4	2.1	2.31×10^5	7.94

参考文献

1 Torre L A, Bray F, Siegel R L, et al. Global cancer statistics 2012. CA Cancer J Clin, 2015, 65: 87–108

2 Zheng Z X, Zheng R S, Chen W Q, et al. Colorectal cancer incidence and mortality in China 2010. Asian Pac J Cancer Prev, 2014, 15: 8455–8460

3 Siegel R, DeSantis C, Virgo K, et al. Cancer treatment and survivorship statistics 2012. CA Cancer J Clin, 2012, 62: 220–241

4 Wanders L K, van Doorn S C, Fockens P, et al. Quality of colonoscopy and advances in detection of colorectal lesions: A current overview. Expert Rev Gastroenterol Hepatol, 2015, 9: 417–430

5 Bressler B, Paszat L F, Vinden C, et al. Colonoscopic miss rates for right-sided colon cancer: A population-based analysis. Gastroenterology, 2004, 127: 452–456

6 Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer, 2002, 2: 161–174

7 Li J, Zhao T F, Duan E K. Matrix metalloproteinases (MMPs) and trophoblast invasion. Chin Sci Bull, 2005, 50: 1169–1173 [李晶, 赵田夫, 段恩奎. 基质金属蛋白酶(MMPs)在人滋养层细胞侵入中的作用. 科学通报, 2005, 50: 1167–1171]

8 Garripelli V K, Kim J K, Son S, et al. Matrix metalloproteinase-sensitive thermogelling polymer for bioresponsive local drug delivery. Acta Biomater, 2011, 7: 1984–1992

9 Roy R, Yang J, Moses M A. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol, 2009, 27: 5287–5297

10 Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: Regulators of the tumor microenvironment. Cell, 2010, 141: 52–67

11 Dai Z F. Applications, opportunities and challenges of molecular probes in the diagnosis and treatment of major diseases (in Chinese). Chin Sci Bull, 2017, 1: 25–35 [戴志飞. 分子探针在重大疾病诊疗中的应用、机遇与挑战. 科学通报, 2017, 1: 25–35]

12 Lee S, Xie J, Chen X Y. Peptides and peptide hormones for molecular imaging and disease diagnosis. Chem Rev, 2010, 110: 3087–3111

13 Hanaoka H, Mukai T, Habashita S, et al. Chemical design of a radiolabeled gelatinase inhibitor peptide for the imaging of gelatinase activity in tumors. Nucleic Med Biol, 2007, 34: 503–510

14 Gallo J, Kamaly N, Lavdas I, et al. CXCR4-targeted and MMP-responsive iron oxide nanoparticles for enhanced magnetic resonance imaging. Angew Chem Int Ed, 2014, 53: 9550–9554

15 Bremer C, Tung C H, Weissleder R. *In vivo* molecular target assessment of matrix metalloproteinase inhibition. Nat Med, 2001, 7: 743–748

16 Shi H, Ryan K T K, Tang B Z, et al. Real-time monitoring of cell apoptosis and drug screening using fluorescent light-up probe with aggregation-induced emission characteristics. J Am Chem Soc, 2012, 134: 17972–17981
17 Shi H, Liu J, Geng J, et al. Specific detection of integrin αVβ3 by light-up bioprobe with aggregation-induced emission characteristics. J Am Chem Soc, 2012, 134: 9569–9572
18 Zhu L, Zhang F, Ma Y, et al. *In vivo* optical imaging of membrane-type matrix metalloproteinase (MT-MMP) activity. Mol Pharmaceutics, 2011, 8: 2331–2338
19 Olsona E S, Jiang T, Aguileraa T A, et al. Activatable cell penetrating peptides linked to nanoparticles as dual probes for *in vivo* fluorescence and MR imaging of proteases. Proc Natl Acad Sci USA, 2010, 107: 4311–4316
20 Le S, Ryu J H, Park K, et al. Polymeric nanoparticle-based activatable near-infrared nanosensor for protease determination *in vivo*. Nano Lett, 2009, 9: 4412–4416
21 Wang Z, Li X H, Feng D, et al. Poly(m-phenylenediamine)-based fluorescent nanoprobe for ultrasensitive detection of matrix metalloproteinase. Anal Chem, 2014, 86: 7719–7725
22 Zhang J, Yuan Z F, Wang Y, et al. Multifunctional envelope-type mesoporous silica nanoparticles for tumor-triggered targeting drug delivery. J Am Chem Soc, 2013, 135: 5068–5073
23 Sun R, Yin L, Zhang S, et al. Simple light-triggered fluorescent labeling of silica nanoparticles for cellular imaging applications. Chem Eur J, 2017, 23: 13893–13896
24 Cheng X, Sun R, Yin L, et al. Light-triggered assembly of gold nanoparticles for photothermal therapy and photoacoustic imaging of tumours *in vivo*. Adv Mater, 2017, doi: 10.1002/adma.201604894
25 Kwong G A, von Maltzahn G, Murugappan G, et al. Mass-encoded synthetic biomarkers for multiplexed urinary monitoring of disease. Nat Biotechnol, 2013, 31: 63–70
26 Xia X H, Yang M X, Oetjen L K, et al. An enzyme-sensitive probe for photoacoustic imaging and fluorescence detection of protease activity. Nanoscale, 2011, 3: 950–953
27 Hou Y, Zhou J, Gao Z Y, et al. Protease-activated ratiometric fluorescent probe for pH mapping of malignant tumors. ACS Nano, 2015, 9: 3199–3205
28 Zhang Y, So M K, Rao J. Protease-modulated cellular uptake of quantum dots. Nano Lett, 2006, 6: 1988–1992
29 Zucker S, Vacirca J. Role of matrix metalloproteinases (MMPs) in colorectal cancer. Cancer Metastasis Rev, 2004, 23: 101–117
30 Kostova E, SlaninkaMiceska M, Labacevski N, et al. Expression of matrix metalloproteinases 2, 7 and 9 in patients with colorectal cancer. Vojnosanit Pregl, 2014, 71: 52–59
31 Langers A M J, Verspaget H W, Hawinkels L J A C, et al. MMP-2 and MMP-9 in normal mucosa are independently associated with outcome of colorectal cancer patients. Br J Cancer, 2012, 106: 1495–1498

Summary for “基质金属蛋白酶 MMP-2/9 分子探针对临床结肠癌组织的检测”

A fluorogenic probe for sensitive detection of MMP-2/9 activities in clinical colorectal cancer tissues

Zuhong Yu[1], Fujuan Luan[1], Xia Leng[1], Yuxue Shi[1], Jie Wang[1], Ling Yin[2,3], Lei He[2,3], Haibin Shi[2,3*] & Weichang Chen[1*]

[1] *Department of Digestion Internal Medicine, the First Affiliated Hospital of Soochow University, Suzhou 215006, China;*
[2] *School of Radiation Medicine and Protection, Medical College of Soochow University, Suzhou 215123, China;*
[3] *School for Radiological & Interdisciplinary Sciences(RAD-X), Soochow University, Suzhou 215123, China*
* Corresponding authors, E-mail: hbshi@suda.edu.cn; weichangchen@126.com

Developing a novel fluorogenic probe which can be specifically recognized by active matrix metalloproteinase-2 and matrix metalloproteinase-9 (MMP-2/9) to rapidly and accurately detect the clinical colorectal cancer is significant but remains a great challenge. Herein, we have designed and synthesized a novel fluorescence resonance energy transfer (FRET)-based probe Dab-GPLGVRGY-FITC for specific detection of MMP-2/9 activity. The probe was prepared by conjugating fluorescein-N_3 (FITC-N_3) and fluorescent quencher dabcyl-NHS to MMP-2/9-responsive peptide substrate GPLGVRGY through copper (I)-catalyzed “click” and amidation reactions, respectively. To assess the specificity and sensitivity of the probe for active MMP-2/9 *in vitro*, the enzymatic assays were firstly performed with recombinant MMP-2/9. Fluorescent signal change of the mixed solution of probe and MMP-2 with and without treatment of MMP-2 specific inhibitor was recorded at 520 nm with fluorescence spectrometer. The capability of probe for specific detection of endogenous MMP-2/9 activity was further evaluated with human colorectal cancer cell LoVo and 30 clinical colorectal cancer tissue samples. The *in vitro* enzymatic assay results indicated that the probe showed good specificity and sensitivity for recombinant MMP-2/9 comparing to other proteins. The detection limit for recombinant MMP-2 is estimated to be 14 ng mL^{-1}. Besides, the confocal laser scanning microscopy (CLSM) imaging results showed that enhanced green fluorescence signals were collected for LoVo cells upon treatment with probe. However, most of the fluorescence was readily competed away by pretreatment of the probe with inhibitor. In sharp contrast, only weak fluorescence was observed for normal gastric mucosa epithelial GES-1 cells. Additionally, the lysates of 15 clinical colorectal tissues displayed ~5 to 9-fold fluorescence enhancements after treatment of the probe in comparison with normal gastritis tissues. However, the fluorescence intensities of 15 peficancerous tissue lysates was only ~0.9 to 3-fold higher than normal tissues. Most notably, in comparison with normal gastritis tissue, strong green fluorescence was observed for both colorectal tissue and peficancerous tissue once the probe was sprayed onto the tissue surface. In summary, this probe shows good specificity and sensitivity for the detection of active MMP-2 both in cancer cell and clinical cancer tissue, which may have great potential for early diagnosis of MMP-2/9 active colorectal cancer in clinic.

fluorogenic probe, fluorescent resonance energy transfer (FRET), matrix metalloproteinases-2/9 (MMP-2/9), colorectal cancer

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