PO Box 2345 Beijing 100023, China
Fax: +86-10-85381893
Email: wcjd@wjgnet.com www.wjgnet.com

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食管鳞癌中COX-2 mRNA的表达以及NSAID对其的影响

刘 萱, 李 鹏, 张澍田, 贾继东, 于中麟

刘萱, 李鹏, 张澍田, 贾继东, 于中麟, 首都医科大学附属北京友谊医院北京市消化疾病中心 北京市 100050
刘萱, 女, 1974-04-22生, 北京市人, 汉族, 2003年首都医科大学硕士, 医师, 主要从事消化系统疾病的治疗和研究.
项目负责人: 张澍田, 100050, 北京市宣武区永安路95号, 首都医科大学附属北京友谊医院, 北京市消化疾病中心. zhangst@bddc_bfh.com.cn
电话: 010-63014411-3339 传真: 010-63020006
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COX-2 mRNA expression in esophageal squamous cell carcinoma before and after treated with non-steroidal anti-inflammatory drugs

Xuan Liu, Peng Li, Shu-Tian Zhang, Ji-Dong Jia, Zhong-Lin Yu

Xuan Liu, Peng Li, Shu-Tian Zhang, Ji-Dong Jia, Zhong-Lin Yu, Beijing Center for Digestive Diseases, Beijing Friendship Hospital, Capital University of Medical Sciences, Beijing 100050, China
Correspondence to: Shu-Tian Zhang, Beijing Center for Digestive Diseases, Beijing Friendship Hospital, Capital University of Medical Sciences, Beijing 100050, China. zhangst@bddc_bfh.com.cn
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Abstract

AIM: To investigate the potential relationship between COX-2 mRNA expression and human esophageal squamous cell carcinoma (ESCC) and to explore the effect of non-steroidal anti-inflammatory drugs (NSAID) on ESCC.

METHODS: Frozen specimens of human esophageal squamous cell carcinoma (n = 22) and adjacent normal esophageal squamous epithelium were examined for COX-2 mRNA expression by reverse-transcription polymerase chain reaction (RT-PCR). After incubated with aspirin or nimesulide, proliferation of human esophageal squamous cancer cells (EC-9706 and EC-109) was quantified by MTT assay, and COX-2 mRNA expression in these cells was detected by RT-PCR.

RESULTS: Of 22 cancer specimens, COX-2 mRNA was expressed in 12 cases(54.5%). However, in all the samples of adjacent normal esophageal squamous epithelium, COX-2 mRNA expression were not detected. Both aspirin and nimesulide inhibited EC-9706 cell proliferation and COX-2 mRNA expression. However, aspirin also inhibited EC-109 cell proliferation and COX-2 mRNA expression while nimesulide did not.

CONCLUSION: COX-2 mRNA is frequently expressed in human ESCC and COX-2 may play an important role in carcinogenesis of ESCC. NSAID may be helpful in prevention and treatment of this cancer.

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摘要

目的: 检测人类食管鳞癌组织和细胞中COX-2 mRNA的表达及NSAID对其的影响, 探讨COX-2与食管鳞癌发病机制之间可能存在的关系.

方法: 用逆转录聚合酶链反应法(RT-PCR)检测22例食管鳞癌患者液氮冻存的食管鳞癌组织和癌周正常食管鳞状上皮组织标本COX-2 mRNA的表达. 将人类食管鳞癌细胞株与阿司匹林或尼美舒利共同孵育后, 用噻唑蓝法定量检测细胞增生情况, 用RT-PCR法检测其COX-2 mRNA的表达情况.

结果: 在22例食管鳞癌组织标本中有12例(54.5%)COX-2 mRNA表达阳性, 但在癌周正常食管鳞状上皮组织标本均未检测到COX-2 mRNA表达. 细胞培养结果表明, 阿司匹林和尼美舒利对EC-9706细胞株的增生和COX-2 mRNA表达均有影响; 但对EC-109细胞株, 仅阿司匹林对细胞的增生和COX-2 mRNA表达有影响.

结论: 人类食管鳞癌常表达COX-2 mRNA, 提示COX-2有可能在食管鳞癌的发生机制中起重要作用, 而且NSAID很可能有助于预防和治疗此病.

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0 引言

食管癌是世界第三大恶性肿瘤, 中国是该病发病率和死亡率较高的国家之一. 每年全世界新增加的30万食管癌患者中, 约有一半发生在中国, 且多为食管鳞癌(esophageal squamous cell cancer, ESCC). 我们通过检测COX-2 mRNA在人类食管鳞癌组织和细胞中的表达, 来探讨COX-2与食管鳞癌发病机制之间可能存在的关系.

1 材料和方法

1.1 材料 液氮冻存食管鳞癌组织和癌周正常食管鳞状上皮组织标本各22例, 2002-03/2002-12首都医科大

学附属北京友谊医院胸心血管外科食管癌切除术标本18对，消化内镜中心内镜下活检标本4对. 人类食管鳞癌细胞株(EC-9706和EC-109)由中国医学科学院肿瘤研究所惠赠. GAPDH(209 bp)上游引物:5'-CCC TTC ATT GAC CTC AAC TAC ATG G-3'; GAPDH(209 bp)下游引物:5'-CAT GGT GGT GAA GAC GCC AG-3'; COX-2(531 bp)上游引物:5'-AAG CCT TCT CTA ACC TCT CC-3'; COX-2(531 bp)下游引物:5'-TAA GCA CAT CGC ATA CTC TG-3'则均由博润基因(北京)有限公司制备合成.

1.2 方法 用逆转录聚合酶链反应法(RT-PCR)检测组织和细胞中COX-2 mRNA的表达. 扩增的反应条件为94 ℃预变性2 min，然后依次是94 ℃ 1 min; 54 ℃ 1 min和72 ℃ 1 min重复33个循环，最后72 ℃后延伸5 min. 用噻唑蓝[3-(4，5-dimethylthiazol-2-yl)-2，5-diphenyltetrazolium bromide，MTT]分析法检测人类食管鳞癌细胞株增生情况. 其原理为MTT可以被活细胞线粒体内的琥珀酸脱氢酶还原成难溶性蓝紫色结晶沉积在细胞体内，DMSO可以溶解这些蓝色结晶，但是在死细胞内MTT却不能被还原为蓝紫色结晶，故通过测定在特定波长下吸光度*A*值的不同可以反映活细胞数. 将(1-2)×10^5个细胞种植在96孔板内，每孔200 μL，加入相应的药物和时间处理后，每孔加入5 g/L MTT 20 μL混匀，在37 ℃，50 mL/L CO_2条件下继续培养4 h，1 000 r/min离心10 min，吸干每孔内的上清，每孔加入DMSO 150 μL震荡使沉淀充分溶解，酶标仪492 nm波长测定吸光度*A*值. 将细胞按所用药物[阿司匹林(Aspirin，A6810)和尼美舒利(Nimesulide，N1016)]及浓度分组，为排除药物溶剂二甲基亚砜(dimethyl sulfoxide，DMSO)的干扰，特设DMSO组作为对照(表1).

表1 各组药物终浓度设置

分组	DMSO终浓度(g/L)	药物浓度
DMSO组	5	0
单纯细胞组	0	0
A5	5	A6810 5 mmol/L
A10	5	A6810 10 mmol/L
A15	5	A6810 15 mmol/L
A20	5	A6810 20 mmol/L
N100	5	N1016 100 μmol/L
N200	5	N1016 200 μmol/L
N400	5	N1016 400 μmol/L
N800	5	N1016 800 μmol/L
N1000	5	N1016 1 000 μmol/L

统计学处理 采用SPSS9.0统计软件进行统计分析. COX-2表达阳性率的比较用χ^2检验; 细胞培养药物干预结果用非参数检验.

2 结果

2.1 食管鳞癌组织标本COX-2表达结果显示，在22例食管鳞癌组织标本中有12例(54.5%)COX-2m RNA表达阳性，但在癌周正常食管鳞状上皮组织标本均未检测到COX-2 mRNA表达(图1，表2).

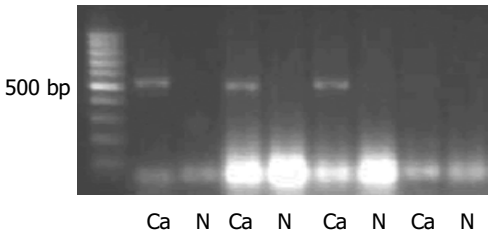

图1 食管鳞癌组织标本COX-2表达. Ca: 食管鳞癌组织; N: 癌周正常食管鳞状上皮组织.

表2 食管鳞癌组织标本COX-2表达

COX-2表达	癌组织(Ca)	癌旁组织(N)	总计
阳性	12[b]	0	12
阴性	10	22	32
总计	22	22	44

[b]$P<0.01$ *vs* 癌旁组织.

2.2 EC-9706细胞株药物干预结果显示，5-20 mmol/L的阿司匹林和100-1 000 μmol/L的尼美舒利对该细胞株增生和COX-2 mRNA表达的影响均具有浓度依赖性(表3，图2).

表3 EC-9706细胞株药物干预结果

分组	mRNA表达	MTT检测
DMSO组	0.9950 ± 0.0294	0.467 ± 0.064
单纯细胞组	1.000 ± 0.0338	0.475 ± 0.047
A5	0.9787 ± 0.0245	0.432 ± 0.056
A10	0.9506 ± 0.0267	0.398 ± 0.046
A15	0.9217 ± 0.0305[a]	0.365 ± 0.031
A20	0.8783 ± 0.0374[b]	0.277 ± 0.030[b]
N100	0.9907 ± 0.0232	0.429 ± 0.032
N200	0.9640 ± 0.0188	0.419 ± 0.027
N400	0.9262 ± 0.0265	0.400 ± 0.018
N800		0.257 ± 0.069[b]
N1000	0.8585 ± 0.0438[b]	0.198 ± 0.021[b]

[a]$P<0.05$ *vs* DMSO组; [b]$P<0.01$ *vs* DMSO组.

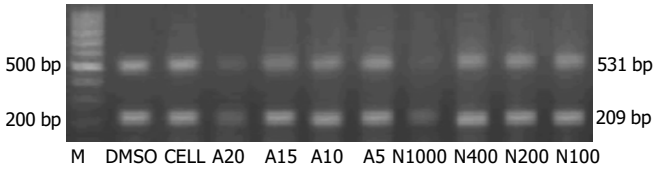

图2 EC-9706细胞株药物干预结果电泳图. M: 标记物，间隔100 bp; 531 bp: COX-2产物; 209 bp: 内对照产物.

2.3 EC-109细胞株药物干预结果显示，5-20 mmol/L的阿司匹林对该细胞株增生的影响与DMSO组之间的差异则均具有极其显著性意义，而对其COX-2 mRNA表达的影响具有浓度依赖性; 但100-800 μmol/L的尼美舒利对其增生和COX-2 mRNA表达的影响与DMSO组之间的差异均无统计学显著性意义(表4，图3).

表4 EC-109细胞株药物干预结果

组别	mRNA表达	MTT检测
DMSO组	0.9 042 ± 0.0 231	0.486 ± 0.056
单纯细胞组	0.9 123 ± 0.0 127	0.495 ± 0.049
A5	0.9 013 ± 0.0 324	0.323 ± 0.053[b]
A10	0.8 636 ± 0.0 312	0.298 ± 0.044[b]
A15	0.8 073 ± 0.0 275[a]	0.305 ± 0.037[b]
A20	0.6 912 ± 0.0 283[b]	0.277 ± 0.034[b]
N100	0.8 590 ± 0.0 103	0.459 ± 0.052
N200	0.8 644 ± 0.0 276	0.426 ± 0.043
N400	0.8 543 ± 0.0 375	0.433 ± 0.027
N800	0.8 612 ± 0.0 251	0.417 ± 0.051

[a]$P<0.05$ *vs* DMSO组; [b]$P<0.01$ *vs* DMSO组.

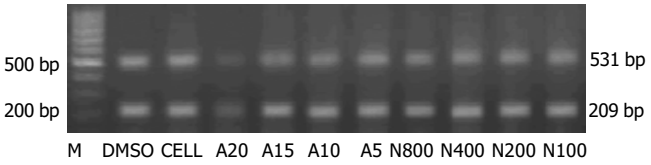

图3 EC-09细胞株药物干预结果电泳图. M: 标记物, 100 bp; 531 bp: COX-2产物; 209 bp: 内对照产物.

3 讨论

食管鳞癌流行病学地区分布差异显著和高发区明显家族聚集现象的突出特征，提示遗传因素在该病的发生中起重要作用. 研究表明,COX-2在食管鳞癌[1-3]等多种消化系统恶性肿瘤[4-5]中表达增高，与其发生和发展密切相关[6-7]，而且是食管鳞癌发生的早期事件[8]. 我们的研究结果为COX-2 mRNA在人类食管鳞癌中的表达情况提供了依据，即约54.5%的人类食管鳞癌存在COX-2 mRNA的表达,而在癌周正常鳞状上皮则未发现表达. 由于本研究标本例数较少,故还有待于扩大样本量,并在不同地区、不同人群中进行更为广泛深入的研究,才能得出COX-2 mRNA在人类食管鳞癌中表达的总体水平和相互差异. 目前研究认为COX-2的致癌机制主要有以下几方面: (1)促进细胞增生，抑制细胞凋亡: COX-2可降低细胞周期素D_1的水平，使细胞G_1期延长，无法进入分裂期，而持续增生，导致细胞发生突变，还可延长异常细胞生存期，拮抗各种刺激诱导的细胞凋亡，增加发生二次突变的机率[8-10]; (2)促进血管形成: 癌细胞在COX-2表达增高的同时，血管内皮生长因子、碱性成纤维细胞生长因子和内皮素-1等促血管生成因子均明显上调，而选择性COX-2抑制剂可显著抑制这些因子的表达[11-13]; (3)促进肿瘤细胞的浸润和转移: COX-2可直接上调肿瘤细胞基质金属蛋白酶-2(MMP-2)和尿激酶型纤溶酶原激活物(uPA)的表达，从而促进肿瘤细胞的浸润和转移[14-15]. 但COX-2的表达与食管鳞癌的发生、发展和预后的关系，仍有待进一步研究[16-18].

非甾体类抗炎药(non-steroidal anti-inflammatory drug，NSAID)通过抑制环氧化酶发挥抗炎作用. 选择性COX-2抑制剂则既能有效地治疗炎症，又能避免或减轻因抑制COX-1而引起的不良反应. 多项研究表明NSAID，特别是选择性COX-2抑制剂，对结肠癌具有防治作用[19-22]，甚至有学者认为对COX-2表达增高的Peutz-Jeghers综合征患者,进行预防性治疗是合理的[23]. 已有的研究亦表明NSAID，特别是选择性COX-2抑制剂，对食管鳞癌具有防治作用[24-26]. 至于NSAID抑制癌症的机制,目前研究认为,NSAID可通过抑制前列腺素合成起到保护p53抑制肿瘤的作用[27]; 抑制COX-2的表达和活性有助于恢复单核-吞噬细胞系统的活性和功能[28-29]; NSAID能够抑制癌细胞端粒酶的活性进而抑制其生长[30]. 在本研究中，非选择性COX-2抑制剂-阿司匹林对食管鳞癌细胞株EC-109和EC-9706的作用均较明显，而选择性COX-2抑制剂-尼美舒利对EC-109的作用不如对EC-9706的作用明显. 这一方面说明人类食管鳞癌的发病机制是多种因素共同作用的结果,另一方面也提示我们选择性COX-2抑制剂对人类食管鳞癌的预防和治疗作用是有限的. 可见，探求COX-2与人类食管鳞癌的关系以及NSAID对人类食管鳞癌的作用仍需要我们更为深入广泛的研究.

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