doi: 10.13241/j.cnki.pmb.2020.10.016

结直肠癌 COX-2 表达及其与临床病理特征的相关性分析 *

刘 芳[1] 周智辉[2] 王志斌[3△] 杜 静[1] 杨 昕[1] 封 茉[1]

（1 陕西中医药大学第二附属医院病理科 陕西 咸阳 712000；2 陕西中医药大学第二附属医院科研科 陕西 咸阳 712000；
3 陕西省核工业二一五医院肿瘤科 陕西 咸阳 712009）

摘要 目的：探讨结直肠癌环氧化酶 -2(cyclooxygenase-2，COX-2)的表达及其与临床病理特征的相关性。**方法：**选择 2017 年 8 月~2019 年 6 月在本院外科手术诊治的结直肠癌患者 60 例，取所有患者的病灶组织标本与癌旁组织标本，采用 PCR 与免疫组化法检测 COX-2 mRNA 与蛋白表达情况，分析其与患者的临床病理特征的相关性。**结果：**直肠癌组织 COX-2 mRNA 与蛋白表达阳性率分别为 63.3 %和 55.0 %，显著高于癌旁组织的 20.0 %和 16.7 %(P<0.05)。随着结直肠癌的病理分期及分化程度的增加、淋巴结转移的发生，直肠癌组织的 COX-2 mRNA、蛋白表达阳性率显著升高(P<0.05)。Spearman 等级相关分析显示直肠癌组织的 COX-2 mRNA、蛋白表达阳性率与临床分期、组织学分化与淋巴结转移都存在显著相关性(P<0.05)。Cox 模型多因素分析显示临床分期、组织学分化与淋巴结转移都是影响 COX-2 蛋白表达的主要因素(P<0.05)。**结论：**结直肠癌 COX-2 的 mRNA 与蛋白都呈现高表达，与患者的临床分期、组织学分化与淋巴结转移显著相关。

关键词：结直肠癌；环氧化酶 -2；临床病理特征；相关性

中图分类号：R735.3 **文献标识码：**A **文章编号：**1673-6273(2020)10-1873-04

Correlation of COX-2 Expression with the Clinicopathological Features of Colorectal Cancer*

LIU Fang[1], ZHOU Zhi-hui[2], WANG Zhi-bin[3△], DU Jing[1], YANG Xin[1], FENG Mo[1]

(1 Department of Pathology, the Second Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine, Xianyang, Shaanxi, 712000, China; 2 Department of Scientific Research, the Second Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine, Xianyang, Shaanxi, 712000, China;

3 Department of Oncology, No.215 Hospital of Shaanxi Nuclear Industry, Xianyang, Shaanxi, 712000, China)

ABSTRACT Objective: To investigate the expression of cyclooxygenase-2 (COX-2) and its correlation with the clinicopathological features of colorectal cancer. **Methods:** From August 2017 to June 2019, 60 patients with colorectal cancer who underwent surgery in our hospital were enrolled. The specimens of the lesions and adjacent tissues were collected from all patients. The expression of mRNA and protein of COX-2 were detected by PCR and immunohistochemistry, the clinicopathological features of the patients were investigated and were given correlation analysis. **Results:** The positive rates of COX-2 mRNA and protein expression in lesion tissues were 63.3% and 55.0%, respectively, which were significantly higher than those in adjacent tissues (20.0% and 16.7%)(P<0.05). With the increased of pathological stage, degree of differentiation and lymph node metastasis of colorectal cancer, the positive rates of COX-2 mRNA and protein expression in lesion tissues were increased significantly (P<0.05). Spearman rank correlation analysis showed that the positive rate of COX-2 mRNA and protein expression in the lesion tissues were correlated with clinical stage, histological differentiation and lymph node metastasis (P<0.05). Multivariate analysis of Cox model showed that clinical stage, histological differentiation and lymph node metastasis were the main factors affectedCOX-2 protein expression (P<0.05). **Conclusion:** The mRNA and protein of COX-2 in colorectal cancer are highly expressed, which aresignificantly correlated with clinical stage, histological differentiation and lymph node metastasis.

Key words: Colorectal cancer; Cyclooxygenase-2; Clinicopathological features; Correlation

Chinese Library Classification(CLC): R735.3 Document code: A

Article ID: 1673-6273(2020)10-1873-04

前言

结直肠癌在我国的发病率和死亡率的都比较高，是导致癌症相关死亡的主要原因之一，也是影响我国健康的重大常见疾病[1,2]。该病患者的 5 年生存率一般不超过 20 %，为此需要有效与特异的生物标志物以早期发现结直肠癌以及评估患者的预后[3,4]。结直肠癌的致病原因复杂，有多种因素参与，目前没有统一的定论[5]。随着分子生物学和遗传学的不断进步，该病在分子

* 基金项目：陕西省教育厅科研基金项目(18JK0218)

作者简介：刘芳(1982-)，女，本科，主治医师，研究方向：胃肠肿瘤病理，电话：13649102109，E-mail：Wangzhibin19800530@163.com

△ 通讯作者：王志斌(1980-)，男，本科，主治医师，研究方向：肿瘤外科，电话：18691060817，E-mail：23715333@qq.com

(收稿日期：2019-09-22 接受日期：2019-10-18)

水平致癌机制研究也越来越深入[6,7]。结直肠癌的发生与发展离不开肿瘤微环境，组织学分化、临床分期、淋巴结转移等病理特征是影响结直肠癌预后的最主要因素之一[8,9]。在癌基因的研究中，抑癌基因的突变失活能够导致许多癌基因的异常激活、过度表达，可能是结直肠癌发生的分子生物学基础[10,11]。

环氧化酶 -2(cyclooxygenase-2，COX-2)的 mRNA 表达和蛋白水平在体内保持稳定，参与前列腺素合成与维持人体正常功能，有炎症和肿瘤发生时，其表达将会升高[12]。现有的研究发现，COX-2 在正常分化组织几乎不表达，而在结直肠癌多种肿瘤组织中特异性表达，因此 COX-2 在结直肠癌中的表达及其与临床分期、组织学分级、淋巴转移等因素密切相关[13,14]。因此，本研究具体分析了结直肠癌 COX-2 表达及其与患者临床病理特征的相关性，以明确 COX-2 在结直肠癌发生、发展中的作用，为结直肠癌的诊治提供参考依据。现将结果总结报道如下。

1 资料与方法

1.1 研究对象

选择 2017 年 8 月到 2019 年 6 月在本院外科手术诊治的结直肠癌患者 60 例，纳入标准：术前均未行放化疗及免疫治疗；术后病理证实为结直肠癌；既往没有进行其他损伤肠系膜的手术史；临床资料完整；患者知情同意本研究且得到医院伦理委员会的批准。排除标准：临床与病理资料缺乏者；标本资料无法进行免疫组化分析与 PCR 分析者。其中，男 35 例，女 25 例；年龄最小 28 岁，最大 57 岁，平均年龄(48.23± 4.11)岁；临床分期：Ⅰ期 34 例，Ⅱ期 20 例，Ⅲ期 6 例；分化程度：高分化 21 例、中分化 19 例、低分化 20 例；淋巴结转移：无转移 45 例，有转移 15 例；病理类型：鳞癌 20 例，腺癌 40 例。

1.2 标本采集

取所有患者的病灶组织标本与癌旁组织标本 (距肿瘤边缘≥ 5 cm 正常结直肠黏膜)。所有样本组织均经 10 %福尔马林固定，常规脱水、浸蜡、包埋，切成 4 μm 厚度切片，然后平分为两份，-80 ℃冰箱冻存。

1.3 COX-2 的表达检测

一份标本采用免疫组化法检测 COX-2 蛋白表达情况，采用 3 %的 H_2O_2 室温孵育 10 min 去除过氧化物酶，微波炉中高温抗原修复 10 min。二甲苯脱蜡后，进行免疫组织化学染色，滴加抗 COX-2 单克隆抗体(稀释度 1:200，北京中杉金桥生物技术有限公司)，阴性对照以 PBS 代替一抗，4 ℃过夜，用 PBS 冲洗 3 次，每次 5 min，后续步骤按照说明书完成，封片镜检。以两名病理科医生判断为阳性作为标准。

另一份标本采用 PCR 检测 COX-2 mRNA 表达情况，按 RNA 抽提试剂盒说明书提取标本组织的总 RNA，以 qPCR 一步法试剂盒(TAKARA OneStep RNA PCR kit)加入外套特异性引物扩增 COX-2 mRNA，内对照为β-actin。PCR 步骤：40 个循环，94℃变性 45 s，57℃退火 5 min，72℃延伸 45 s。COX-2 上游引物序列为 5"-GCCAGTAGCACTCACCATAGCTCG-3"，下游引物序列为 5"-GAC CCAAATGTCGCAGTCAG-3"；β-actin 上游引物序列为 5"-AATCCCATCACCATCCCCA-3"，下游引物序列为 5"TCACAGCACTTCTCCCCAGTTGTCT"。以 Ct 值≤ 27 判断为阳性。

1.4 资料调查

调查患者的性别、年龄、病理分期、分化程度等病理特征。

1.5 统计学分析

应用 SPSS 22.00 对数据进行统计学分析，计量数据以均数± 标准差表示，组间比较采用 t 检验；计数数据采用百分比、率表示，组间比较行 x^2 检验，相关性分析采用 Spearman 等级相关分析与 Cox 模型多因素分析，以 P<0.05 为差异有统计学意义。

2 结果

2.1 结直肠癌 COX-2 mRNA 与蛋白表达

结直肠癌病灶组织的 COX-2 mRNA 与蛋白表达阳性率分别为 63.3 %和 55.0 %，显著高于癌旁组织 (20.0 %和 16.7 %，P<0.05)。见表 1。

表 1 不同组织的 COX-2 mRNA 与蛋白表达阳性率对比(例，%)

Table 1 Comparison of the positive rate of COX-2 mRNA and protein expression between different tissues (n, %)

Tissue	n	Positive expression rate of mRNA	Protein expression positive rate
Lesion tissue	60	38(63.3%)*	33(55.0%)*
Paracancer tissue	60	12(20.0%)	10(16.7%)

Note: Compared with the paracancer, *P<0.05.

2.2 COX-2 mRNA、蛋白表达与结直肠癌临床病理特征的关系

随着结直肠癌的病理分期、分化程度的增加、淋巴结转移的发生，结直肠癌病灶组织的 COX-2 mRNA、蛋白表达阳性率显著升高(P<0.05)。见表 2。

2.3 相关性分析

在 60 例患者中，Spearman 等级相关分析显示结直肠癌病灶组织的 COX-2 mRNA、蛋白表达阳性率与临床分期、组织学分化与淋巴结转移都存在显著相关性(P<0.05)。见表 3。以病灶组织的 COX-2 蛋白表达阳性作为因变量，以临床分期、组织学分化与淋巴结转移作为自变量，Cox 模型多因素分析显示临床分期、组织学分化与淋巴结转移都是影响 COX-2 蛋白表达的主要因素(P<0.05)。见表 4。

3 讨论

结直肠癌约占所有恶性肿瘤 10 %左右，随着筛查技术的普及，近年来年轻人群结直肠癌的发病率在逐渐增加[15]。早期发现的结直肠癌患者预后较好，但多数患者发现已处于晚期，预后差，当其发生转移时，无法彻底根治，预后效果差[16]。为此需要进行合理的判断分期、评估高危因素，从而为结直肠癌患者制定个体化的治疗方案提供参考。

表 2 COX-2 mRNA、蛋白表达与结直肠癌的临床病理特征关系(例,%)

Table 2 Relationship between COX-2 mRNA and protein expression and clinicopathological features of colorectal cancer (n, %)

Pathological features	n	Positive expression rate of mRNA(n=38)	x^2	*P*	Protein expression positive rate(n=33)	x^2	*P*
Pathological staging-Ⅰ	34	16(47.1%)	9.744	0.008	16(47.1%)	4.014	0.045
Ⅱ	20	16(80.0%)			12(60.0%)		
Ⅲ	6	6(100.0%)			5(83.3%)		
Degree of differentiation - poor differentiation	21	10(47.6%)	4.626	0.041	8(38.1%)	6.244	0.044
Medium differentiation	19	12(63.2%)			10(52.6%)		
Highly differentiated	20	16(80.0%)			15(75.0%)		
Lymph node metastasis No	45	26(57.8%)	5.896	0.031	20(44.4%)	8.103	0.004
Yes	15	12(80.0%)			13(86.7%)		

表 3 COX-2 mRNA、蛋白表达与结直肠癌临床病理特征的相关性(n=60)

Table 3 Correlation between COX-2 mRNA and protein expression and clinicopathological features of colorectal cancer (n=60)

Index	Clinical stage	Histological differentiation	Lymph node metastasis
COX-2 mRNA-r	0.542	0.613	0.557
P	0.003	0.000	0.002
COX-2 protein-r	0.489	0.592	0.611
P	0.008	0.000	0.000

表 4 影响结直肠癌患者 COX-2 蛋白表达阳性的主要因素(n=60)

Table 4 Main factors affecting COX-2 protein expression in colorectal cancer patients (n=60)

Index	β	SE	Wald	*P*	OR(95%CI)
Clinical stage	1.862	0.524	12.556	0.000	10.593(4.986-14.591)
Histological differentiation	1.811	0.683	6.982	0.008	6.194(2.533-8.145)
Lymph node metastasis	1.498	0.561	6.093	0.014	3.255(2.093-4.781)

当前已有研究从从原癌基因激活、抑癌基因突变、细胞异常增殖中分析结直肠癌的发生与发展机制,目前多数研究仅局限于单一指标与单一维度,导致分析结果存在偏倚性。COX-2 能够促进肿瘤细胞增殖、增强侵袭转移能力,抑制机体的抗肿瘤免疫反应,在肿瘤组织和转移灶的血管上均表达[17,18]。COX-2 还可以刺激肿瘤细胞释放前列腺素,促使内皮细胞生长,诱导血管生成;并且通过上调影响血管生成的因子 VEGF 等诱导肿瘤血管形成[19,20]。本研究显示结直肠癌病灶组织的 COX-2 mRNA 与蛋白表达显著高于癌旁组织,提示其可能参与结直肠癌的发生和发展。

结直肠癌的总体预后随着外科治疗手段的不断创新,取得了很大的改善。但是多数患者术后复发,影响预后[21,22]。结直肠癌的转移由一系列复杂步骤形成,涉及肿瘤细胞的黏附改变、细胞增殖改变、肿瘤细胞的迁移等过程[24]。淋巴结微转移是一般指常规检测手段(临床体检、病理检查、影像学)不能发现的肿瘤转移灶,可为临床分期、辅助治疗、预后判断提供更多的依据[23]。本研究显示随着结直肠癌的病理分期、分化程度的增加、淋巴结转移的发生,病灶组织的 COX-2 mRNA、蛋白表达阳性率显著上升。Spearman 等级相关分析显示病灶组织的 COX-2 mRNA、蛋白阳性率与临床分期、组织分化与淋巴结转移有一定的关系;Cox 模型多因素分析显示临床分期、组织学分化与淋巴结转移都是影响 COX-2 蛋白表达的主要因素。从机制上分析,COX-2 与缺氧诱导肿瘤血管、淋巴管的生成相关,而恶性肿瘤的侵袭、转移与肿瘤血管、淋巴管的生成及 COX-2 的失调密切相关[25,26]。COX-2 也可通过多种方式影响肿瘤细胞黏附分子的表达,参与细胞与基质间的黏附和细胞伪足的形成,进而调节细胞骨架结构,促进细胞侵袭和转移[27,28]。而更多的抑癌基因失活,癌基因被激活,加之外界刺激,共同加快了结直肠癌的发生、发展[29,30]。本研究也存在一定的不足,选择病例数少,统计分析可能存在一定的偏移。同时,随访研究 3 年,患者的预后情况还没有得到全部的数据,将在后续不断的追踪,进一步分析其影响因素。

总之,结直肠癌 COX-2 的 mRNA 与蛋白都呈现高表达,与患者的临床分期、组织学分化与淋巴结转移显著相关,后三者也是影响 COX-2 蛋白表达的主要因素。

参 考 文 献(References)

[1] Arisan ED, Ergul Z, Bozdag G, et al. Diclofenac induced apoptosis via altering PI3K/Akt/MAPK signaling axis in HCT 116 more efficiently compared to SW480 colon cancer cells[J]. Mol Biol Rep, 2018, 45(6): 2175-2184

[2] Arya M, Singh P, Tripathi CB, et al. Pectin-encrusted gold nanocomposites containing phytic acid and jacalin: 1,2-dimethylhydrazine-induced colon carcinogenesis in Wistar rats, PI3K/Akt, COX-2, and serum metabolomics as potential targets [J]. Drug Deliv Transl Res, 2019, 9(1): 53-65

[3] Bajpai M, Seril DN, Van Gurp J, et al. Effect of Long-Term Mesalamine Therapy on Cancer-Associated Gene Expression in Colonic Mucosa of Patients with Ulcerative Colitis [J]. Dig Dis Sci, 2019, 64(3): 740-750

[4] Buzharevski A, Paskas S, Sarosi MB, et al. Carboranyl Analogues of Celecoxib with Potent Cytostatic Activity against Human Melanoma and Colon Cancer Cell Lines [J]. ChemMedChem, 2019, 14 (3): 315-321

[5] Cai J, Huang L, Huang J, et al. Associations between the cyclooxygenase-2 expression in circulating tumor cells and the clinicopathological features of patients with colorectal cancer [J]. J Cell Biochem, 2019, 120(4): 4935-4941

[6] Fiegle E, Doleschel D, Koletnik S, et al. Dual CTLA-4 and PD-L1 Blockade Inhibits Tumor Growth and Liver Metastasis in a Highly Aggressive Orthotopic Mouse Model of Colon Cancer [J]. Neoplasia, 2019, 21(9): 932-944

[7] Gaballah HH, Gaber RA, Elrashidy MA, et al. Expression of long non-coding RNA CCHE1 in colorectal carcinoma: correlations with clinicopathological features and ERK/COX-2 pathway [J]. Mol Biol Rep, 2019, 46(1): 657-667

[8] Ganaie MA, Al Saeedan A, Madhkali H, et al. Chemopreventive efficacy zingerone (4-[4-hydroxy-3-methylphenyl] butan-2-one) in experimental colon carcinogenesis in Wistar rats[J]. Environ Toxicol, 2019, 34(5): 610-625

[9] Garcia VB, De Carvalho TG, Da Silva Gasparotto LH, et al. Environmentally compatible bioconjugated gold nanoparticles as efficient contrast agents for inflammation-induced cancer imaging [J]. Nanoscale Res Lett, 2019, 14(1): 166-184

[10] He P, Yang C, Ye G, et al. Risks of colorectal neoplasms and cardiovascular thromboembolic events after the combined use of selective COX-2 inhibitors and aspirin with 5-year follow-up: a meta-analysis [J]. Colorectal Dis, 2019, 21(4): 417-426

[11] Huang H, Aladelokun O, Ideta T, et al. Inhibition of PGE2/EP4 receptor signaling enhances oxaliplatin efficacy in resistant colon cancer cells through modulation of oxidative stress [J]. Sci Rep, 2019, 9 (1): 4954-4972

[12] Jaen RI, Prieto P, Casado M, et al. Post-translational modifications of prostaglandin-endoperoxide synthase 2 in colorectal cancer: An update[J]. World J Gastroenterol, 2018, 24(48): 5454-5461

[13] Jeong JW, Park C, Cha HJ, et al. Cordycepin inhibits lipopolysaccharide-induced cell migration and invasion in human colorectal carcinoma HCT-116 cells through down-regulation of prostaglandin E2 receptor EP4[J]. BMB Rep, 2018, 51(10): 532-537

[14] Khan R, Rehman MU, Khan AQ, et al. Glycyrrhizic acid suppresses 1,2-dimethylhydrazine-induced colon tumorigenesis in Wistar rats: Alleviation of inflammatory, proliferation, angiogenic, and apoptotic markers[J]. Environ Toxicol, 2018, 33(12): 1272-1283

[15] Kojima Y, Kondo Y, Fujishita T, et al. Stromal iodothyronine deiodinase 2 (DIO2) promotes the growth of intestinal tumors in Apc (Delta716) mutant mice[J]. Cancer Sci, 2019, 110(8): 2520-2528

[16] Kosumi K, Hamada T, Zhang S, et al. Prognostic association of PTGS2 (COX-2) over-expression according to BRAF mutation status in colorectal cancer: Results from two prospective cohorts and CALGB 89803 (Alliance) trial[J]. Eur J Cancer, 2019, 111: 82-93

[17] Li B, Wang Y, Yin L, et al. Glucocorticoids promote the development of azoxymethane and dextran sulfate sodium-induced colorectal carcinoma in mice[J]. BMC Cancer, 2019, 19(1): 94-112

[18] Lin YM, Lu CC, Hsiang YP, et al. c-Met inhibition is required for the celecoxib-attenuated stemness property of human colorectal cancer cells[J]. J Cell Physiol, 2019, 234(7): 10336-10344

[19] Mantso T, Vasileiadis S, Lampri E, et al. Hyperthermia Suppresses Post - In Vitro Proliferation and Tumor Growth in Murine Malignant Melanoma and Colon Carcinoma [J]. Anticancer Res, 2019, 39 (5): 2307-2315

[20] Monteleone NJ, Moore AE, Iacona JR, et al. miR-21-mediated regulation of 15-hydroxyprostaglandin dehydrogenase in colon cancer[J]. Sci Rep, 2019, 9(1): 5405-5425

[21] Naji S, Issa K, Eid A, et al. Cadmium Induces Migration of Colon Cancer Cells: Roles of Reactive Oxygen Species, P38 and Cyclooxygenase-2[J]. Cell Physiol Biochem, 2019, 52(6): 1517-1534

[22] Negi RR, Rana SV, Gupta V, et al. Over-Expression of Cyclooxygenase-2 in Colorectal Cancer Patients [J]. Asian Pac J Cancer Prev, 2019, 20(6): 1675-1681

[23] Rodriguez-Miguel A, Garcia-Rodriguez LA, Gil M, et al. Population-based case-control study: chemoprotection of colorectal cancer with non-aspirin nonsteroidal anti-inflammatory drugs and other drugs for pain control [J]. Aliment Pharmacol Ther, 2019, 50 (3): 295-305

[24] Soares PV, Kannen V, Jordao Junior AA, et al. Coffee, but Neither Decaffeinated Coffee nor Caffeine, Elicits Chemoprotection Against a Direct Carcinogen in the Colon of Wistar Rats[J]. Nutr Cancer, 2019, 71(4): 615-623

[25] Sui X, Guo Y, Ni W, et al. Molecular profiling analysis for colorectal cancer patients with Pi-Xu or Shi-Re syndrome [J]. Integr Med Res, 2019, 8(1): 21-25

[26] Tseng TH, Lin WL, Chang CK, et al. Protective Effects of Morus Root Extract (MRE) Against Lipopolysaccharide-Activated RAW264. 7 Cells and CCl4-Induced Mouse Hepatic Damage [J]. Cell Physiol Biochem, 2018, 51(3): 1376-1388

[27] Tupa V, Drahosova S, Grendar M, et al. Expression and association of carbonic anhydrase IX and cyclooxygenase-2 in colorectal cancer [J]. Pathol Res Pract, 2019, 215(4): 705-711

[28] Xie R, Yang Y, Zhang H, et al. c-Myb and its Effector COX-2 as an Indicator Associated with Prognosis and Therapeutic Outcome in Colorectal Cancer[J]. J Cancer, 2019, 10(7): 1601-1610

[29] Xu Y, Yang X, Gao D, et al. Dihomo-gamma-linolenic acid inhibits xenograft tumor growth in mice bearing shRNA-transfected HCA-7 cells targeting delta-5-desaturase [J]. BMC Cancer, 2018, 18 (1): 1268-1291

[30] Zhang Y, Weng Y, Gan H, et al. Streptococcus gallolyticus conspires myeloid cells to promote tumorigenesis of inflammatory bowel disease[J]. Biochem Biophys Res Commun, 2018, 506(4): 907-911