

DOI: 10.3872/j.issn.1007-385x.2019.05.012

· 临床研究 ·

环加氧酶-2通过调控EMT促进乳腺癌MDA-MB-231细胞的迁移和侵袭

谭林彦, 刘敏[△], 葛菲, 陈文林, 黄赛君, 李云茜, 叶优男, 王曦, 张勇(昆明医科大学第三附属医院暨云南省肿瘤医院 乳腺外一科, 云南昆明 650118)

[摘要] **目的:**探讨环加氧酶-2(COX-2)在乳腺癌转移中的作用及其可能的机制。**方法:**收集从2015年10月至2018年4月在云南省肿瘤医院接受乳腺切除术的患者中获得的原发乳腺癌组织和脑转移乳腺癌组织临床病理样本共45例,其中原发30例、脑转移15例。采用qPCR检测COX-2在原位乳腺癌和脑转移乳腺癌组织中的表达。将COX-2过表达重组病毒(LV6-COX2)或敲减COX-2重组病毒(LV3-COX2 shRNA1、LV3-COX2 shRNA2)感染人乳腺癌MDA-MB-231细胞并获得稳转细胞株后,CCK-8法检测COX-2表达对MDA-MB-231细胞增殖的影响,划痕实验和Transwell法检测对MDA-MB-231细胞迁移和侵袭的影响。qPCR和WB实验分析各组细胞中COX-2 mRNA和蛋白的表达水平,qPCR检测COX-2表达对MDA-MB-231细胞内EMT相关基因表达的影响。**结果:**COX-2表达水平在脑转移乳腺癌患者组织中显著高于原位乳腺癌组织($P<0.01$);并且与乳腺癌患者肿瘤TMN分期有关。成功构建稳定过表达/敲减COX-2的MDA-MB-231细胞株。过表达COX-2促进MDA-MB-231细胞的迁移和侵袭(均 $P<0.01$),同时显著提高MMP2、MMP1、N-cadherin和vimentin的表达(均 $P<0.01$),但对细胞增殖无明显影响;而沉默COX-2则有相反的作用,且可促进细胞增殖($P<0.05$)。**结论:**COX-2在脑转移乳腺癌组织中高表达,其可能通过调控EMT过程促进乳腺癌MDA-MB-231细胞的迁移和侵袭。

[关键词] 乳腺癌;MDA-MB-231细胞;环加氧酶-2;迁移;侵袭;上皮间质转化

[中图分类号] R737.9; R730.2 **[文献标识码]** A **[文章编号]** 1007-385X(2019)05-0557-06

Cyclo-oxygenase-2 promotes migration and invasion of breast cancer MDA-MB-231 cells by regulating EMT

TAN Linyan, LIU Min[△], GE Fei, CHEN Wenlin, HUANG Saijun, LI Yunqian, YE Younan, WANG Xi, ZHANG Yong (First Department of Breast Surgery, the Third Affiliated Hospital of Kunming Medical University & Yunnan Cancer Hospital, Kunming 650118, Yunnan, China)

[Abstract] Objective: To investigate the role of cyclo-oxygenase-2 (COX-2) in breast cancer metastasis and its possible mechanism. **Methods:** A total of 45 cases of primary breast cancer tissues and brain metastatic breast cancer tissues were collected from patients, who underwent mastectomy in Yunnan Cancer Hospital from October 2015 to April 2018, including 30 cases of primary lesions and 15 cases of brain metastasis. qPCR was used to detect the expression of COX-2 in breast cancer tissues and brain metastatic breast cancer tissues. Recombinant viruses with COX-2 over-expression (LV6-COX2) or COX-2 knockdown (LV3-COX2 shRNA1, LV3-COX2 shRNA2) were transfected into human breast cancer MDA-MB-231 cells; After obtaining the stable expression cell lines, the effect of COX-2 expression on the proliferation of MDA-MB-231 cells was detected by CCK-8, and the effects of COX-2 expression on the migration and invasion of MDA-MB-231 cells were detected by scratch test and Transwell assay, respectively. The mRNA and protein expressions of COX-2 in each group were examined by qPCR and WB, respectively. The effect of COX-2 expression on the expression of EMT-related genes in MDA-MB-231 cells was analyzed by qPCR. **Results:** The expression of COX-2 in tissues of patients with brain metastases was significantly higher than that in patients with primary breast cancer tissues ($P<0.01$), and it was correlated with tumor TMN stage in breast cancer patients. MDA-MB-231 cell lines with stable COX-2 over-expression/knockout were successfully construct-

[基金项目] 国家自然科学基金地区项目资助(No.81660437, No.81660438);云南省医疗卫生单位内设研究机构项目资助(No.2014N5021);国家留学基金委西部人才项目资助(No.201608535050)。Project supported by the Regional Project of National Natural Science Foundation of China(No. 81660437, No.81660438), the Project of Internal Institutions of Medical and Health Units from Yunnan(No.2014N5021), and the Western Talents Project of the State Overseas Study Fund Committee of China(No.201608535050)

[作者简介] 谭林彦(1993-),女,硕士生,主要从事乳腺癌临床研究,E-mail:1325421224@qq.com;刘敏(1991-),女,硕士生,主要从事乳腺癌基础研究,E-mail:xixo816@sina.com。[△]为共同第一作者

[通信作者] 陈文林(CHEN Wenlin, corresponding author),硕士,副教授,硕士生导师,主要从事三阴性乳腺癌发病机制及治疗的研究,E-mail:chenwenlin@aliyun.com;葛菲(GE Fei,co-corresponding author),主治医师,主要从事乳腺癌发生发展的分子机制研究,E-mail:ajqnadjd@hotmail.com

ed. Over-expression of COX-2 promoted the migration and invasion of MDA-MB-231 cells (all $P < 0.01$), and significantly increased the expressions of MMP2, MMP1, N-cadherin and vimentin (all $P < 0.01$), but exerted insignificant effect on cell proliferation. The effect of COX-2 silence exerted the opposite effect and promoted cell proliferation ($P < 0.05$). **Conclusion:** COX-2 is highly expressed in brain metastatic breast cancer tissues, which may promote the migration and invasion of breast cancer MDA-MB-231 cells by regulating EMT processes.

[Key words] breast cancer; MDA-MB-231 cell; cyclo-oxygenase-2 (COX-2); migration; invasion; EMT

[Chin J Cancer Biother, 2019, 26(5): 557-562. DOI: 10.3872/j.issn.1007-385X.2019.05.012]

乳腺癌是世界范围内妇女最常见的恶性肿瘤之一^[1],其中癌细胞的转移和不受控制的增殖是导致患者死亡的主要原因。因此,了解乳腺癌进展的分子机制,开发适合关键靶点的药物,对于预防和治疗乳腺癌具有重要作用。环加氧酶-2(cyclo-oxygenase-2, COX-2)可以诱导血管生成,并且与肿瘤的生长、侵袭和转移有关^[2-8],在预后不良的乳腺癌中常见COX-2表达升高。目前,在人类结直肠癌^[2,9]、头颈部癌^[10]、子宫内膜癌^[11]、乳腺癌^[12]和肝癌^[13]中证实COX-2参与了肿瘤的血管生成和疾病进展,其机制可能是COX-2通过VEGF途径促进血管生成。但COX-2导致乳腺癌患者预后不良的机制还没有完全阐明。本研究旨在探索COX-2在乳腺癌细胞增殖、迁移和侵袭中的作用和可能的机制。

1 材料与方法

1.1 临床资料

收集从2015年10月至2018年4月在云南省肿瘤医院接受乳腺切除术的患者中获得的原发乳腺癌组织和脑转移乳腺癌组织临床病理样本共45例,其中原发30例、脑转移15例。研究样本乳腺癌患者年龄40~55岁,中位年龄47.5岁;肿瘤组织直径1.5~4.5 cm;浸润性导管癌43例、浸润性小叶癌2例。没有患者在手术前接受化疗或放疗。本研究方案经医院伦理委员会批准,所有患者均签署知情同意书。所有样本均根据世界卫生组织分类进行病理诊断^[14]。

1.2 细胞培养和病毒感染

人乳腺癌MDA-MB-231细胞由中国科学院昆明动物研究所提供。COX-2过表达病毒和COX-2敲减病毒购自上海吉玛制药技术有限公司。COX-2敲减病毒靶向序列分别为COX-2 shRNA1: GGAAC-GTTGTGAATAACATTC, COX-2 shRNA2: GCTTTA-TGCTGAAGCCCTATG。MDA-MB-231细胞用含10% FBS(购自Cellmax公司)和1%双抗的DMEM高糖(购自Hyclone公司)培养基培养。细胞置于37 °C、5% CO₂的细胞培养箱中培养。将MDA-MB-231细胞按照 $1 \times 10^4/\text{cm}^2$ 的密度铺到6孔板里培养24 h。按病毒感染说明书操作,用5 μg/ml聚凝胺将COX-2过表达病毒(LV6-COX2)、阴性对照病毒(NC-LV6)、COX-2敲减病毒(LV3-COX2 shRNA1和LV3-COX2 shRNA2)和阴性对照病毒(NC-LV3)转染到相应的细胞中,通过抗药筛选获得稳转细胞株。

1.3 qPCR检测样本中目的基因表达水平

用TRNzol试剂(购自天根生化科技有限公司)从乳腺癌组织或细胞系中提取总RNA,用逆转录试剂盒FastQuant RT Kit (With gDNase)(购自天根生化科技有限公司)将2 μg mRNA逆转录成cDNA。用荧光定量试剂盒SuperReal PreMix Plus (SYBR Green)(购自天根生化科技有限公司)以cDNA为模板在Rotor-Gene Q荧光定量PCR仪(购自美国Qiagen公司)扩增目的基因。以 $2^{-\Delta\Delta Ct}$ 法计算目的基因相对表达量。所用引物见表1。

表1 qPCR引物
Tab. 1 Primers of qPCR

Gene	Forward Primer	Reverse Primer
COX-2	5'-ACTTTTGGTGGAGAAGTGGGTT-3'	5'-AGTGTGGGTGGGGATGAGTT-3'
MMP2	5'-GTGAAGTATGGGAACGCCGA-3'	5'-AGAAGCCGTACTTGCCATCC-3'
MMP1	5'-ACTCGGCCATTCTCTTGGAC-3'	5'-CGATGGGCTGGACAGGATTT-3'
N-cadherin	5'-ACCCTGGAGACATTGGGGAC-3'	5'-TGCTCACCACCCTACTTGA-3'
Vimentin	5'-GGACCAGCTAACCAACGACA-3'	5'-AAGGTCAAGACGTGCCAGAG-3'

1.4 CCK-8法检测COX-2表达对MDA-MB-231细胞增殖的影响

MDA-MB-231细胞转染LV6-COX2、NC-LV6、NC-LV3、LV3-COX2 shRNA1和LV3-COX2 shRNA2

病毒并获得稳转细胞株后,在96孔板中接种 3.0×10^3 个/孔,每组细胞设3个复孔。接种24、48、72、96 h后向每孔加入10 μl CCK-8试剂,37 °C孵育4 h。使用SpectraMax M5酶标仪(购自美国分子仪器公司)测定

波长在 450 nm 处的光密度(D)值,以 D 值代表细胞增殖水平。

1.5 划痕实验检测 COX-2 表达对 MDA-MB-231 细胞迁移的影响

转染相应病毒的各组细胞在 6 孔板中铺板,培养至 100% 汇合。汇合的单层细胞以 100 μ l 吸头垂直划线,再用 PBS 将划掉的细胞洗净,加入新鲜培养基,于 37 $^{\circ}$ C、5%CO₂ 培养箱培养。在 0 和 24 h 对划痕进行拍照,用 Image J 软件处理图像,通过对初始划线(0 h)和后期观察点(24 h)划痕的距离对比来计算划痕愈合率。

1.6 Transwell 侵袭实验检测 COX-2 表达对 MDA-MB-231 细胞侵袭的影响

各组细胞分别取 5×10^3 个,用无血清的 DMEM 高糖培养基重悬后接种到涂有基质凝胶的上部过滤器(购自美国 Corning 公司)中,下室加入 600 μ l 完全培养基培养液刺激迁移。于 5% CO₂ 培养箱 37 $^{\circ}$ C 培养 48 h 后,移除上室细胞。将细胞固定后用棉签擦拭清除上室中的细胞,对迁移到下室中的细胞进行结晶紫色,使用光学显微镜(购自日本尼康公司;放大倍数 400 倍)进行观察,随机取 5 个视野计数穿膜细胞,计算穿膜的相对细胞数量。

1.7 WB 实验检测细胞内目的蛋白的表达水平

用冷 PBS 洗涤乳腺癌细胞,用加入新鲜蛋白酶抑制剂(购自 Sigma 公司)的细胞裂解缓冲液(RIPA)在冰上裂解 30 min,离心后收集总蛋白,用 BCA 蛋白浓度检测试剂盒(购自天根生化科技有限公司)检测蛋白浓度。各组分别取 20 μ g 蛋白样品用 10% SDS-PAGE 分离不同相对分子质量的蛋白,然后电转移到 PVDF 膜上。用 5% 脱脂牛奶封闭,加入一抗:兔源 KIF26b 抗体(1:1 000,购自美国 Proteintech 公司)、兔源 COX-2 抗体(1:1 000,购自美国 CST 公司)、兔源 β -actin 抗体(1:1 000,购自美国 CST 公司)在 4 $^{\circ}$ C 孵育过夜,用 Peroxidase-conjugated 山羊抗兔 IgG(1:3 000,购自美国 CST 公司)二抗识别一抗后,用鲁米诺增强剂(购自美国 Bio-Rad 公司)化学发光检测条带的变化。

1.8 统计学处理

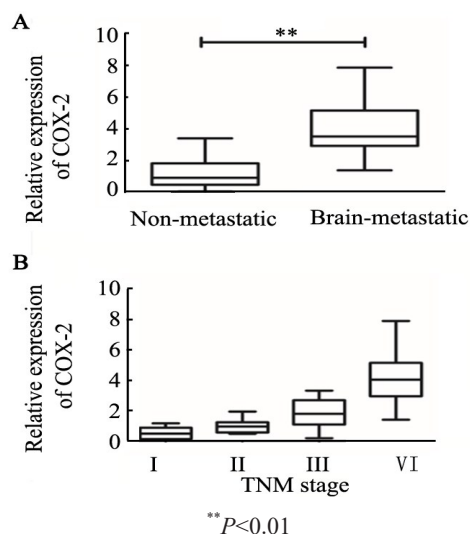
应用 SPSS19.0 软件,各组实验数据用 $\bar{x} \pm s$ 表示,两组间比较用 t 检验,以 $P < 0.05$ 或 $P < 0.01$ 表示差异具有统计学意义。

2 结果

2.1 COX-2 在脑转移乳腺癌组织中高表达

qPCR 检测结果显示,COX-2 在脑转移患者的乳腺癌组织中表达显著升高($P < 0.01$,图 1A)。此外,为进一步探讨 COX-2 的表达是否与 TNM 分期相关,将 45 例乳腺癌患者按 TNM 分期进行分组,分别为 I 期、II 期、III

期和 IV 期,分析不同 TNM 分期的乳腺癌患者肿瘤组织中的 COX-2 表达情况,结果显示,TNM 分期越高 COX-2 的表达水平也越高,但差异无统计学意义(图 1B)。



A: qPCR was used to detect the expressions of COX-2 in non-metastatic breast cancer ($n=30$) and brain metastatic breast cancer ($n=15$); B: COX-2 expressions in breast cancer tissues of patients with different TNM stages, I($n=9$), II($n=9$), III($n=8$), IV($n=19$)

图 1 COX-2 在原位及脑转移乳腺癌组织中的表达情况

Fig. 1 Expressions of COX-2 in non-metastatic and brain-metastatic breast cancer tissues

2.2 COX-2 影响 MDA-MB-231 细胞的增殖

WB 检测结果(图 2A)显示,感染 LV6-COX2 病毒的 MDA-MB-231 细胞中 COX-2 的蛋白表达水平显著上调($P < 0.05$),而感染 LV3-COX2 siRNA1 和 LV3-COX2 siRNA2 能显著下调 COX-2 蛋白水平的表达(均 $P < 0.01$),并且 LV3-COX2 siRNA1 对 COX-2 的敲减效果优于 LV3-COX2 siRNA2。另一方面,qPCR 在 mRNA 水平验证了 COX-2 的过表达水平和敲减效率,其结果与蛋白表达水平一致(图 2B)。

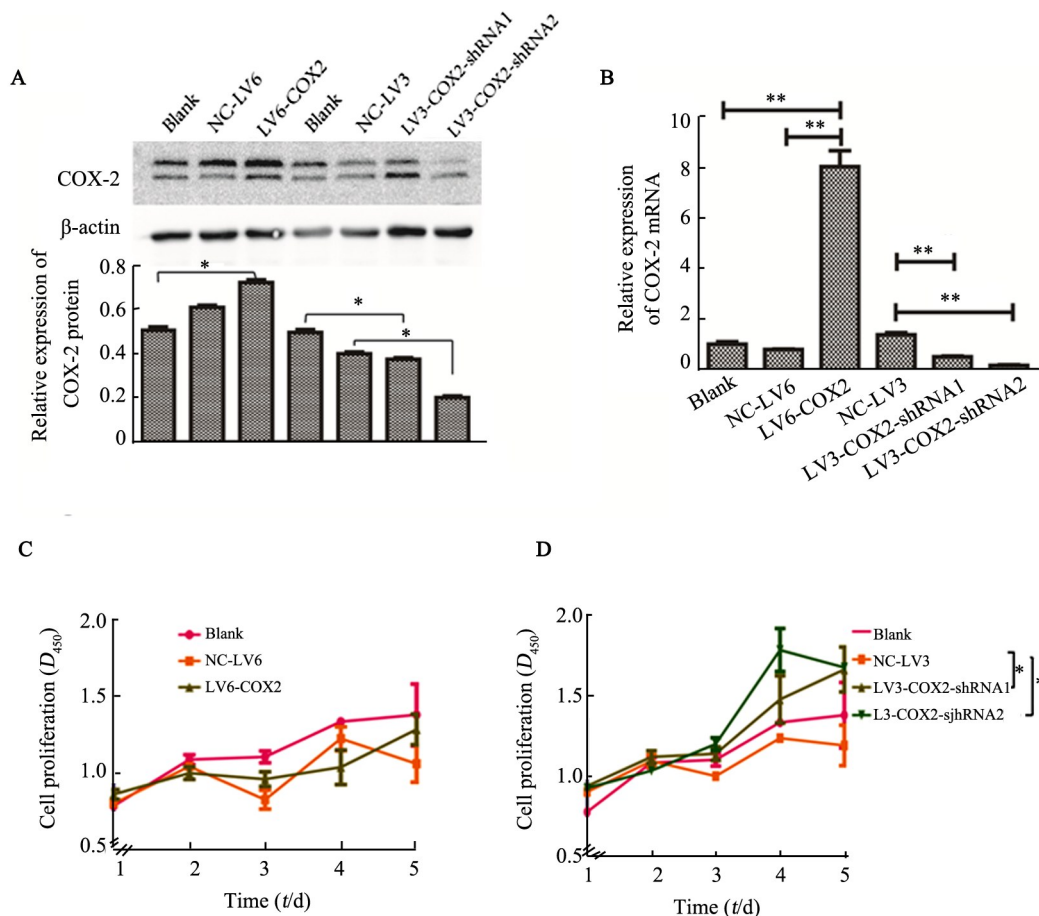
CCK-8 法检测结果(图 2C、D)显示,COX-2 过表达对细胞增殖没有明显影响,但敲减 COX-2 却能促进细胞增殖(均 $P < 0.05$)。

2.3 COX-2 过表达促进 MDA-MB-231 细胞的侵袭和转移

划痕实验结果(图 3A)显示,LV6-COX2 过表达组细胞划痕愈合率显著高于空白对照组[(51.3 \pm 4.6)% vs (32.7 \pm 5.5)%, $P < 0.05$],LV3-COX2-shRNA2 组划痕愈合率显著低于 LV3 组[(25.4 \pm 0.7)% vs (40.3 \pm 8.2)%, $P < 0.05$]。

Transwell 侵袭实验结果(图 3B)显示,LV6-COX2 过表达组相对侵袭比显著高于 LV6 对照组(3.475 \pm 0.197 vs 1.039 \pm 0.010, $P < 0.01$),干扰细胞内 COX-2 表达的 LV3-COX2-shRNA1 组、LV3-COX2-

shRNA2 组侵袭比显著低于 LV3 组 (0.438 ± 0.079 、 0.342 ± 0.021 vs 0.907 ± 0.079 , 均 $P < 0.01$)。



* $P < 0.05$, ** $P < 0.01$

A: COX-2 protein expression in cells infected with different viruses was detected by WB, β -Actin was used as internal reference; B: qPCR was used to verify the expression of COX-2 mRNA in cells infected with COX-2 overexpression virus and knockout virus; C: CCK-8 was used to detect the effect of COX-2 overexpression on the proliferation of breast cancer cells; D: CCK-8 was used to detect the effect of COX-2 knockdown on the proliferation of breast cancer cells. The significant difference analysis is the comparison of growth curves of different groups, that is, the difference of overall data at all time points

图2 COX-2 过表达或敲减对MDA-MB-231 细胞增殖活性的影响

Fig. 2 Effects of COX-2 over-expression or knockdown on the proliferation activity of MDA-MB-231 cells

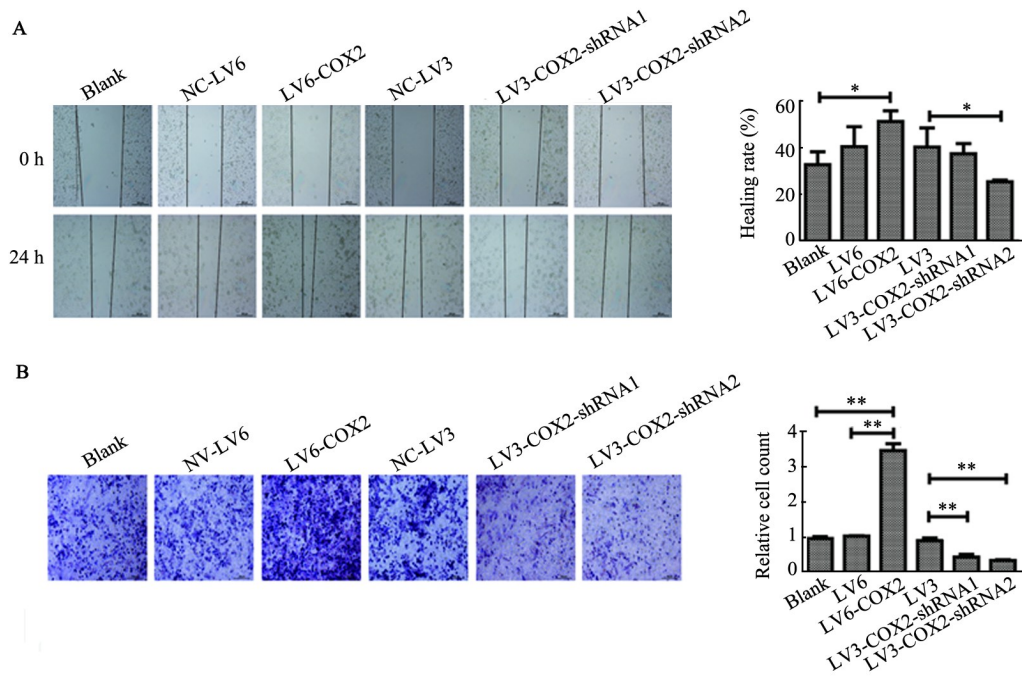
2.4 COX-2 可调控 EMT 相关基因的表达

qPCR 检测结果(图4)显示, LV6-COX-2 过表达组中 MMP2、MMP1、N-cadherin 和 vimentin 的表达显著高于其余 5 组(均 $P < 0.01$), 而 LV3-COX2-shRNA1 组、LV3-COX2-shRNA2 组细胞中 N-cadherin 和 vimentin 的表达水平显著低于 LV3 组(均 $P < 0.01$)。

3 讨论

乳腺癌的发生和转移是涉及基因表达、信号通路和表观遗传学变化的复杂过程^[15-16], 基因表达的异常变化导致正常生物活性的变化, 可能导致细胞的恶性转化, 核心基因的异常表达通常导致细胞生长、分化、凋亡和迁移的失调。因此, 寻找乳腺癌异常表达的功能重要基因是研究乳腺癌发生机制的重要途

径。TANG 等^[17]发现, COX-2 在肝细胞癌中高度表达, 其可通过上调 VEGF 来增强肿瘤血管生成, 进而促进癌细胞的侵袭。VAN REES 等^[18]发现, COX-2 的表达与胃肠道腺癌的晚期、分化程度低、生存率低有关。值得注意的是, 有研究^[19]发现 COX-2 在乳腺癌中高表达, 并且 COX-2 阳性与乳腺癌侵袭性类型的几个参数相关, 如肿瘤体积大、腋窝淋巴结转移、组织学分级高、激素受体阴性、高增殖率、高 p53 表达、HER-2 扩增等。本研究发现, COX-2 在乳腺癌脑转移组织中表达更高, 并且 COX-2 过表达显著促进乳腺癌细胞的迁移和侵袭, 而 COX-2 敲低在体外有相反的结果, 并且影响细胞增殖。上述结果表明, COX-2 是乳腺癌中一个重要的致癌基因, 它可以调节肿瘤细胞的恶性生物学行为。

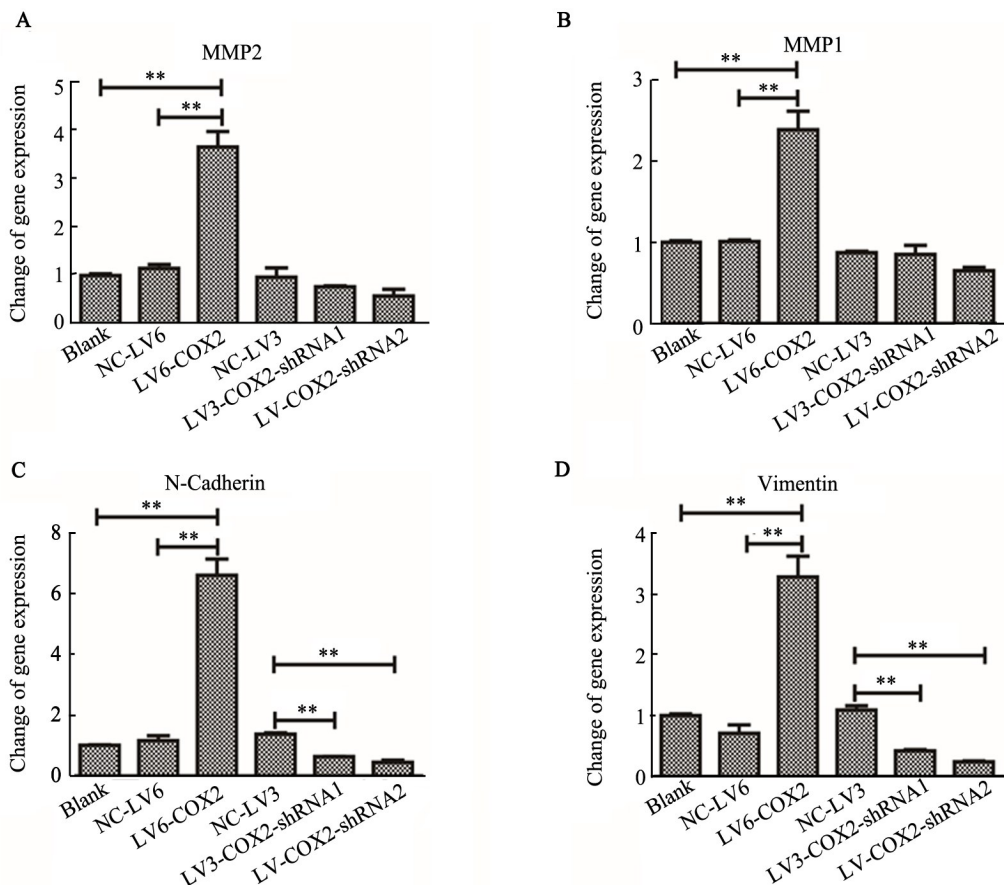


*P<0.05, **P<0.01

A: Cell scratch test and repair area statistics 24 h after scratch; B: Number of cells transferred to the bottom of Transwell after 24 h and the relative number of cells in each group (×200)

图3 COX-2对乳腺癌MDA-MB-231细胞迁移和侵袭的影响

Fig. 3 Effect of COX-2 on the migration and invasion of breast cancer MDA-MB-231 cells



**P<0.01

图4 COX-2过表达和COX-2敲减的MDA-MB-231细胞中EMT相关基因的表达情况

Fig. 4 Expressions of EMT-related genes in MDA-MB-231 cells with COX-2 over-expression/knockdown

乳腺癌患者病死的主要原因是乳腺癌细胞的转移和不受控制的增殖。其中,脑是乳腺癌转移的主要部位之一,有研究^[20]证实 COX-2 在脑转移细胞中高度上调,并且 COX-2 诱导的前列腺素能够直接促进 MMP1 的表达,随后增强脑转移。之前的报道^[21-22]也表明,COX-2 能够刺激血管生成,与肿瘤的生长、侵袭和转移有关。本研究对 EMT 相关基因(MMP2、MMP1、N-cadherin 和 vimentin 等)进行基因表达检测,发现 COX-2 过表达促进乳腺癌细胞中 MMP2、MMP1、N-cadherin 和 vimentin 的表达,而 COX-2 敲减可有相反的结果,说明 COX-2 可能通过调控 EMT 过程调控乳腺癌细胞迁移和侵袭。

总之,本研究发现 COX-2 在乳腺癌细胞和脑转移乳腺癌组织中上调,并可通过调控 EMT 过程调控乳腺癌细胞迁移和侵袭,为乳腺癌转移的分子机制提供新认识,并可能为治疗乳腺癌提供新策略。

[参考文献]

- [1] MITRA S, DASH R. Natural products for the management and prevention of breast cancer[J/OL]. *Evid Based Complement Alternat Med*, 2018, 2018: 8324696[2018-11-10]. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5846366/>. DOI:10.1155/2018/8324696.
- [2] XIE W Y, HE R H, ZHANG J, et al. B-blockers inhibit the viability of breast cancer cells by regulating the ERK/COX-2 signaling pathway and the drug response is affected by ADRB2 single-nucleotide polymorphisms[J]. *Oncol Rep*, 2019, 41(1): 341-350. DOI:10.3892/or.2018.6830.
- [3] GALLO O, MASINI E, BIANCHI B, et al. Prognostic significance of cyclooxygenase-2 pathway and angiogenesis in head and neck squamous cell carcinoma[J]. *Hum Pathol*, 2002, 33(7): 708-714. DOI:10.1053/hupa.2002.125376.
- [4] SUNITA B S, SEN A, SUHAG V. To evaluate immunoreactivity of cyclooxygenase-2 in cases of endometrial carcinoma and correlate it with expression of p53 and vascular endothelial growth factor[J]. *J Cancer Res Ther*, 2018, 14(6): 1366-1372. DOI: 10.4103/0973-1482.202890.
- [5] CHIKMAN B, VASYANOVICH S, LAVY R, et al. COX2 expression in high-grade breast cancer: evidence for prognostic significance in the subset of triple-negative breast cancer patients[J]. *Med Oncol*, 2014, 31(6): 989. DOI:10.1007/s12032-014-0989-1.
- [6] 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/OL]. *Eur J Cancer*, 2019, 111: 82-93[2018-11-10]. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6436990/>. DOI:10.1016/j.ejca.2019.01.022.
- [7] HALDAR R, SHAASHUA L, LAVON H, et al. Perioperative inhibition of β -adrenergic and COX2 signaling in a clinical trial in breast cancer patients improves tumor Ki-67 expression, serum cytokine levels, and PBMCs transcriptome[J]. *Brain Behav Immun*, 2018, 73(10): 294-309. DOI:10.1016/j.bbi.2018.05.014.
- [8] CHRESTELLA J, FARHAT F, DAULAY E R, et al. Cyclooxygenase-2 expression and its correlation with primary tumor size and lymph node involvement in nasopharyngeal carcinoma[J/OL]. *Open Access Maced J Med Sci*, 2018, 6(11): 2001-2005[2018-11-10]. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6290448/>. DOI: 10.3889/oamjms.2018.356.
- [9] CIANCHI F, CUZZOCREA S, VINCI M C, et al. Heterogeneous expression of cyclooxygenase-2 and inducible nitric oxide synthase within colorectal tumors: correlation with tumor angiogenesis[J]. *Dig Liver Dis*, 2010, 42(1): 20-27. DOI:10.1016/j.dld.2009.04.010.
- [10] SOLANKI R, AGRAWAL N, ANSARI M, et al. COX-2 expression in breast carcinoma with correlation to clinicopathological parameters[J/OL]. *Asian Pac J Cancer Prev*, 2018, 19(7): 1971-1975[2018-11-10]. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6165637/>. DOI:10.22034/APJCP.2018.19.7.1971.
- [11] WANG K H, KAO A P, CHANG C C, et al. Bisphenol A-induced epithelial to mesenchymal transition is mediated by cyclooxygenase-2 up-regulation in human endometrial carcinoma cells[J]. *Reprod Toxicol*, 2015, 58(10): 229-233. DOI:10.1016/j.reprotox.2015.10.011.
- [12] SOLANKI R, AGRAWAL N, ANSARI M, et al. COX-2 expression in breast carcinoma with correlation to clinicopathological parameters[J/OL]. *Asian Pac J Cancer Prev*, 2018, 19(7): 1971-1975[2018-11-10]. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6165637/>. DOI:10.22034/APJCP.2018.19.7.1971.
- [13] NASSAR A, RADHAKRISHNAN A, CABRERO I A, et al. COX-2 expression in invasive breast cancer: correlation with prognostic parameters and outcome[J]. *Appl Immunohistochem Mol Morphol*, 2007, 15(3): 255-259. DOI:10.1097/01.pai.0000213130.63417.b3.
- [14] WAN G X, TIAN L, YU Y D, et al. Overexpression of Pofut1 and activated Notch1 may be associated with poor prognosis in breast cancer[J]. *Biochem Biophys Res Commun*, 2017, 491(1): 104-111. DOI:10.1016/j.bbrc.2017.07.053.
- [15] APONTE-LÓPEZ A, FUENTES-PANANÁ E M, CORTES-MUÑOZ D, et al. Mast cell, the neglected member of the tumor microenvironment: role in breast cancer[J/OL]. *J Immunol Res*, 2018, 2018: 2584243[2018-11-10]. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5832101/>. DOI:10.1155/2018/2584243.
- [16] SPRONK I, SCHELLEVIS F G, BURGERS J S, et al. Incidence of isolated local breast cancer recurrence and contralateral breast cancer: a systematic review[J]. *Breast*, 2018, 39(6): 70-79. DOI: 10.1016/j.breast.2018.03.011.
- [17] TANG T C. Tumor cyclooxygenase-2 levels correlate with tumor invasiveness in human hepatocellular carcinoma[J]. *WJG*, 2005, 11(13): 1896-1902. DOI:10.3748/wjg.v11.i13.1896.
- [18] VAN REES B P, RISTIMÄKI A. Cyclooxygenase-2 in carcinogenesis of the gastrointestinal tract[J]. *Scand J Gastroenterol*, 2001, 36(9): 897-903.
- [19] RISTIMÄKI A, SIVULA A, LUNDIN J, et al. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer[J]. *Cancer Res*, 2002, 62(3): 632-635.
- [20] WU K R, FUKUDA K, XING F, et al. Roles of the cyclooxygenase 2 matrix metalloproteinase 1 pathway in brain metastasis of breast cancer[J/OL]. *J Biol Chem*, 2015, 290(15): 9842-9854[2018-11-10]. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4392281/>. DOI:10.1074/jbc.M114.602185.
- [21] HOSOMI Y, YOKOSE T, HIROSE Y, et al. Increased cyclooxygenase 2 (COX-2) expression occurs frequently in precursor lesions of human adenocarcinoma of the lung[J]. *Lung Cancer*, 2000, 30(2):73-81. DOI:10.1016/s0169-5002(00)00132-x.
- [22] CLARK B L, MURPHY M A, KAMDEM L K. COX2 induction: a mechanism of endocrine breast cancer resistance?[J]. *Breast Cancer Res Treat*, 2017, 165(2): 383-389. DOI:10.1007/s10549-017-4284-7.

[收稿日期] 2018-11-21

[修回日期] 2019-04-22

[本文编辑] 黄静怡