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***Retrospective Study***

**Cyclooxygenase-2 expression is associated with initiation of hepatocellular carcinoma, while****prostaglandin receptor-1 expression predicts survival**

Yang HJ *et al*. prostaglandin receptor-1 expression predicts survival

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**Abstract**

***AIM***

To explore how cyclooxygenase-2 (COX-2) and prostaglandin E2 receptor (EP1) contribute to disease and whether they help predict prognosis.

***METHODS***

We retrospectively reviewed the records of 116 patients with HCC who underwent surgery between 2008 and 2011 at our hospital. Expression of COX-2 and EP1 receptor was examined by immunohistochemistry of formalin-fixed, paraffin-embedded tissues using polyclonal antibodies. Possible associations were explored between immunohistochemical scores and survival.

***Results***

Factors associated with poor overall survival (OS) were identified to be alpha-fetoprotein (AFP) > 400 ng/mL, tumor size ≥ 5 cm, and high EP1 receptor expression, but not high COX-2 expression. The disease free survival (DFS) was not significantly different between patients with low or high levels of COX-2 or EP1. COX-2 immunoreactivity was significantly higher in well-differentiated HCC tissues (Edmondson grade I-II) than in poorly differentiated tissues (Edmondson grade III–IV) (*p* = 0.003). EP1 receptor immunoreactivity was significantly higher in poorly differentiated tissue than in well-differentiated tissue (*p* = 0.001).

***Conclusion***

COX-2 expression appears linked to early HCC events (initiation), while EP1 receptor expression may participate in tumor progression and predict survival.

**Key words**: Cyclooxygenase-2; Hepatocellular carcinoma; Liver resection; Prognosis; prostaglandin E2 receptor

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**Core tip**: We retrospectively reviewed the records of 116 patients with HCC who underwent surgery between 2008 and 2011 at our hospital. Our results suggest that factors associated with poor overall survival (OS) were identified to be alpha-fetoprotein (AFP) > 400 ng/mL, tumor size ≥ 5 cm, and high EP1 receptor expression, but not high COX-2 expression. Disease-free survival (DFS) did not differ significantly between patients with low or high levels of COX-2 or EP1. COX-2 immunoreactivity was significantly higher in well-differentiated HCC tissues (Edmondson grade I-II) than in poorly differentiated tissues (Edmondson grade III–IV) (*p* = 0.003). EP1 receptor immunoreactivity was significantly higher in poorly differentiated tissue than in well-differentiated tissue (*p* = 0.001).

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**Introduction**

Hepatocellular carcinoma (HCC) is one of the most aggressive tumors and the third most frequent cause of cancer-related death in the world[1,2]. Although early diagnosis and treatment of HCC have improved substantially, prognosis remains unsatisfactory. HCC often involves highly malignant tumors that respond poorly or not at all to adjuvant systemic and local therapies. This highlights the need for new approaches to prevent and treat the disease.

Two molecules that may be involved with HCC at different stages, and that therefore may be useful for understanding the pathogenesis and progression of the disease, are cyclooxygenase-2 (COX-2) and prostaglandin E2 receptor (EP1 receptor). These molecules have already been shown to play important roles in onset of various cancers, including HCC[3-9].

COX-2 inhibits apoptosis and increases proliferation in various type of tumors[10-12]. It also triggers production of vascular endothelial growth factor and activates metalloproteinases, substantially altering the tumor microenvironment of various cancers[13-15]. The precise role of COX-2 in HCC remains unclear. Its expression decreases with extent of de-differentiation, and it does not appear to be associated with prognosis[16-19]. It may be involved in HCC initiation, though direct evidence of this is lacking.

COX-2 catalyzes the conversion of arachidonic acid to prostaglandin E2, which promotes progression of various types of tumors by binding to G-protein-coupled EP1 receptor. This led us to wonder whether EP1 receptor expression might correlate with HCC progression and might even serve as a prognostic indicator of survival. In fact, in a mouse model of chemically induced colon cancer, administration of selective EP1 receptor antagonists or knockout of the EP1 receptor gene led to nearly 60% fewer precancerous lesions and lower overall colon cancer incidence[20,21]. EP1 receptor antagonists have also been reported to block the progression of other types of tumor[22,23], including HCC[18,24].

To begin to clarify the potential roles of COX-2 and EP1 receptor in HCC, as well as explore the potential prognostic value of EP1 receptor expression, we examined relative expression levels in tissues taken from HCC patients treated at our hospital, and we correlated these levels with survival.

**MATERIALS AND METHODS**

This research was approved by the Ethics Committee of the Tumor Hospital of Guangxi Medical University, and patients provided informed consent for their data and tissue to be used for research purposes when they were admitted for treatment at our hospital.

***Patients***

This study involved retrospective analysis of HCC patients treated by curative hepatectomy at Tumor Hospital of Guangxi Medical University between May 2008 and May 2011. To be included in our study, patients needed to have pathology-confirmed HCC and no history of antitumor therapies before hepatic resection. They also needed to satisfy the following curative hepatectomy criteria: (1) the tumor removed by hepatectomy was solitary; (2) the surgery margin was greater than 1 cm; (3) there was no residual tumor[25], portal tumor thromboses[26] or extrahepatic metastases based on post-surgical imaging; and (4) patients with high levels of alpha-fetoprotein (AFP) before surgery showed normal levels within two months after surgery. Patients were excluded from the study if they had multiple tumors, extrahepatic metastases or macroscopic intrahepatic metastases adjacent to the primary tumor.

***Follow-up***

All HCC patients were followed up at 1 month after resection, then at 3-month intervals in the first year, then at 3-6 month intervals thereafter until 60 mo after resection or death. During each follow-up visit,routine investigations including AFP level, liver function, chest X-ray, ultrasound, CT or MRI were conducted.

***Immunohistochemistry of COX-2 and EP1 receptor***

Tumor specimens were fixed in 10% formalin, embedded in paraffin, cut into 3-μm sections, de-paraffinized with xylene and rehydrated by stepping through decreasing concentrations of ethanol. Antigen retrieval was performed for 10 min at 95 ºC in citrate buffer (pH 6.0) in a microwave oven. Sections were immersed in 3% hydrogen peroxide for 15 min to block endogenous peroxidases, then incubated at 37 ºC for 1 h with rabbit anti-human COX-2 polyclonal antibody (1:400; Abcam, United Kingdom) or rabbit anti-human EP1 receptor polyclonal antibody (1:200; Abcam). Sections were rinsed in phosphate-buffered saline (PBS), incubated with biotinylated anti-rabbit immunoglobulin for 20 min at room temperature, then rinsed again with PBS. Sections were incubated with anti-horseradish peroxidase conjugate for 10 min, rinsed with PBS, and incubated with diaminobenzidine for 10 min. Finally, sections were counterstained with hematoxylin. As a negative control, tissues were treated as described above, except they were incubated with PBS instead of primary antibodies.

Immunohistochemical staining results were independently evaluated by three authors (Hao-jie Yang, Zhe Guo and Yu-ting Yang) and an experienced hepatopathologist (Chun-jun Li) from the Department of Pathology of Guangxi Tumor Hospital. The percentages of cells positive for COX-2 or EP1 receptor as well as relative staining intensity were determined. Percentages of positive cells were categorized as follows: 0 (no positive tumor cells), 1 (1%-25% positive), 2 (26%-50% positive), 3 (51%-75% positive), and 4 (76%-100% positive). Staining intensity was categorized as follows: 0 (no staining), 1 (weak, light yellow), 2 (moderate, yellow-brown), and 3 (strong, brown)[27]. The scores for positive cell percentages and for staining intensity were multiplied together to yield a single immunohistochemical staining index from 0 to 12. Sections with an index of 0-5 were defined as showing low expression, while those with an index of 6-12 were defined as showing high expression.

***Statistical analysis***

All statistical analyses were performed using SPSS 19.0 (IBM, United States). Inter-group differences for categorical variables were assessed for significance using the chi-squared test; differences for continuous variables were assessed using the Mann-Whitney *U* test or *t*-test. OS and DFS were analyzed using the Kaplan-Meier method, and differences between curves were assessed for significance using the log-rank test. Multivariate Cox proportional hazards modeling was used to identify independent prognostic factors. The threshold for significance was defined as *p* < 0.05.

**Results**

***Patient characteristics***

During the research, 748 patients with HCC were scheduled for hepatectomy in my center. Of these, 221 (29.5%) were prevent from entering owing to receiving initial HCC treatment in other hospitals. Of the rest of 527 patients, 161 (30.5%) had solitary nodular tumors without portal tumor thromboses or extrahepatic metastases. We reject 33 (20.4%) because they underwent only transarterial chemoembolization, local ablation therapy, or ethanol injection, and we reject 12 (7.4%) because of lacking complete follow-up. In the end, 116 (72%) patients were included in the final analysis (93 men, 23 women) with a median age of 67 (range, 39-83) (Table 1). Immunohistochemistry showed low COX-2 expression in 62 patients (53.4%) and low EP1 receptor expression in 73 (62.9%).

***EP1 receptor expression is a prognostic predictor of OS***

OS was significantly lower among patients with high expression of EP1 receptor than among those with low expression (Figure 1). OS did not differ significantly between patients with low or high COX-2 expression. DFS did not differ significantly between patients with low or high expression of COX-2 or EP1 receptor.

Cox hazards model shown 3 independent predictors of poor OS: tumor size ≥ 5 cm,high expression of EP1 receptor and AFP ≥ 400 ng/mL (Table 2).

***COX-2 and EP1 receptor expression correlates with tumor differentiation***

COX-2 immunoreactivity was significantly higher in well-differentiated HCC tissues (Edmondson grade I-II) than in poorly differentiated tissues (Edmondson grade III–IV) (*p* = 0.003). EP1 receptor immunoreactivity was significantly higher in poorly differentiated tissue than in well-differentiated tissue (Figure 2, *p* = 0.001). Figure 3shows representative examples of different staining results in tissues of different histology grade.

**Discussion**

HCC is one of the most aggressive tumors and it features poor prognosis. Some patients can undergo curative resection, but this treatment is associated with a high rate of intrahepatic recurrence. Most patients with HCC are ineligible for resection because their disease has already reached an advanced stage by the time it is diagnosed. These patients are treated with local or systemic adjuvant modalities that provide only short-term regression, stabilizations, or symptomatic control. Therefore new therapeutic strategies are needed that may improve long-term survival.

Our results implicate the EP1 receptor in HCC progression, suggesting that it may be a reasonable therapeutic target. High expression of EP1 receptor was associated with poor prognosis in our patients, and expression was significantly higher in poorly differentiated tissue than in well-differentiated tissue. The observed correlation of higher expression with poorer differentiation is consistent with a previous study[18]. These results suggest that targeting the EP1 receptor may provide a more selective approach to treating HCC than using COX inhibitors to block prostaglandin E2 synthesis, which increases risk of cardiovascular events[28].

Although studies have associated COX-2 expression with differentiation, invasion and metastasis in HCC[3,18,24], they have not linked it with survival. In the present study, we failed to find an influence of COX-2 expression on survival when patients were dichotomized into groups showing low or high expression. While it is possible that a more quantitative approach may turn up associations between COX-2 levels and survival, we believe it is more likely that expression of COX-2 may be important only during initiation of HCC, whereas the EP1 receptor, which is the downstream target of the prostaglandin E2 generated by COX-2, may be involved in disease progression. This may help explain why we observed a different relationship between expression of COX-2 and EP1 receptor: patients with high expression of one showed low expression of the other. This may also explain why we found that expression of EP1 receptor, but not COX-2 receptor, predicted OS in our cohort.

Several factors may help explain why high expression of EP1 receptor predicts poor survival. The receptor enhances tumor cell proliferation, invasion and migration[4,6,29,30], as well as adaptation to hypoxic conditions[6,31]. The receptor has also been reported to inhibit immune function and promote progression of tumor[32]. The EP1 receptor can even induce prostaglandin E2 production by binding to the receptor of Fas ligand[32-34].

In this article, there are some limitations. First, in this draft, the authors used only HE method, more accurate and quantitative method, such as Western blot, polymerase chain reaction *etc.*, should be applied in future studies. Secondly, as we known, other cytokines except COX-2, EP1 have been reported to be tightly connected with HCC. These cytokines may involve in different aspects of the pathogenesis of HCC and should be explored as a network pattern in future studies.

In conclusion, our results suggest that COX-2 expression correlates with an early event during initiation of HCC, while EP1 receptor expression plays an important role in tumor progression and predicts OS.

**COMMENTS**

***Background***

Hepatocellular carcinoma (HCC) is one of the most aggressive tumors and the third most frequent cause of cancer-related death in the world. Although early diagnosis and treatment of HCC have improved substantially, prognosis remains unsatisfactory. HCC often involves highly malignant tumors that respond poorly or not at all to adjuvant systemic and local therapies. This highlights the need for new approaches to prevent and treat the disease.

***Research frontiers***

Two molecules that may be involved with HCC at different stages, and that therefore may be useful for understanding the pathogenesis and progression of the disease, are cyclooxygenase-2 (COX-2) and prostaglandin E2 receptor (EP1 receptor). These molecules have already been shown to play important roles in onset of various cancers, including HCC.

***Innovations and breakthroughs***

The authors retrospectively reviewed the records of 116 patients with HCC who underwent surgery between 2008 and 2011 at their hospital, and found COX-2 expression appears linked to early HCC events (initiation), while EP1 receptor expression may participate in tumor progression and predict survival.

***Applications***

These results suggest that targeting the EP1 receptor may provide a more selective approach to treating HCC than using COX inhibitors to block prostaglandin E2 synthesis, which increases risk of cardiovascular events.

***Terminology***

Percentages of positive cells were categorized as follows: 0 (no positive tumor cells), 1 (1%-25% positive), 2 (26%-50% positive), 3 (51%-75% positive), and 4 (76%-100% positive). Staining intensity was categorized as follows: 0 (no staining), 1 (weak, light yellow), 2 (moderate, yellow-brown), and 3 (strong, brown). The scores for positive cell percentages and for staining intensity were multiplied together to yield a single immunohistochemical staining index from 0 to 12. Sections with an index of 0-5 were defined as showing low expression, while those with an index of 6-12 were defined as showing high expression.

***Peer review***

The paper is a good study on COX-2 and EP1 receptor immunoreactivity in patients with HCC. The investigators shown that COX-2 immunoreactivity was higher in well-differentiated HCC tissues and EP1 receptor immunoreactivity was significantly higher in poorly differentiated tissue than in well-differentiated tissue.

**References**

1 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]

2 **Yang HJ**, Guo Z, Yang YT, Jiang JH, Qi YP, Li JJ, Li LQ, Xiang BD. Blood neutrophil-lymphocyte ratio predicts survival after hepatectomy for hepatocellular carcinoma: A propensity score-based analysis. *World J Gastroenterol* 2016; **22**: 5088-5095 [PMID: 27275101 DOI: 10.3748/wjg.v22.i21.5088]

3 **Guo Z**, Jiang JH, Zhang J, Yang HJ, Yang FQ, Qi YP, Zhong YP, Su J, Yang RR, Li LQ, Xiang BD. COX-2 Promotes Migration and Invasion by the Side Population of Cancer Stem Cell-Like Hepatocellular Carcinoma Cells. *Medicine (Baltimore)* 2015; **94**: e1806 [PMID: 26554780 DOI: 10.1097/MD.0000000000001806]

4 **Zhang H**, Cheng S, Zhang M, Ma X, Zhang L, Wang Y, Rong R, Ma J, Xia S, Du M, Shi F, Wang J, Yang Q, Bai X, Leng J. Prostaglandin E2 promotes hepatocellular carcinoma cell invasion through upregulation of YB-1 protein expression. *Int J Oncol* 2014; **44**: 769-780 [PMID: 24378923 DOI: 10.3892/ijo.2013.2234]

5 **Jin J**, Chang Y, Wei W, He YF, Hu SS, Wang D, Wu YJ. Prostanoid EP1 receptor as the target of (-)-epigallocatechin-3-gallate in suppressing hepatocellular carcinoma cells in vitro. *Acta Pharmacol Sin* 2012; **33**: 701-709 [PMID: 22555372 DOI: 10.1038/aps.2012.13]

6 **Kim SH**, Park YY, Kim SW, Lee JS, Wang D, DuBois RN. ANGPTL4 induction by prostaglandin E2 under hypoxic conditions promotes colorectal cancer progression. *Cancer Res* 2011; **71**: 7010-7020 [PMID: 21937683 DOI: 10.1158/0008-5472.CAN-11-1262]

7 **Bai XM**, Jiang H, Ding JX, Peng T, Ma J, Wang YH, Zhang L, Zhang H, Leng J. Prostaglandin E2 upregulates survivin expression via the EP1 receptor in hepatocellular carcinoma cells. *Life Sci* 2010; **86**: 214-223 [PMID: 20035770 DOI: 10.1016/j.lfs.2009.12.009]

8 **Han C**, Michalopoulos GK, Wu T. Prostaglandin E2 receptor EP1 transactivates EGFR/MET receptor tyrosine kinases and enhances invasiveness in human hepatocellular carcinoma cells. *J Cell Physiol* 2006; **207**: 261-270 [PMID: 16331686 DOI: 10.1002/jcp.20560]

9 **Hull MA**, Ko SC, Hawcroft G. Prostaglandin EP receptors: targets for treatment and prevention of colorectal cancer? *Mol Cancer Ther* 2004; **3**: 1031-1039 [PMID: 15299086]

10 **Zhang P**, Luo HS, Li M, Tan SY. Artesunate inhibits the growth and induces apoptosis of human gastric cancer cells by downregulating COX-2. *Onco Targets Ther* 2015; **8**: 845-854 [PMID: 25945055 DOI: 10.2147/OTT.S81041]

11 **Qian M**, Yang X, Li Z, Jiang C, Song D, Yan W, Liu T, Wu Z, Kong J, Wei H, Xiao J. P50-associated COX-2 extragenic RNA (PACER) overexpression promotes proliferation and metastasis of osteosarcoma cells by activating COX-2 gene. *Tumour Biol* 2016; **37**: 3879-3886 [PMID: 26476537 DOI: 10.1007/s13277-015-3838-8]

12 **Lv P**, Zhang P, Li X, Chen Y. Micro ribonucleic acid (RNA)-101 inhibits cell proliferation and invasion of lung cancer by regulating cyclooxygenase-2. *Thorac Cancer* 2015; **6**: 778-784 [PMID: 26557918 DOI: 10.1111/1759-7714.12283]

13 **Lee JH**, Piao MS, Choi JY, Yun SJ, Lee JB, Lee SC. Up-regulation of cyclooxygenase 2 and matrix metalloproteinases-2 and -9 in cutaneous squamous cell carcinoma: active role of inflammation and tissue remodeling in carcinogenesis. *Ann Dermatol* 2013; **25**: 145-151 [PMID: 23717003 DOI: 10.5021/ad.2013.25.2.145]

14 **Mohammad MA**, Zeeneldin AA, Abd Elmageed ZY, Khalil EH, Mahdy SM, Sharada HM, Sharawy SK, Abdel-Wahab AH. Clinical relevance of cyclooxygenase-2 and matrix metalloproteinases (MMP-2 and MT1-MMP) in human breast cancer tissue. *Mol Cell Biochem* 2012; **366**: 269-275 [PMID: 22527932 DOI: 10.1007/s11010-012-1305-z]

15 **Li Y**, Li S, Sun D, Song L, Liu X. Expression of 15-hydroxyprostaglandin dehydrogenase and cyclooxygenase-2 in non-small cell lung cancer: Correlations with angiogenesis and prognosis. *Oncol Lett* 2014; **8**: 1589-1594 [PMID: 25202373 DOI: 10.3892/ol.2014.2371]

16 **Yang Y**, Zhu J, Gou H, Cao D, Jiang M, Hou M. Clinical significance of Cox-2, Survivin and Bcl-2 expression in hepatocellular carcinoma (HCC). *Med Oncol* 2011; **28**: 796-803 [PMID: 20401641 DOI: 10.1007/s12032-010-9519-y]

17 **Yildirim Y**, Ozyilkan O, Bilezikci B, Akcali Z, Haberal M. Lack of influence of cyclooxygenese-2 expression in hepatocellular carcinomas on patient survival. *Asian Pac J Cancer Prev* 2008; **9**: 295-298 [PMID: 18712978]

18 **Breinig M**, Rieker R, Eiteneuer E, Wertenbruch T, Haugg AM, Helmke BM, Schirmacher P, Kern MA. Differential expression of E-prostanoid receptors in human hepatocellular carcinoma. *Int J Cancer* 2008; **122**: 547-557 [PMID: 17918156 DOI: 10.1002/ijc.23098]

19 **Koga H**, Sakisaka S, Ohishi M, Kawaguchi T, Taniguchi E, Sasatomi K, Harada M, Kusaba T, Tanaka M, Kimura R, Nakashima Y, Nakashima O, Kojiro M, Kurohiji T, Sata M. Expression of cyclooxygenase-2 in human hepatocellular carcinoma: relevance to tumor dedifferentiation. *Hepatology* 1999; **29**: 688-696 [PMID: 10051469 DOI: 10.1002/hep.510290355]

20 **Watanabe K**, Kawamori T, Nakatsugi S, Ohta T, Ohuchida S, Yamamoto H, Maruyama T, Kondo K, Narumiya S, Sugimura T, Wakabayashi K. Inhibitory effect of a prostaglandin E receptor subtype EP(1) selective antagonist, ONO-8713, on development of azoxymethane-induced aberrant crypt foci in mice. *Cancer Lett* 2000; **156**: 57-61 [PMID: 10840160 DOI: 10.1016/S0304-3835(00)00440-7]

21 **Watanabe K**, Kawamori T, Nakatsugi S, Ohta T, Ohuchida S, Yamamoto H, Maruyama T, Kondo K, Ushikubi F, Narumiya S, Sugimura T, Wakabayashi K. Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis. *Cancer Res* 1999; **59**: 5093-5096 [PMID: 10537280]

22 **Kawamori T**, Uchiya N, Nakatsugi S, Watanabe K, Ohuchida S, Yamamoto H, Maruyama T, Kondo K, Sugimura T, Wakabayashi K. Chemopreventive effects of ONO-8711, a selective prostaglandin E receptor EP(1) antagonist, on breast cancer development. *Carcinogenesis* 2001; **22**: 2001-2004 [PMID: 11751431 DOI: 10.1093/carcin/22.12.2001]

23 **Tober KL**, Wilgus TA, Kusewitt DF, Thomas-Ahner JM, Maruyama T, Oberyszyn TM. Importance of the EP(1) receptor in cutaneous UVB-induced inflammation and tumor development. *J Invest Dermatol* 2006; **126**: 205-211 [PMID: 16417238 DOI: 10.1038/sj.jid.5700014]

24 **Cusimano A**, Fodera D, Lampiasi N, Azzolina A, Notarbartolo M, Giannitrapani L, D'Alessandro N, Montalto G, Cervello M. Prostaglandin E2 receptors and COX enzymes in human hepatocellular carcinoma: role in the regulation of cell growth. *Ann N Y Acad Sci* 2009; **1155**: 300-308 [PMID: 19250221 DOI: 10.1111/j.1749-6632.2009.03701.x]

25 **Fan ST**, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Hepatectomy for hepatocellular carcinoma: toward zero hospital deaths. *Ann Surg* 1999; **229**: 322-330 [PMID: 10077043 DOI: 10.1097/00000658-199903000-00004]

26 **Xia Y**, Qiu Y, Li J, Shi L, Wang K, Xi T, Shen F, Yan Z, Wu M. Adjuvant therapy with capecitabine postpones recurrence of hepatocellular carcinoma after curative resection: a randomized controlled trial. *Ann Surg Oncol* 2010; **17**: 3137-3144 [PMID: 20602260 DOI: 10.1245/s10434-010-1148-3]

27 **Baker AM**, Bird D, Welti JC, Gourlaouen M, Lang G, Murray GI, Reynolds AR, Cox TR, Erler JT. Lysyl oxidase plays a critical role in endothelial cell stimulation to drive tumor angiogenesis. *Cancer Res* 2013; **73**: 583-594 [PMID: 23188504 DOI: 10.1158/0008-5472.CAN-12-2447]

28 **Narumiya S**, FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. *J Clin Invest* 2001; **108**: 25-30 [PMID: 11435452 DOI: 10.1172/JCI13455]

29 **Bai X**, Wang J, Zhang L, Ma J, Zhang H, Xia S, Zhang M, Ma X, Guo Y, Rong R, Cheng S, Shu W, Wang Y, Leng J. Prostaglandin E₂ receptor EP1-mediated phosphorylation of focal adhesion kinase enhances cell adhesion and migration in hepatocellular carcinoma cells. *Int J Oncol* 2013; **42**: 1833-1841 [PMID: 23525457 DOI: 10.3892/ijo.2013.1859]

30 **Yang SF**, Chen MK, Hsieh YS, Chung TT, Hsieh YH, Lin CW, Su JL, Tsai MH, Tang CH. Prostaglandin E2/EP1 signaling pathway enhances intercellular adhesion molecule 1 (ICAM-1) expression and cell motility in oral cancer cells. *J Biol Chem* 2010; **285**: 29808-29816 [PMID: 20647315 DOI: 10.1074/jbc.M110.108183]

31 **Kaidi A**, Qualtrough D, Williams AC, Paraskeva C. Direct transcriptional up-regulation of cyclooxygenase-2 by hypoxia-inducible factor (HIF)-1 promotes colorectal tumor cell survival and enhances HIF-1 transcriptional activity during hypoxia. *Cancer Res* 2006; **66**: 6683-6691 [PMID: 16818642 DOI: 10.1158/0008-5472.CAN-06-0425]

32 **O'Callaghan G**, Ryan A, Neary P, O'Mahony C, Shanahan F, Houston A. Targeting the EP1 receptor reduces Fas ligand expression and increases the antitumor immune response in an in vivo model of colon cancer. *Int J Cancer* 2013; **133**: 825-834 [PMID: 23390011 DOI: 10.1002/ijc.28076]

33 **Zhang Y**, Liu Q, Zhang M, Yu Y, Liu X, Cao X. Fas signal promotes lung cancer growth by recruiting myeloid-derived suppressor cells via cancer cell-derived PGE2. *J Immunol* 2009; **182**: 3801-3808 [PMID: 19265159 DOI: 10.4049/jimmunol.0801548]

34 **O'Callaghan G**, Kelly J, Shanahan F, Houston A. Prostaglandin E2 stimulates Fas ligand expression via the EP1 receptor in colon cancer cells. *Br J Cancer* 2008; **99**: 502-512 [PMID: 18648368 DOI: 10.1038/sj.bjc.6604490]

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Grade C (Good): C, C

Grade D (Fair): 0

Grade E (Poor): 0

**Table 1** **Clinicopathological characteristics of 116 hepatocellular carcinoma patients treated by curative resection, stratified by COX-2 and EP1 receptor expression**

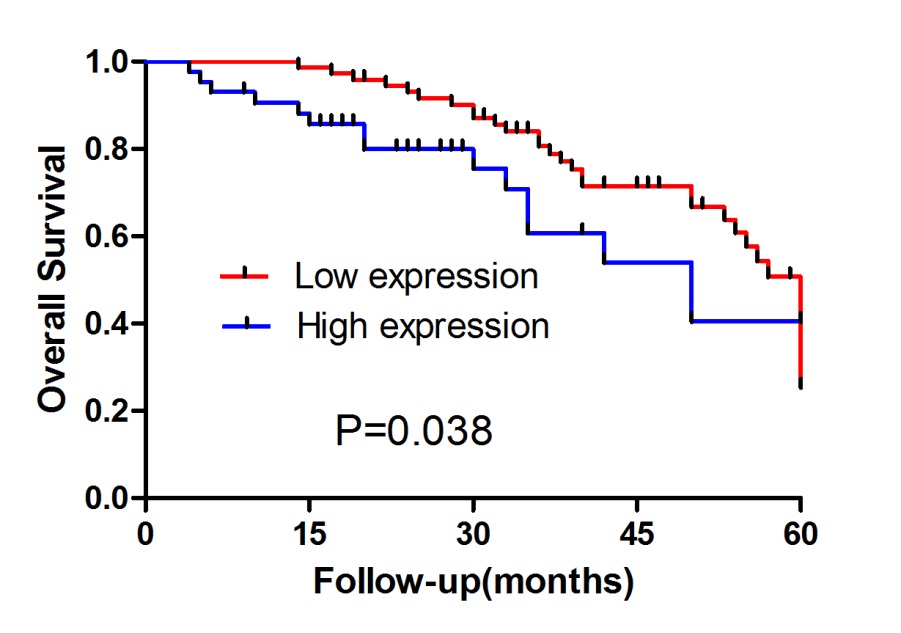
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **COX-2** | | ***p* value** | **EP1 receptor** | | ***p* value** |
| **Low expression (*n* = 62)** | **High expression (*n* = 54)** | **Low expression**  **(*n* = 73)** | **High expression**  **(*n* = 43)** |
| Gender, M/F | 48/14 | 45/9 | 0.426 | 60/13 | 33/10 | 0.477 |
| Age (yr) | 46.9 ± 10.8 | 47.9 ± 11.5 | 0.308 | 46.6 ± 11.1 | 47.3 ± 11.2 | 0.486 |
| HbsAg |  |  |  |  |  |  |
| Negative | 10 | 10 | 0.734 | 11 | 9 | 0.420 |
| Positive | 52 | 44 |  | 62 | 34 |  |
| Liver cirrhosis |  |  |  |  |  |  |
| No | 10 | 10 | 0.734 | 14 | 6 | 0.472 |
| Yes | 52 | 44 |  | 59 | 37 |  |
| AFP (ng/mL) |  |  |  |  |  |  |
| < 400 | 41 | 44 | 0.062 | 56 | 29 | 0.276 |
| ≥ 400 | 21 | 10 |  | 17 | 14 |  |
| Edmondson grade |  |  |  |  |  |  |
| I-II | 26 | 37 | 0.004 | 46 | 17 | 0.014 |
| III–IV | 36 | 17 |  | 27 | 26 |  |
| Child-Pugh class |  |  |  |  |  |  |
| A | 43 | 48 | 0.773 | 60 | 31 | 0.201 |
| B | 11 | 14 |  | 13 | 12 |  |
| Tumor capsule |  |  |  |  |  |  |
| Complete | 37 | 34 | 0.717 | 44 | 27 | 0.788 |
| Incomplete | 25 | 20 |  | 29 | 16 |  |
| Tumor size (cm) | 5.7 (3-7) | 6.6 (4-8.6) | 0.134 | 5 (3.5-7) | 6 (3.7-8.5) | 0.207 |
| Albumin (g/L) | 41.1 ± 4.4 | 40.4 ± 4.6 | 0.434 | 41.5 ± 4.3 | 40.2 ± 4.5 | 0.134 |
| Platele count (109/L) | 167.9 (107.3-205.3 | 186.6 (136.3-230) | 0.136 | 178.9 (107.3-205.3 | 186.6 (136.3-230) | 0.833 |
| AST (U/L) | 56.6 (28.3-61) | 55.6 (27-60) | 0.924 | 38 (27-60) | 44 (30.5-61.5) | 0.737 |
| ALT (U/L) | 54.8 (28-56) | 62.7 (24.8-57.9) | 0.539 | 37(27-56) | 38(27-55) | 0.917 |
| Total bilirubin (μmol/L) | 14.08 (8.9-18.4) | 14.3 (9.4-15.3) | 0.912 | 11.9(9.35-16.6) | 13.4(9-17.7) | 0.580 |

AFP: alpha-fetoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HBsAg: hepatitis B surface antigen.

**Table 2** **Multivariable analysis to identify predictors of overall survival in 116 hepatocellular carcinoma patients**

|  |  |  |  |
| --- | --- | --- | --- |
| **Factors** | **Hazard ratio** | **95%CI** | ***p* value** |
| AFP ≥ 400 ng/mL | 1.691 | 1.094-2.614 | 0.018 |
| Incomplete tumor capsule | 0.979 | 0.659-1.454 | 0.915 |
| Tumor size ≥ 5 cm | 1.582 | 1.027-2.438 | 0.038 |
| Edmondson grade III–IV | 1.149 | 0.663-1.992 | 0.621 |
| EP1 receptor expression | 2.318 | 1.190-4.516 | 0.014 |

AFP: alpha-fetoprotein.



**Figure 1 Overall survival of hepatocellular carcinoma patients stratified by low or high expression of EP1 receptor.**

C:\Users\Administrator\Desktop\COX-2 EP1.tif

**Figure 2 COX-2 and EP1 receptor immunoreactivity scores in hepatocellular carcinoma tissues at different histological grades.** COX-2 expression was higher in well-differentiated tissue (Edmondson grade I-II), while EP1 receptor expression was higher in poorly-differentiated tissue (Edmondson grade III–IV). WD: well-differentiated; PD: poorly differentiated.

COX-2

1COX-2.tif

**COX-2**

EP1 receptor

1EP1.tif

**Figure 3 Representative micrographs showing different intensities of immunohistochemical stain against COX-2 and EP1 receptor in tissues with different histological grades (Edmondson grade I–IV).** Magnification, 100 ×. COX-2 expression level decreased with lower grade of differentiation, while EP1 receptor expression increased with lower grade of differentiation.