The effect of the expression of cyclooxygenase-2 (COX-2) and prostaglandin E₂ (PGE₂) synthesis on cell migration, the secretion of matrix metalloproteinases (MMPs) and the adhesion of human hepatoma cell lines has been investigated. A close correlation was observed between the expression of COX-2 under basal conditions and the secretion of MMP-2 and MMP-9. Cell migration in HuH-7 cells, which express high constitutive levels of COX-2 was significantly inhibited by selective inhibitors of COX-2 and enhanced by exogenous addition of PGE₂.

Hepatocellular carcinoma (HCC) cells expressed β1 and αvβ3 integrins, exhibiting an increase in cell adhesion onto fibronectin and vitronectin. Moreover, addition of PGE₂ increased the β1 integrin levels and adhesion on vitronectin in HuH-7 cells. Inhibitors of MEK/ERK, p38 MAPK, protein kinases A and C impaired the migration of HuH-7 cells induced by PGE₂, indicating the involvement of multiple pathways in the process. Taken together, these results support the existence of a relationship between COX-2-derived PGE₂ synthesis, and migration and adhesion through an integrin-dependent pathway in HCC cells.

Introduction

Cyclooxygenase-1 (COX-1) and COX-2 catalyze the first step in the biosynthesis of various prostaglandins (PGs) and thromboxanes (1,2). COX-1 is constitutively expressed in many tissues and seems to be involved in the housekeeping functions of PGs (3). COX-2, the inducible isoform of COX, accounts for the elevated production of PGs in response to various pro-inflammatory stimuli and other cellular stresses, such as endotoxemia and septic shock (2,4,5). The role of COX-2 and the PGs resulting from its enzymatic activity in modulating cell growth and neoplasia is well established, particularly in colon cancer (6,7). In addition to colon cancer, different authors have reported that the increased production of PGs may contribute to the development of other cancers, including hepatocellular carcinoma (HCC) (8). Expression of COX-2 has been demonstrated in liver regeneration after partial hepatectomy (9), in different cirrhosis animal models (10,11), in human hepatoma cell lines (12,13), in chronic hepatitis C infection (14,15) and in HCC (16,17). Moreover, in vitro pharmacological inhibition of COX-2 by non-steroidal anti-inflammatory drugs (NSAIDs) effectively inhibits the growth of cell lines, which is mediated by a decrease in the rate of cell proliferation and increase in apoptosis (12,13), suggesting a potential therapeutic role for COX-2 inhibitors in chronic liver disease.

With respect to the mechanisms by which COX-2 contributes to tumorigenesis, an increase in angiogenesis through the production of vascular endothelial growth factor (VEGF) and PGs has been proposed (18,19), in addition to an increased invasiveness via the activation of several matrix metalloproteinases (MMPs) (20,21).

In addition to MMPs, integrins play a central role in the process of cell migration and invasion. Indeed, integrins are the regulators of the expression of MMPs, secretion and activation of the latent protease at the cell surface (22). Recognition of matrix molecules by cell surface integrins and the subsequent degradation of the matrix are important mechanisms in cell invasion. The integrin family of cell adhesion receptors are heterodimeric transmembrane receptors consisting of α and β subunits that render >20 different heterodimers, which bind to matrix proteins, while others have cell surface proteins as receptors (23). Each subunit is a glycoprotein with a large extracellular domain that mediates the cell–matrix and cell–cell contacts and a relatively small cytoplasmic domain that associates with the cytoskeleton and various kinases involved in signaling pathways, such as focal adhesion kinase (FAK). Integrons are capable of transducing information in a bidirectional manner to control the cell movement, morphology, cell growth and gene expression from the outside of the cell and to modulate their binding affinity to ligands from the inside of the cell (22). Changes in the expression of integrins have been linked to both tumor suppression and progression in different human malignancies (24,25). For example, several studies have described a reduced expression of α2, α3 and α5 subunits (26) and an overexpression of α6 subunits and β1 integrins in HCC with aggressive phenotypes (27,28). Moreover, αv integrins through its binding to vitronectin play an important role in the development of liver metastasis (29,30). The relationship between COX-2 expression and integrin function has been clearly established in endothelial cells where PGE₂ accelerated αvβ3-mediated human umbilical vein endothelial cells (HUVEC) adhesion and promoted Rac activation and cell-spreadng, protein kinase A (PKA) activity being critical in this process (31,32).
The present study analyzes the relationship between PGs produced by COX-2 in different cell lines with migratory capacity, secretion of MMPs and the expression of adhesion molecules. We show that hepatoma cell lines exhibit a constitutive and functional COX-2 expression, which is related to the migratory capacity, secretion of MMPs and cellular adhesion through an integrin-dependent pathway.

**Materials and methods**

**Chemicals**

Antibodies were from Santa Cruz Laboratories (Santa Cruz, CA), Chemicon International Inc. (Temecula, CA) and Cayman Chemical Co. Inc. (Ann Arbor, MI). 5,5-Dimethyl-3(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furanone (DFU) was from Merck (Rahway, NJ). Lipopolysaccharides (LPS), cytokines, fibronectin, vitronectin and other reagents were from Roche.

**Fig. 1.** COX-2 expression in HCC cells. CHL, HuH-7 and HTB-52 cells were grown in DMEM supplemented with 10% FCS and antibiotics. THLE-2 cells were grown in the same conditions but with BEBM medium supplemented with Bullet kit CC3170. The culture medium was replaced with fresh medium containing 1% FCS, 18 h before the experiment. A representative western blot of COX-2 and COX-1 in total cellular extracts is shown. Blots were normalized by measuring the amount of β-actin. Immunocytochemical analysis of COX-2 (extranuclear staining) by confocal microscopy. The nuclei were visualized by treatment with Hoechst 33258. Results show a representative experiment out of four. The PGE2 levels were determined by EIA in the culture medium. Effect of treatment with 5 μM of the COX-2 selective inhibitor DFU on PGE2 levels. Results are mean ± SD of three different experiments. *P < 0.05 versus the THLE-2 control cells. #P < 0.05 versus cells without DFU treatment. See online supplementary material for a colour version of this figure.
Diagnosics (Mannheim, Germany) or Sigma Chemical Co. (St Louis, MO). PGs and pharmacological inhibitors were from Calbiochem EMD Biosciences Inc. (San Diego, CA). Tissue culture dishes were from Falcon (Lincoln Park, NJ). Transwells were from Costar Corp. (Cambridge, MA). Tissue culture media were from BioWhittaker (Walkersville, MD) and American Type Culture Collection (ATCC) (Manassas, VA). Reagents for electrophoresis were from Bio-Rad (Hercules, CA).

Cell culture and treatments

The human liver cell lines THLE-2, CCL-13 and the hepatoma cell line SK-HEP-1 (HTB-52) were purchased from the ATCC. THLE-2 was derived from primary normal liver cells. The ATCC catalog states that the CCL-13 (Chang liver, CHL) cell line was derived from normal liver tissue but has HeLa markers. To evaluate the hepatocyte nature of the clones, we analyzed the expression of the hepatocyte markers α-fetoprotein and albumin, which are highly expressed (data not shown) as described previously (33). HuH-7 was kindly provided by Dr P. Schirmacher (Institute of Pathology, University of Cologne, Germany) and is considered a HCC cell line. HTB-52 is a liver adenocarcinoma cell line. CHL, HuH-7 and HTB-52 were grown in DMEM supplemented with 10% fetal calf serum (FCS) and antibiotics (50 μg/ml each of penicillin, streptomycin and gentamicin). THLE-2 were grown in the same conditions but with BEBM medium supplemented with Bullet kit CC3170 (ATCC). The culture medium was replaced with fresh medium containing 1% FCS, 18 h before the experiment.

Cell migration assays

Cell migration was assayed in 24-well transwell units with 8-μm porosity polycarbonate filters coated with 50 μg/ml of Type I collagen. Cells (10^5) were added to the upper chamber and 600 μl of medium with 0.5% FCS to the lower chamber of the transwell. After 4 h, the cells were incubated with 5 μM PGE2, 2 μM 15-deoxy-Δ12,14-PGJ2 and 5 μM DFU for 18 h. After this incubation period, the filters were fixed with 70% ethanol at −20°C and washed with phosphate-buffered saline (PBS). The filters were treated with 1 mg/ml of RNase for 30 min at 37°C, stained with 0.003% propidium iodide and counted under the microscope.

Adhesion assays

Adhesion assays were performed in 96-well plates coated with fibronectin (10 μg/ml), vitronectin (5 μg/ml) or gelatin (0.5%) diluted in PBS. The plates were incubated at 37°C for 60 min and washed three times with PBS. Unbound sites were blocked for 1 h at 37°C with 1% bovine serum albumin (BSA) heat inactivated previously for 60 min at 56°C. Control wells were coated with 1% heat-inactivated BSA. A total of 50 × 10^3 cells resuspended in serum-free medium were allowed to adhere in each well and incubated at 37°C. Immunocytochemical agents were added at the time of plating and after 30 min, the unattached cells were removed by washing with PBS. The attached cells were fixed with 4% paraformaldehyde, pH 7.0 for 15 min and stained with 0.5% crystal violet for 1 h at RT. After washing the wells with PBS, the adherent cells were solubilized with 36% acetic acid and quantified by the absorbance at 620 nm. Specific adhesion was defined as the adhesion on an extracellular matrix protein subtracting the adhesion on BSA.

Immunocytochemistry

Cells (50 × 10^3) were cultured in 24-multiwell plates on glass coverslips and maintained overnight with 1% FCS. After that period, the cells were fixed for 15 min with 4% paraformaldehyde, pH 7.0, washed with PBS and permeabilized with methanol at −20°C for 10 min. After blocking with 3% BSA for 1 h at RT, the cells were incubated with the corresponding monoclonal or polyclonal antibodies in a 1:150 dilution in PBS with 1% BSA for 2 h at RT. After washing three times with PBS, the coverslips were incubated with fluorescent secondary antibodies: goat anti-rabbit IgG-Cy3 and goat anti-mouse IgG FITC, each diluted 1:150 with 1% BSA for 1 h at RT. The cells were mounted with 1% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/0.1% (w/v) 4’-6-diamidino-2-phenylindole (DAPI) in 50% glycerol. After airdrying, the analyses were performed with a MRC 1024 microscope (Bio-Rad) using Lasersharp software (34).

Preparation of total cell extracts

Cells (2 × 10^6) were homogenized in a medium containing 10 mmol/l Tris–HCl, pH 7.5, 1 mmol/l MgCl2, 1 mmol/l EDTA, 10% glycerol, 0.5% 3-(3-cholamidopropyl) dimethylammonio)-1-propanesulfonate (CHAPS), 1 mmol/l β-mercaptoethanol and 0.1 mmol/l PMSF. The extracts were vortexed for 30 min at 4°C and after centrifuging for 20 min at 13000 g, the supernatants were stored at −20°C. For western blot analysis, total proteins were boiled in Laemmli sample buffer and equal amounts of the protein (20–30 μg) were loaded on to a 10–12% SDS–PAGE.

Western blotting analysis

The amount of COX-2 (Cayman, cat # 160107), COX-1 (Santa Cruz, sc 1752), integrin β1 (Santa Cruz, sc 6622) and integrin αvβ3 (Chemicon, LM609) were determined in total cellular extracts by immunoblot using the commercial Abs given in parentheses. The amount of MMP-2 and MMP-9 (Chemicon, AB805 and 809) was determined in total extracts and in the culture medium. To determine the secreted MMP-2 and MMP-9, 500 μl of the medium was treated with 25 mmol/l EDTA and 0.05% SDS and concentrated by a Centricon 10. After digestion for 18 h at 4°C, the samples were denatured and resolved by electrophoresis. Equal amounts of protein were size-fractionated in 8–10% SDS–PAGE.

Fig. 2. Effect of PGE2 on HCC cell migration and MMP secretion. Migration of cells (10^5) was assayed in 24-well transwells with 8.0-μm polycarbonate filters coated with Type I collagen. Results are mean ± SD of three different experiments and expressed as percentage of the control value for THLE-2 (A). Effect on cell migration of the incubation for 18 h with 5 μM PGE2, 2 μM 15-deoxy-Δ12,14-PGJ2 and 5 μM DFU. Results are mean ± SD of four different experiments and expressed versus their respective control conditions without treatment (B). Effect of 5 μM arachidonic acid challenge on HuH-7 and HTB-52 cell migration (C). The protein levels of MMP-9 and MMP-2 secreted in the cultured medium. MMP-9 and MMP-2 were determined by western blot. A representative western blot is shown in (D). Results are the mean ± SD of three different experiments and expressed as percentage with respect to the control value for THLE-2 control cells. *P < 0.05 versus THLE-2 control cells. #P < 0.05 versus cells without treatment.

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SDS-PAGE, transferred to a Hybond P membrane (Amersham) and after blocking with 5% non-fat dry milk, incubated with the corresponding polyclonal or monoclonal antibodies. The blot was revealed after incubation with the anti-rabbit or anti-mouse HRP conjugated following the ECL protocol as recommended (Amersham). Blots were normalized by measuring the amount of β-actin. Different exposition times were performed for each blot to ensure the linearity of the band intensities. Densitometric analysis of the bands was performed using a scanner (Amersham) and the results were expressed in arbitrary units.

**Determination of metabolites**

PGE2 levels were determined in the culture medium using a specific immunoassay system (Amersham). Protein levels were determined with Bradford reagent.

**Data analysis**

Data are expressed as mean ± standard deviation (SD). Statistical significance was estimated with the Student’s t-test for unpaired observation. The results were considered significant at $P < 0.05$. Data were analyzed by SPSS for Windows statistical package version 9.0.1.

**Results**

**COX-2 expression in HCC cells**

COX-2 expression was analyzed in control-like liver cells (THLE-2 and CHL) and HCC cells (HuH-7 and HTB-52) by western blot and immunocytochemistry. As shown in

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Fig. 3. Expression of αVβ3 and β1 integrins in HCC cells. Total cellular extracts were analyzed to determine αVβ3 protein levels by western blot in THLE-2 and HCC cells. A representative western blot (n = 3) is shown in (A). Effect of incubation for 18 h with 5 μM PGE2 and 5 μM DFU on αVβ3 protein levels in THLE-2 and HuH-7 cells (B). Immunocytochemical analysis of β1 integrin in HCC cells (C). Protein levels (D) and effect of PGE2 and DFU on the amount of β1 integrin (E). Results are mean ± SD of three different experiments and expressed versus THLE-2 cells as percentage of the control situation. *$P < 0.05$ versus the THLE2 control cells. **$P < 0.05$ versus cells without treatment. See online supplementary material for a colour version of this figure.
Figure 1A. COX-2 protein was absent in THLE-2 cells, but significant levels were detected in HuH-7 and HTB-52 cells. CHL cells display an intermediate phenotype, with a slight band of COX-2 protein. Treatment of the cells with LPS, pro-inflammatory cytokines or phorbol esters did not enhance the COX-2 protein levels (data not shown). These results were confirmed by immunocytochemistry (Figure 1B), with a clear staining for COX-2 in the endoplasmic reticulum and perinuclear membranes. COX-1 and COX-2 expressed in the HCC cells are functional as demonstrated in Figure 1C by the presence of PGE2 in the culture medium. Incubation with the COX-2 selective inhibitor DFU revealed the contribution of each isoenzyme to PGE2 release (Figure 1D).

**Effect of PGE2 on cell migration and MMP secretion**

As shown in Figure 2A, the number of cells that migrated through the filters coated with Type I collagen was much higher in HuH-7 and HTB-52, the cells with constitutive COX-2 expression, than in the control cells THLE-2. It has been described that PGE2, produced by COX-2, was implicated in cell-spreading and migration in HUVECs (31). To test this possibility, the control and HCC cells were incubated with PGE2 and DFU for 18 h and as shown in Figure 2B, PGE2 promoted a significant increase in the number of migrated HuH-7 and HTB-52 cells, but not in control cells. Moreover, treatment with DFU reduced ~60% of the basal migration of HCC cells. We also evaluated the effect of the cyclopentenone 15-deoxy-

**Expression of αVβ3 and β1 integrins in HCC cells**

Once the relationship between PGE2 produced by COX-2 with the migratory capacity and MMP secretion in HCC cells was established, the levels of αVβ3 and β1 integrins and the effect of PGE2 on integrin-mediated cell adhesion were analyzed. Our results show that the levels of αV integrin were significantly higher in HCC cells when compared with the control cells (Figure 3A). However, they were unaffected after the treatment with PGE2 or DFU (Figure 3B). The amount of β1 integrin was also analyzed by immunocytochemistry (Figure 3C). The results show that the β1 subunit is localized at the plasma membrane with no redistribution in hepatoma cell lines. Evaluation of the total amount of integrin β1 by immunoblot showed higher levels in the control cells (Figure 3D). Treatment with PGE2 enhanced the levels in HuH-7 cells considerably, reaching the levels measured in THLE-2 cells (Figure 3D and E).

**Effect of PGE2 on HCC cell adhesion**

In addition to constitutive integrin levels, cell migration and adhesion to a given substratum depends on several variables, including the substratum concentration, binding affinity and integrin activation. Hepatocytes are the main source of vitronectin and the capacity to synthesize and secrete vitronectin is usually retained in HCC cells (30). To determine the correlation between integrin levels and cell adhesion, we analyzed the adhesion to gelatin, vitronectin and fibronectin and the effect of PGE2 in the control and HCC cells. Our results show a significant increase of HCC adhesion onto fibronectin, gelatin and vitronectin versus the control cells (Figure 4), suggesting that the α5β1 and αVβ3 integrins are implicated in HCC adhesion. The addition of PGE2 during adhesion to fibronectin slightly increased the cell attachment of the control or HCC cells (Figure 5A and B); however, a clear increase of HuH-7 cell adhesion owing to the effect of PGE2 was observed when the matrix was vitronectin (Figure 5B) and a decrease was observed when the cells were treated with DFU. Figure 5C shows the cells fixed and stained for 30 min after plating, exhibiting important differences between the control and HuH-7 cells owing to the effect of PGE2 treatment.

To evaluate the contribution of various intracellular signaling pathways to the migration of HCC cells in response to the treatment with PGE2, we performed a series of experiments as described in the Materials and methods section. The results are presented in Figure 6, where we show that the effect of PGE2 on cell migration and adhesion was significantly inhibited by the treatment with the COX-2 selective inhibitor DFU.

Fig. 4. Adhesion of HCC cells. Cell adhesion (5 × 10⁴ cells) was performed in 96-well plates coated with fibronectin (10 μg/ml), vitronectin (5 μg/ml) and gelatin (0.5%) as described in Materials and methods. After incubation for 30 min at 37°C, the attached cells were fixed, stained with 0.5% crystal violet and quantified by optical density (OD) reading at 620 nm. Specific adhesion is equal to the adhesion on an extracellular matrix protein subtracting the adhesion on BSA. Results are given as OD values and are the mean ± SD of three different experiments. *P < 0.05 versus the THLE2 control cells.
to COX-2 dependent metabolites, the cells were challenged with PGE2 and treated with pharmacological inhibitors of phosphatidylinositol-3-kinase (LY204002), mitogen activated protein kinase/extracellular signal-regulated kinase (MEK/ERK) (PD98059), p38 mitogen-activated protein kinase (MAPK) (SB202190), PKA (4-cyano-3-methyl-isoquinoline, CMI) and PKC (Gö 6850, Gö 6976). As shown in Figure 6 the treatment of HuH-7 cells with LY204002 did not affect the migration induced by PGE2; however, PD98059, SB202190, CMI and PKC inhibitors suppressed the migration of HuH-7 cells at different degrees, indicating that the MEK/ERK, p38 MAPK, PKA and PKC signaling pathways are involved in the process.

Discussion

The present study has shown that the PGs produced by COX-2 are implicated in migration, secretion of MMP and cell adhesion mainly through integrin αVβ3 in HCC cells and these effects of PGs are mediated by different signaling pathways, including MAPKs, PKA and PKC. The role of COX-2 in

Fig. 5. Effect of PGE2 and DFU on cell adhesion and spreading in HCC cells. Cell adhesion assays were performed as in Figure 4 but 5 µM PGE2 and 5 µM DFU were added to the cells at the time of plating. The attached cells were fixed, stained and quantified. THLE-2 and HuH-7 cells were plated under the same conditions as described for panels (A and B), but treated with 5 µM PGE2, fixed and photographed 30 min after plating. A representative experiment out of two is shown in (C). Results are given as OD values and are the mean ± SD of four different experiments. *P < 0.05 versus the cells without treatment.
modulating cell growth and tumor progression was well established in colon cancer (6,7), and recent studies have reported the COX-2 expression in different human tumors, including HCC (16,35). Indeed, COX-2 is expressed in different hepatoma cell lines and the treatment of these cells with COX-2 inhibitors impaired cell growth, invasiveness and proliferation, and induced apoptosis (12,13,17).

The process of tumor cell invasion requires the ability of the cells to interact with endothelial cells and extracellular matrix to release MMPs. A good correlation has been found between the expression of MMP and the invasive ability of malignant tumors, including HCC (36,37). Our previous results demonstrated that PGs produced by COX-2 are implicated in the release of MMP-2 and MMP-9 through the activation of PKA and p38 MAPK in fetal hepatocytes (21). Moreover, the increased invasive capacity induced by hepatitis B virus X protein (HBx) in liver cells, is mediated by an upregulation of MT-1-MMP and MMP-2 expression and this effect is dependent on COX-2 activity (38). The present results show a relationship between COX-2 expression, cell migration and the secretion of MMPs. In this context, the HuH-7 and HTB-52 cell lines, which express high levels of COX-2, exhibited a higher migration on collagen filters and secretion of MMP-2 (45). The expression of MMP and the invasive ability of malignant tumors, including HCC (36,37). Our previous results demonstrated that COX-2 expression in different human tumors, including HCC (16,35). Indeed, COX-2 is expressed in different hepatoma cell lines and the treatment of these cells with COX-2 inhibitors impaired cell growth, invasiveness and proliferation, and induced apoptosis (12,13,17).

In addition to MMPs, integrins play a central role in the process of cell migration and invasiveness. A clear relationship between integrins and the expression of MMPs has been established through the process denominated as integrin-guided proteolysis (22). For example, signal transduction through β1 integrins induces the production of MMP and activation of MMP proenzymes in rabbit synovial fibroblasts (39). Furthermore, the activated MMPs can be localized with β1 integrins at the focal contacts on the surface of invasive cells (40).

In invasive melanoma cells, activation of the αvβ3 integrin results in an increase of MMP secretion (41).

Several studies have described the altered expression of β1, α subunits and αv integrins in HCC both in vivo and in vitro. It has been reported that in HCC cells, the levels of constitutive activity of β1 integrin correlated with the invasive capacity (42). Moreover, β1 integrin overexpression converted the phenotype of HepG2 cells induced by hepatocyte growth factor (HGF) from inhibition to growth stimulation (28). Indeed, HCC coexpressing αv integrin chain and vitronectin are characterized by a high frequency of adverse histological factors and αv integrins contribute to mediate cell adhesion and migration onto vitronectin and fibronectin in hepatoma cells. In cells expressing the HBx protein from the HBV, the levels of the β1 subunit and αvβ3 integrin were similar in the control and HBx cell types, but activated β1 integrin was redistributed to the tips of the pseudopodial protrusions of HBx cells, thus contributing to the migratory phenotype and tumor-spreading of HBV (43). The relationship between COX-2 expression and integrin function has been clearly established in endothelial cells where PGE2 accelerated αvβ3-mediated HUVEC adhesion and promote Rac activation and cell-spreading through the PKA signaling pathway (31,32). In addition to endothelial cells, it has been described that in colon cancer cells, PGE2 mediates the adhesion through the upregulation of αvβ1 integrin (44).

Our results show that the integrin β1 subunit is localized at the plasma membrane with no redistribution in the hepatoma cell lines, but it was present in both the HCC and control liver cells. Cell adhesion assays on fibronectin exhibited an increase in HCC cells with respect to the control cells, indicating that the fibronectin receptor α5β1 is altered in HCC cells rather than the β1 subunit. However, PGE2 increased, although not significantly, the adhesion of the cells onto fibronectin, in line with the results obtained in endothelial cells (31) and suggesting that the αvβ1-mediated HCC adhesion on fibronectin was independent of COX-2-derived PGE2. With respect to αvβ3 integrin, there was a clear increase in protein levels determined by western blot in HuH-7 cells, which correlates with COX-2 expression, the migratory capacity and protein levels of MMPs of this hepatoma cell line. Cell adhesion onto vitronectin and gelatin showed an important increase in HuH-7 cell line, demonstrating that αvβ3-dependent adhesion is increased in the HCC cells. PGE2 induced a significant increase in the adhesion of HuH-7 cells onto vitronectin, without changes in the total amount of αvβ3. The addition of DFU decreased the adhesion of the cells to vitronectin, suggesting that PGE2 promotes the integrin αvβ3-dependent HCC cell adhesion.

With respect to the mechanism implicated in integrin αvβ3-dependent HCC cell adhesion induced by COX-2/PGE2 pathway, Dormond et al. (31) reported that in endothelial cells, αvβ3-dependent adhesion, spreading and migration is mediated through cAMP/PKA-dependent signaling pathway. Our previous results (21) demonstrated that PGE2 promotes the release of MMPs in hepatocytes and that inhibitors of PKA, p38 MAPK and NF-xB impaired this process. However, in colon cancer cells, PGE2 regulates the cell migration via a transactivation of the epidermal growth factor receptor (EGFR) and HGF receptor, activation of Akt and nuclear accumulation of β-catenin (45,46). Our results show that in HCC cells, different signaling pathways, MEK/ERK, p38 MAPK, PKA and PKC are implicated in the migration induced by PGE2.
In summary, our results demonstrate that COX-2 dependent PGE$_2$ is implicated in migration, secretion of MMPs and adhesion of human hepatoma cell lines; hence it is apparent that it is a common mechanism by which the COX-2 expression modulates the development of tumor progression and suggests new targets for therapeutic options in human HCC.

Supplementary material

Supplementary material can be found at: http://www.carcin.oupjournals.org

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