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Protein Kinase CK2 Promotes Cancer Cell Viability Via Up-Regulation of Cyclooxygenase-2 Expression and Enhanced Prostaglandin E2 Production

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ABSTRACT

Augmented expression of protein kinase CK2 is associated with hyperproliferation and resistance to apoptosis in cancer cells. Effects of CK2 are at least partially linked to signaling via the Wnt/β-catenin pathway, which is dramatically enhanced in colon cancer. Cyclooxygenase-2 (COX-2), a Wnt/β-catenin target gene, has been associated with enhanced cancer progression and metastasis. However, the possibility that a connection may exist between CK2 and COX-2 has not been explored previously. Here we investigated changes in COX-2 expression and activity upon CK2 modulation and evaluated how these changes affected cell viability. COX-2 expression and cell viability decreased upon selective inhibition of COX-2 with SC-791 or CK2 with 2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole (DMAT), both in human colon (HT29-ATCC, HT29-US, DLD-1) and breast (ZR-75) cancer cells, as well as in human embryonic kidney (HEK-293T) cells. On the other hand, ectopic CK2α expression promoted up-regulation of COX-2 by activating the Wnt/β-catenin pathway in HEK-293T cells. Noteworthy, over-expression of either CK2α, β-catenin or COX-2, as well as supplementation of the medium with prostaglandin E2 (PGE2), all were individually sufficient to overcome limitations in cell viability triggered by CK2 inhibition either upon addition of DMAT or over-expression of a dominant negative CK2α variant. Altogether, these findings provide new insight to the role of CK2 in cancer by up-regulating COX-2 expression and thereby PGE2 production. J. Cell. Biochem. 112: 3167–3175, 2011.

KEY WORDS: CK2; β-CATENIN; CYCLOOXYGENASE-2; PROSTAGLANDIN E2; CANCER

Canonical Wnt (or Wnt/β-catenin) signaling is essential in development, stem cell maintenance, and tissue regeneration. Also, enhanced signaling via this pathway is associated with the onset and progression of multiple cancers in humans [Beachy et al., 2004]. Activation of the pathway is linked to hypophosphorylation of β-catenin, its stabilization and accumulation in the cytosol and nucleus followed by increased gene expression [Heeg-Truesdell and LaBonne, 2006; Kimelman and Xu, 2006]. Augmented cytoplasmic β-catenin levels and subsequent nuclear import permit association with transcription factors of the Tcf/Lef family that control the expression of genes, including *cyclin D1*, *vegf*, and *survivin*, all of which contribute to cell survival and metastasis [Altieri, 2004; Nagy et al., 2007; Kerbel, 2008]. To preclude activation of these events, cytosolic β-catenin is assembled into a multi-protein complex including axin, the adenomatous polyposis coli (APC) protein and GSK3β. This protein kinase

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phosphorylates several sites at the N-terminal end of β-catenin, which targets it for proteasomal degradation [Kimelman and Xu, 2006]. Mutations of APC and/or β-catenin are frequently found in cancer cells, increasing β-catenin levels in the cytoplasm and its transcriptional activity, thereby promoting the expression of survivin and consequently increasing cancer cell survival [Zhang et al., 2001; Segditsas and Tomlinson, 2006].

Many other kinases in addition to GSK3β have been implicated in the regulation of these events. In particular, protein kinase CK2 participates as a critical component of the canonical Wnt pathway by interacting with and phosphorylating APC, as well as β-catenin at residue Thr-393 [Seldin et al., 2005; Gao and Wang, 2006]. CK2 is a ubiquitous heterotetrameric eukaryotic Ser/Thr-kinase formed by catalytic (α or α′) and regulatory (β) subunits in the combinations $\alpha_2\beta_2$, $\alpha'_2\beta_2$, or $\alpha\alpha'\beta_2$ [Pinna, 2002]. When over-expressed, CK2 behaves as an oncogene and induces neoplastic growth [Guerra and Issinger, 1999; Tawfic et al., 2001]. This enzyme phosphorylates over 300 proteins participating in many processes like replication, transcription, translation, signal transduction, and cell death [Ahmed et al., 2002; Litchfield, 2003; Meggio and Pinna, 2003]. Furthermore, elevated CK2 levels are associated with increased mammary cell proliferation and hyperplasia in vitro and in vivo [Landesman-Bollag et al., 2001a,b]. The ability of CK2 to promote proliferation is generally associated with phosphorylation of cell cycle regulatory proteins, such as p53, MDM-2, $p34^{cdc2}$, and $p27^{KIP1}$ [Pinna, 2002; Litchfield, 2003; Tapia et al., 2004]. CK2 promotes the expression of the inhibitor of apoptosis protein (IAP) survivin, a known β-catenin-Tcf/Lef target gene, in colon cancer cells. Indeed, 4,5,6,7-tetrabromobenzotriazole (TBB) and 2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole (DMAT) have been shown to reduce expression of β-catenin and survivin, a known anti-apoptotic protein that has also been implicated in cell cycle progression [Altieri, 2004; Tapia et al., 2006]. In addition, co-expression of CK2α and c-Rel in mammary tumor cells is sufficient to up-regulate slug, a metastasis-related repressor of E-cadherin expression and thereby favor cell invasiveness [Belguise et al., 2007].

Cyclooxygenase-2 (COX-2) is also thought to represent a target of the Wnt/β-catenin signaling pathway in articular chondrocytes, as well as in colon and liver cancer cells [Gupta and Dubois, 2001; Liu et al., 2001; Kim et al., 2002; Araki et al., 2003]. Two distinct genes encode COX enzymes, *cox-1* and *cox-2* [Hla et al., 1999]. COX-1 is constitutive in most tissues, but COX-2 expression is induced by growth factors, inflammation, and carcinogenic signals [Telliez et al., 2006]. Enhanced COX-2 mRNA and protein levels observed in colon cancer are associated with augmented cancer development and progression, as well as phenotypic changes that favor cancer metastasis [DuBois et al., 1996; Kutchera et al., 1996; Tsujii et al., 1997; Kanaoka et al., 2007]. Cyclooxygenases (COXs) catalyze the conversion of arachidonic acid to prostaglandin H2 (PGH2), which is then processed to prostaglandin I2 (PGI2), prostaglandin D2 (PGD2), tromboxane A2 (TXA2), and prostaglandin E2 (PGE2) by specific synthases [Wang et al., 2007]. PGE2, a secreted product generated as a consequence of COX-2 activity, has been observed to increase β-catenin protein levels as well as colon cancer cell proliferation and viability [Shao et al., 2003, 2005; Castellone et al., 2005, 2006]. Interestingly, COX-2 expression and PGE2 production have been suggested to stabilize survivin protein and promote resistance to apoptosis [Krysan et al., 2004a,b].

In this study, we investigated whether protein kinase CK2 is involved in the up-regulation of COX-2 through the Wnt/β-catenin signaling pathway. Our results demonstrated that CK2-mediated up-regulation of COX-2 involves activation of β-catenin-Tcf/Lef-dependent transcription in human colon (HT29-ATCC, HT29-US, DLD-1) and breast (ZR-75) cancer cell lines, as well as human embryonic kidney (HEK-293T) cells. Additionally, ectopic expression of the CK2α catalytic subunit, β-catenin or COX-2, as well as supplementation of the medium with PGE2, all prevented decreased cell survival observed following inhibition of CK2. Taken together, these data provide important new insights to the role of protein kinase CK2 as an oncogene by implicating it as a positive regulator of COX-2-dependent PGE2 production.

MATERIALS AND METHODS

MATERIALS

Cell medium, antibiotics, and the TriZOL® reagent were from Invitrogen (Paisley, Scotland, UK). Fetal bovine serum (FBS) was from HyClone (Logan, UT). The COX-2 inhibitor SC-791 was from Pharmacia (St. Louis, MO). CK2 inhibitor DMAT, COX-2 inhibitor SC-791, and PGE2 were from Calbiochem (San Diego, CA). MTS® and CaspaseGlo® assays were from Promega (Madison, WI). COX-2 Activity Assay kit was from Cayman Chemical Company (Ann Arbor, MI). Monoclonal anti-β-catenin antibody was from Becton Dickinson (Lexington, KY). Goat polyclonal anti-human COX-2 antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal anti-survivin was from R&D Systems (Minneapolis, MN). Polyclonal anti-actin antibody and anti-mouse IgG antibody coupled to HRPO were from Sigma (St. Louis, MO). Anti-rabbit IgG coupled to HRPO was from Bio-Rad Laboratories (Hercules, CA). Protran® membrane was from PerkinElmer (Boston, MA). BCA protein determination kit was from Thermo Scientific (Rockford, IL). Superfect® and the Plasmid Midiprep kit were from Qiagen (Valencia, CA). EZ-ECL Chemiluminescence kit was from Biological Industries (Kibbutz Beit, Haemek, Israel). Luciferin was from USBiological (Swampscott, MA). Buffers and all other reagents used, but not specified, were from Sigma or the highest grade available.

CELL CULTURE AND TRANSFECTION

Culture of human HT29-ATCC, HT29-US and DLD-1 colon, and ZR-75 breast cancer cell lines, as well as human HEK-293T embryonic kidney cells were maintained at 37°C and 5% CO_2 in DMEM-HG supplemented with 10% FBS and antibiotics (10,000 U/ml penicillin, 10 μg/ml streptomycin). Superfect® transfection reagent was used following instructions provided by the manufacturer. Efficiency was roughly 50% in HEK-293T cells, as assessed by flow cytometry analysis using pEGFP-C1 empty vector (data not shown).

PLASMIDS

Wild-type CK2α and a dominant negative variant (D156A) have been described elsewhere [Tapia et al., 2002, 2006]. The specific

COX-2 reporter plasmid pGL3-COX-2 (containing a functional Tcf/Lef site) was kindly provided by Dr. Ying Zhu (Laboratory of Virology, Wuhan University, Wuhan, China). Plasmid pOSML-COX-2 encoding human wild-type COX-2 was kindly provided by Dr. Tim Hla (Center for Vascular Biology, University of Connecticut Health Center, Farmington). Plasmids pEGFP-β-catenin, encoding wild-type GFP-β-catenin, pCMV5-myr-HA-AKT-CA and pCMV5-HA-AKT-DN, encoding constitutively active or dominant negative Akt mutants, respectively, have been described elsewhere [Ponce et al., 2011].

PROLIFERATION ASSAY

Previously transfected HEK-293T or cancer cells were seeded in 96-well plates at a density of 1×10^4 cells/well. After 24 h, cells were treated with the compounds (i.e., DMAT or PGE2) at the indicated concentrations for an additional 16–20 h. Proliferation was measured using the MTS® assay according to the manufacturer's instructions.

APOPTOSIS ANALYSIS

Apoptotic cells were quantified following propidium iodide (PI) staining by flow cytometry, essentially as described [Torres et al., 2006]. Briefly, viable, apoptotic, and necrotic fractions were determined in roughly 2×10^4 cells by plotting PI fluorescence versus the forward scatter parameter using the Cell Quest program. When indicated, caspase-dependent apoptosis was also measured by the chemiluminescence-based Caspase Glo® assay following instructions provided by the manufacturer.

COX-2 ACTIVITY ASSAY

COX-2 activity was determined with the COX Activity Assay kit according to the manufacturer (Cayman Chemical Company). Briefly, peroxidase activity of COXs in crude cell lysates is measured colorimetrically by monitoring the appearance of oxidized *N,N,N′,N′*,-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm, using isozyme-specific COX inhibitors to distinguish COX-2 from COX-1.

ANALYSIS OF mRNA LEVELS

Total RNA was isolated with TriZOL® following instructions provided by manufacturer. RNA templates were used to generate cDNAs by PCR for COX-2 and actin using the primers and protocols described elsewhere [Rodriguez et al., 2009].

PROTEIN DETECTION BY WESTERN BLOTTING

Cell lysates were prepared in RIPA buffer and separated by SDS–PAGE on acrylamide minigels. Proteins were transferred to nitrocellulose and subsequently blocked with 5% milk in 0.1% Tween/PBS. Blots were probed with specific primary antibodies, which were then detected with secondary antibodies conjugated to HRPO. Bands were visualized using the EZ-ECL® chemiluminescence kit.

LUCIFERASE REPORTER ASSAY

HEK-293T cells were co-transfected with 2 μg of either the plasmid pGL3-COX-2 (containing the COX-2's promoter) or pGL3 (empty vector) together with other plasmids as indicated. Cells were treated after transfection with the specific CK2 inhibitor DMAT (40 μM) for 16–24 h and then were lysed in a buffer containing 100 mM KH_2PO_4 (pH 7.9), 1 mM DTT, and 0.5% Triton X-100. Supernatants were either analyzed by Western blotting or luciferase activity was measured following manufacturer's instructions. Reported values for luciferase activity were used for calculating the pGL3-COX-2/pGL3 activity ratios.

STATISTICAL ANALYSIS

All results were obtained of at least three independent experiments. When required, data were compared using the Dunnett's or Student's methods after ANOVA, except for Figure 1 where the Wilcoxon method was applied. A value for $P \leq 0.05$ was considered significant.

RESULTS

CYCLOOXYGENASE-2 OR CK2 INHIBITION DECREASE VIABILITY OF CANCER CELLS

The effects of a selective COX-2 inhibitor, SC-791, were evaluated in HT29-ATCC, HT29-US (a cell line derived from HT29-ATCC with higher metastatic potential [Torres et al., 2007]) and DLD-1 colon, as well as ZR-75 breast cancer cell lines. Decreases in proliferation were similar in HT29-ATCC and DLD-1 cells, but more pronounced in ZR-75 cells (Fig. 1A). Interestingly, these changes in cell proliferation were inversely correlated with basal COX-2 activity. Highest COX-2 activity was observed in HT29-US followed by HT29-ATCC and DLD-1 colon cancer cells, while ZR-75 breast cancer cells had the lowest levels (Fig. S1). The opposite was observed for apoptosis in the presence of SC-791, since decreased proliferation (and basal COX-2 activity) coincided with increased apoptosis in all cells (Fig. 1B). However, although SC-791 had essentially no effect on the proliferation of HT29-US cells, apoptosis was significantly increased in the presence of this inhibitor. In addition, inhibition of CK2 with the most specific inhibitor available, DMAT, yielded changes in proliferation that were similar to those observed with SC-791 (Fig. 1C). Furthermore, DMAT-induced decreases in proliferation correlated with increased apoptosis levels in all cells (Fig. 1D).

CK2 UP-REGULATES CYCLOOXYGENASE-2 EXPRESSION AND THEREBY ENHANCES VIABILITY OF CANCER CELLS

Since ectopic COX-2 augments expression of survivin, a known β-catenin target [Rodriguez et al., 2009], we anticipated that survivin may be also down-regulated by SC-791 in cancer cells. Indeed, decreased cell viability was paralleled by reduced expression of survivin, as well as COX-2 (Fig. 2A). This result suggested that SC-791-induced decreases in viability of cancer cells may be linked to down-regulation of COX-2 and survivin by a common transcriptional mechanism. Additionally, CK2 inhibition with 40 μM DMAT reduced COX-2 mRNA and protein levels in all cancer cells (Fig. 2B). In fact, DMAT decreased in a dose-dependent manner COX-2 mRNA and protein levels ($IC_{50} \sim 40$ μM) in HT29-US colon cancer cells (Fig. 2C), as well as in DLD-1 colon and ZR-75 breast cancer cells (data not shown). Decreased COX-2 expression was paralleled by diminished enzymatic activity (Fig. S2) and reduced viability of

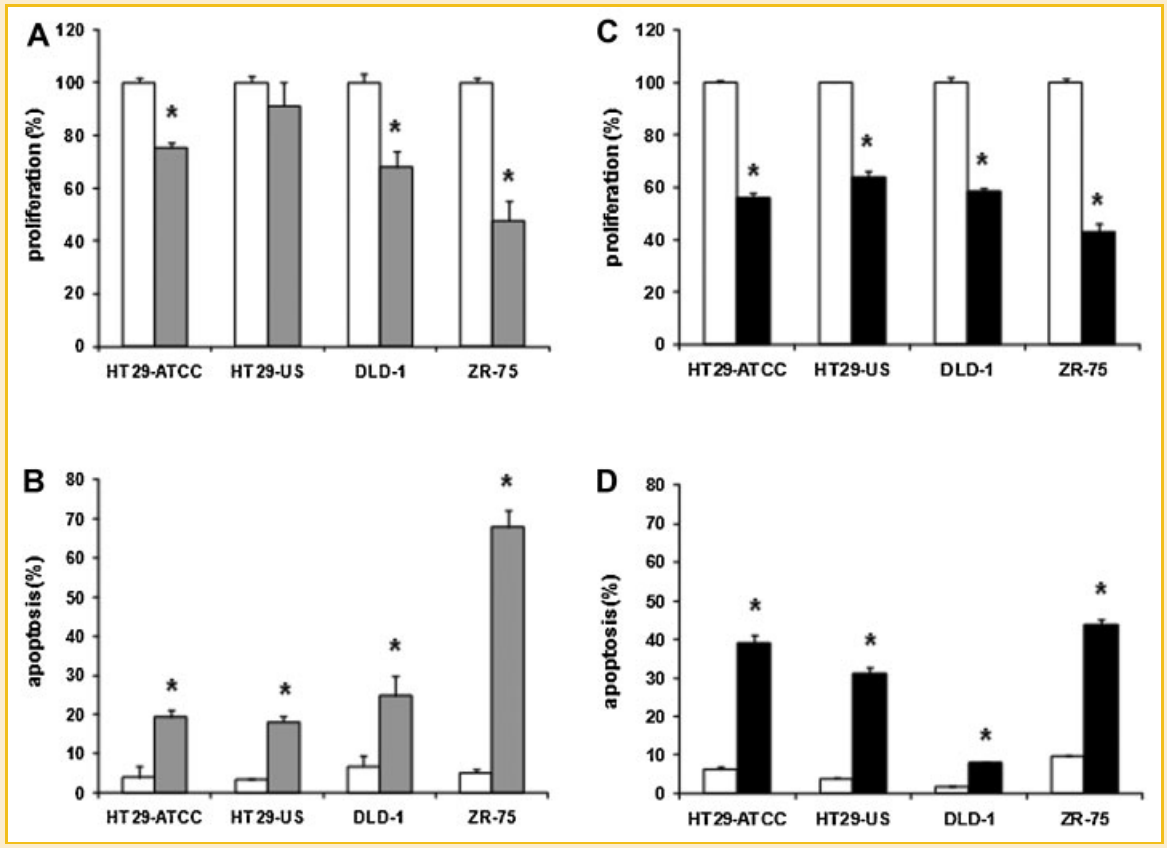

Fig. 1. COX-2 or CK2 inhibitors decreased viability of human colon and breast cancer cells. A: Cancer cells were grown in the absence (white bars) or presence (gray bars) of SC-791 (10 μM) for 16–20 h and proliferation was measured using the MTS® assay. B: Cells were grown as in (A) and apoptosis after treatment with selective SC-791 (gray bars) was detected by flow cytometry. Samples containing roughly 2×10^4 cells were evaluated. C: Cells were grown in the absence (white bars) or presence (black bars) of DMAT (40 μM) for 16–20 h and proliferation was determined by the MTS® assay. D: Cells were grown as in (C) and apoptosis was determined by flow cytometry after treatment with DMAT (black bars). Note that proliferation in the absence of inhibitor was considered as the reference value (100%) for each cell line. On an average, 6.0 ± 1.8% of necrotic cells was observed in all experimental settings. Statistically significant differences for data in the absence or presence of inhibitors were calculated by the Wilcoxon method (mean ± SEM; $^*P < 0.05$).

HT29-US cells, since both decreases in proliferation (Fig. S3) and augmented apoptosis (Fig. 2D) were observed with the specific CK2 inhibitor DMAT. Altogether, these results suggest that CK2 activity may regulate COX-2 expression, and that down-regulation of COX-2 by inhibiting CK2 can lead to diminished viability of colon and breast cancer cells.

CK2 REGULATES CYCLOOXYGENASE-2 EXPRESSION VIA MODULATION OF β-CATENIN ACTIVITY IN HUMAN EMBRYONIC KIDNEY CELLS

The effect of DMAT on COX-2 expression cells was evaluated in HEK-293T human embryonic kidney cells. Dose-dependent reductions in COX-2 mRNA and protein levels were detected with DMAT concentrations beyond 40 μM (Fig. 3A). Likewise, β-catenin protein levels were substantially reduced in a manner similar to what we previously reported with TBB, another CK2 inhibitor [Tapia et al., 2006]. A promoter specific reporter assay was employed to evaluate changes in *cox-2* gene transcription. DMAT led to a dose-dependent decrease in COX-2 transcription, which was significant at concentrations beyond 20 μM (Fig. 3B). Moreover, DMAT concentrations above 20 μM significantly decreased cell viability by augmenting apoptosis (Fig. 3C). Finally, GFP-CK2α or GFP-β-catenin was ectopically expressed and reporter activity was evaluated in the absence or presence of DMAT. Both CK2α and β-catenin expression almost duplicated COX-2 transcription; however, the increments were essentially ablated by CK2 inhibition (Fig. 3D). Taken together, these data demonstrate that CK2 up-regulates COX-2 expression by modulating the Wnt/β-catenin signaling pathway and, in doing so, promotes viability of embryonic cells.

CK2-DEPENDENT UP-REGULATION OF COX-2 EXPRESSION IS LINKED TO THE EXPRESSION OF KEY CANCER-RELATED GENES

Taking into consideration the above results and that we have recently described that the up-regulation of β-catenin activity also requires hyperactivation of Akt/PKB following phosphorylation at Ser-129 by CK2 in HEK-293T cells [Ponce et al., 2011], we ectopically expressed dominant negative forms of either CK2α (i.e., CK2α-DN) or Akt (i.e., Akt-DN), or treated cells with DMAT to down-regulate β-catenin, COX-2, and survivin protein levels. As observed in Figure 4, inhibition of CK2 with DMAT led to decreased β-catenin, COX-2, and survivin protein levels. However, although the levels of

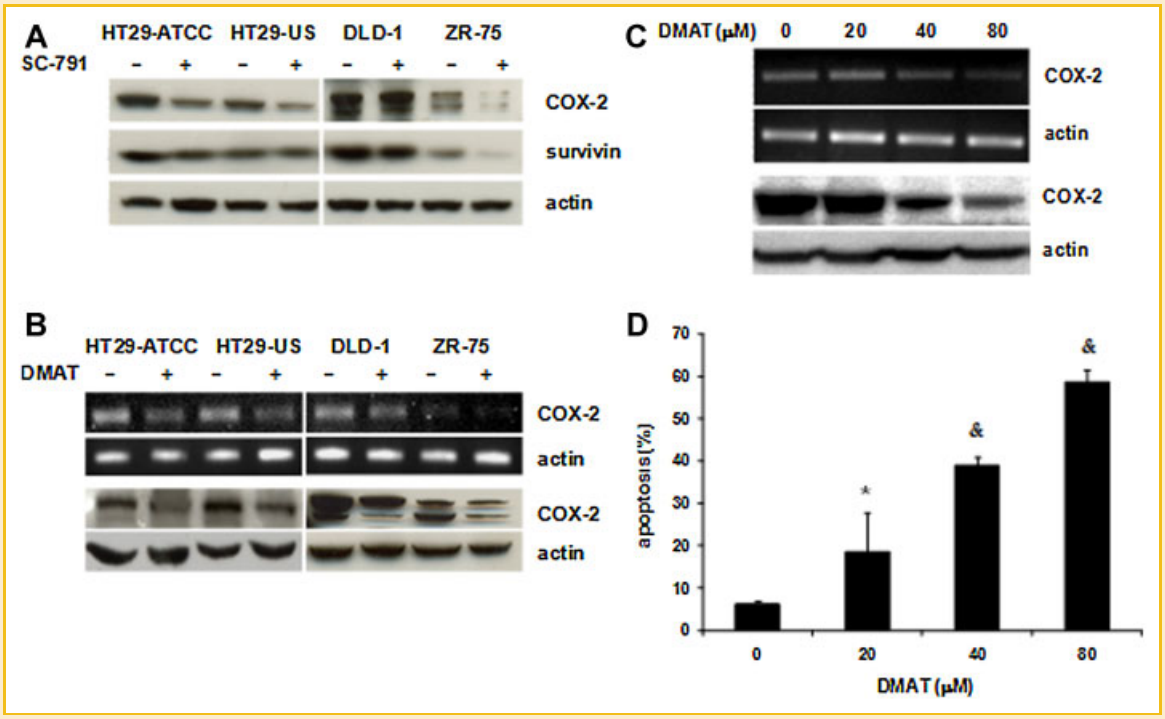

Fig. 2. COX-2 or CK2 inhibition down-regulated COX-2 and survivin expression in cancer cells. A: Cells were grown in the absence (−) or presence (+) of SC-791 (10 μM) for 16–20 h. Then, protein lysates (30 μg for HT29-ATCC and HT29-US cells; 60 μg for DLD-1 and ZR-75 cells) were separated by SDS–PAGE and COX-2, as well as survivin protein levels were analyzed by Western blotting with specific antibodies. B: Cancer cells were grown in the absence (−) or presence (+) of DMAT (40 μM) for 16–20 h. COX-2 mRNA (upper panel) and protein (same amounts as in (A), lower panel) levels were evaluated. C: HT29-US cells were grown for 16–20 h in the presence of increasing concentrations of DMAT (0–80 μM) and COX-2 mRNA levels were evaluated by conventional RT-PCR (upper panel). Protein lysates were separated by SDS–PAGE and analyzed by Western blotting with the indicated antibodies (lower panel). Actin mRNA and protein levels were used as an internal control. D: Cells were grown for 16–20 h as in (C) and apoptosis was analyzed by flow cytometry. Samples containing roughly 2×10^4 cells were evaluated. Statistically significant differences compared to non-treated cells were calculated by the Dunnett's method (mean ± SEM; $^{*}P < 0.05$, $^{\&}P < 0.005$).

COX-2 and survivin after ectopic expression of CK2α-DN were quite similar to DMAT treatment, this CK2α mutant did not decrease β-catenin levels in a same extent. Likewise, survivin levels were differentially affected upon Akt-DN expression, despite both β-catenin and COX-2 levels were indeed reduced (compare Fig. 4A and B). Nevertheless, these findings were mostly consistent with the results obtained by using the COX-2 reporter assay (see Fig. 3D). In addition, given that down-regulation of β-catenin activity can be prevented by ectopically expressing COX-2 or incubating cells with a downstream product, PGE2 [Rodriguez et al., 2009], we evaluated the effect of COX-2 expression or PGE2 supplementation on the CK2α (and Akt)-dependent loss of β-catenin transcriptional activity. As expected, decreased β-catenin, COX-2, and survivin protein levels observed upon these conditions were blocked either by ectopically expressing COX-2 or PGE2 supplementation of the media (Fig. 4A and B, respectively). Altogether, these results suggest that the CK2-dependent up-regulation of COX-2 expression and subsequent PGE2 production are linked to aberrant expression of important cancer-related genes, including *cox-2* itself.

PROSTAGLANDIN E2 SUPPLEMENTATION PRECLUDES LOSS OF CELL VIABILITY DUE TO CK2 INHIBITION

Next, we explored whether DMAT-dependent reduction of cell viability may be prevented by increased presence of intermediates of the Wnt/β-catenin pathway that promote COX-2 activity. To that end, HEK-293T cells were transfected with plasmids encoding CK2α, constitutively active Akt, β-catenin, or COX-2. Indeed, CK2α, Akt, β-catenin, or COX-2 expression overcame limitations in proliferation imposed by DMAT (Fig. 5A). Importantly, PGE2 (5 μM) supplementation also prevented the observed reduction in proliferation by DMAT. As anticipated, CK2α, Akt, β-catenin, and COX-2 expression, as well as PGE2 supplementation reduced significantly DMAT-dependent increments in apoptosis (Fig. 5B). Taken together, these results indicate that CK2 promotes cell viability via a mechanism involving β-catenin-dependent increments in COX-2 expression and activity and, as a consequence, augmented PGE2 production.

PROSTAGLANDIN E2 IS A KEY MEDIATOR OF THE CK2-DEPENDENT REGULATION OF VIABILITY IN COLON AND BREAST CANCER CELLS

Finally, we evaluated whether supplementation of culture media with PGE2 was sufficient to overcome limitations in cell viability imposed by DMAT in cancer cells. We focused our analysis on HT29-US and ZR-75 cells due to the observed differences in cell viability following either COX-2 or CK2 inhibition (see Fig. 1). As was expected from the experiments with HEK-293T cells, PGE2 treatment enhanced β-catenin and COX-2 protein levels in both cancer cell lines (Fig. 6A). Noteworthy, PGE2 supplementation

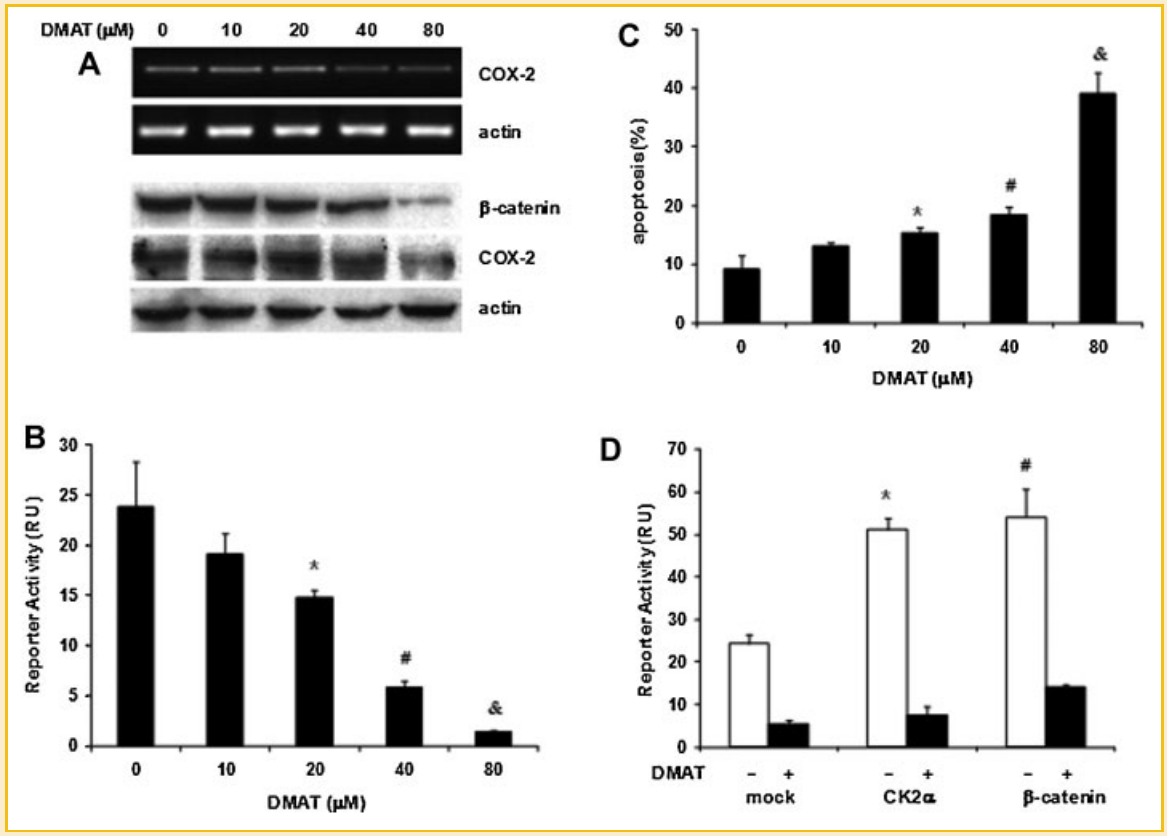

Fig. 3. CK2 regulated cell viability via modulation of β-catenin-Tcf/Lef-dependent COX-2 expression in HEK-293T cells. A: Cells were grown for 16–20 h in the presence of DMAT (0–80 μM). Total RNA was isolated to analyze COX-2 mRNA levels by conventional RT-PCR (upper panel). Cell lysates (100 μg) were also analyzed by Western blotting using antibodies to detect β-catenin, COX-2, and actin protein levels (lower panel). Actin mRNA and protein levels were used as an internal control. B: Cells were transfected with the *cox-2* promoter-specific reporter (pGL3-COX-2) or control (pGL3) vectors. After 24 h, cells were incubated for another 16–20 h with increasing concentrations of DMAT as in (A). Luciferase activity was measured as indicated in the Materials and Methods Section. C: Cells were grown 24 h in the presence of increasing concentrations of DMAT as indicated. Then, the increment in the number of apoptotic cells was determined by flow cytometry. Samples containing roughly 2×10^4 cells were evaluated. D: Cells were co-transfected with 2 μg of plasmids encoding either GFP-CK2α, GFP-β-catenin, or GFP alone (mock) together with the reporter constructs mentioned in (B). After 24 h following transfection, cells were incubated for another 16–20 h with 40 μM DMAT (black bars). Then, COX-2 reporter activity was measured as in (B). Statistically significant differences are indicated for results compared to non-treated cells, which were calculated by the Dunnett's (B,C) or the Student's (D) methods (mean ± SEM; $^{*}P < 0.05$, $^{\#}P < 0.01$, $^{\&}P < 0.005$).

prevented the decrease in COX-2 protein levels following DMAT treatment in HT29-US and ZR-75 cells (Fig. 6A, compare lanes 2 and 4). Consequently, these augmented COX-2 levels correlated with a significantly augmented proliferation (Fig. S4) as well as decreased apoptosis of HT29-US colon cancer cells (Fig. 6B). However, DMAT-induced apoptosis was greater in ZR-75 breast cancer cells, where lower levels of endogenous COX-2 protein and enzymatic activity could be detected (Figures 2A and S1, respectively). In summary, these results implicate CK2 as a key regulator of COX-2 expression in colon and breast cancer cells and suggest that PGE2 represents an important intermediate in CK2 function as an oncogene.

DISCUSSION

The COX-2 inhibitor SC-791 has been successfully employed in in vitro as well as in animal studies [Blomme et al., 2003; Hardy et al., 2003; Frolov et al., 2010]. Moreover, we have reported that it reduces proliferation of HT29-ATCC and DLD-1 colon cancer cells [Rodriguez et al., 2009]. Here, we show that SC-791 reduced colon and breast cancer cell viability, whereby susceptibility to SC-791-induced death correlated inversely with the amount of COX-2 protein present in different cell lines. Interestingly, COX-2 expression was down-regulated in cancer cells upon inhibition with either SC-791 or the CK2 inhibitor DMAT. Moreover, COX-2 over-expression significantly increased β-catenin-Tcf/Lef dependent transcriptional activity, cell viability, as well as survivin and COX-2 expression in the presence of SC-791 or DMAT. These findings confirm previous data showing that COX-2 regulates its own expression, as well as that of survivin, and, importantly, also indicate that CK2 plays an important role in regulating these events.

Indeed, DMAT reduced cancer cell viability in a way resembling effects observed previously with TBB, another CK2 inhibitor [Tapia et al., 2006]. Furthermore, microarray data revealed a marked reduction in mRNA levels of several known β-catenin targets, including survivin and COX-2, following CK2 inhibition with TBB in

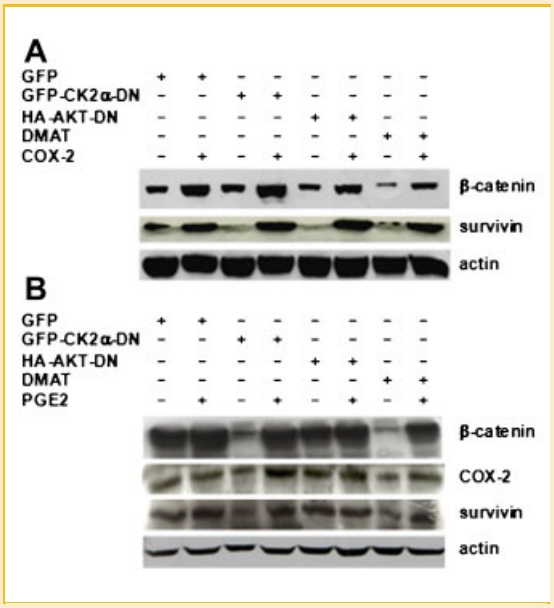

Fig. 4. COX-2 over-expression or PGE2 supplementation prevented DMAT-dependent decreases in COX-2 and survivin expression in HEK-293T cells. A: Cells were transfected with plasmids (2 μg) encoding dominant negative forms for CK2α (GFP-CK2αDN) or Akt/PKB (HA-Akt-DN) or treated with DMAT. After 24 h, cells were transfected or not with a plasmid encoding COX-2 (pOSML-COX-2) and after another 16–20 h lysates (100 μg) were analyzed for the presence of β-catenin and survivin by Western blotting with specific antibodies. B: Cells were treated as mentioned in (A), and 24 h post-treatment culture media were supplemented or not with 5 μM prostaglandin E2 (PGE2) for another 24 h. Cell lysates were analyzed for the presence of β-catenin, COX-2, and survivin by Western blotting with specific antibodies.

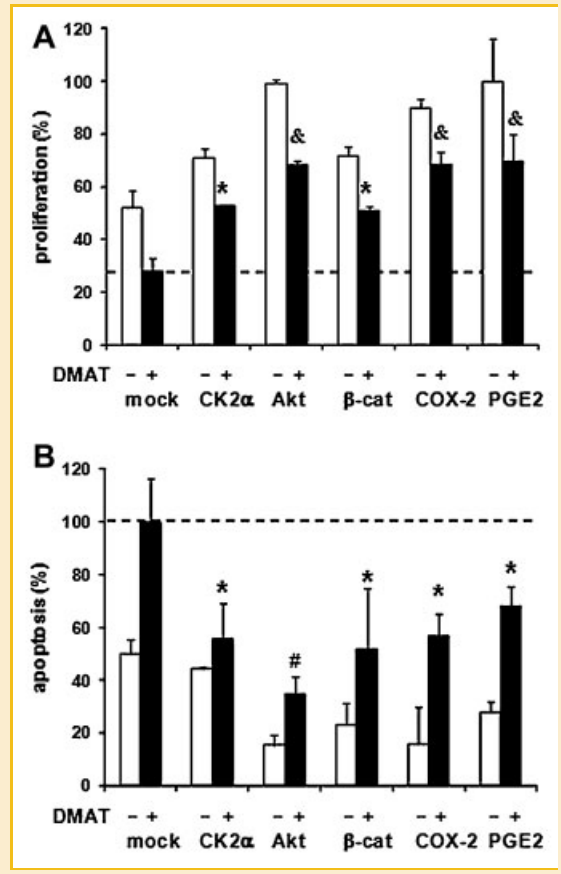

Fig. 5. PGE2 prevented DMAT-dependent decreases in viability of HEK-293T cells. A: Cells were transfected with plasmids (2 μg) encoding CK2α, Akt-CA, β-catenin, or COX-2. Alternatively, media were supplemented with 5 μM PGE2 in cells previously transfected with empty vector (pEGFP-C1). After 24 h, cells were incubated (black bars) or not (white bars) with 40 μM DMAT for 16–20 h. Then, proliferation was measured by the MTS® assay. Values obtained were compared to those of control cells transfected with the empty vector pEGFP-C1 (mock). Note that proliferation of mock cells was reduced by 50% compared with non-transfected cells. This decrease in proliferation represents a normal response of this cell line to transfection. B: Cells were treated as in (A) and apoptosis levels were measured using the Caspase Glo® assay. Statistically significant differences with respect to results obtained in DMAT-treated mock cells were calculated by the Student's method (mean ± SEM; $^{*}P < 0.05$, $^{\#}P < 0.01$, $^{\&}P < 0.005$).

HT29-US colon cancer cells [Tapia et al., 2006]. Thus, CK2 appears to behave as a master regulator of the Wnt/β-catenin pathway and the expression of several genes involved in cancer progression and metastasis.

Molecular details concerning the role of CK2 in this loop remain unclear, but some insights are beginning to emerge. A major downstream product of COX-2 activity is PGE2, which promotes nuclear import of β-catenin and augmented transcriptional activity, thereby favoring colon cancer progression [Castellone et al., 2005, 2006; Shao et al., 2005]. Here, we focused on the possibility that COX-2 down-regulation by DMAT was responsible for reduced PGE2 production, although we cannot exclude the participation of cytosolic prostaglandin E synthase, as suggested elsewhere [Kobayashi et al., 2004].

PGE2 binding to EP2 receptor leads to activation of the PI3K-Akt pathway and, subsequently, GSK3β inhibition by Akt-dependent phosphorylation [Castellone et al., 2005, 2006]. Moreover, up-regulation of β-catenin activity also involves CK2-mediated phosphorylation of Akt, although this event does not affect cytosolic stability of β-catenin per se, but rather helps the protein bypass the axin/APC/GSK3β complex [Di Maira et al., 2005; Ponce et al., 2011]. Therefore, these observations together with our results indicate that cell-type dependent differences in COX-2 expression (and PGE2 production) may arise from distinct modes of Akt activation by CK2. Despite such differences, our results confirm the notion that Akt is an important target downstream of CK2 in the sequence of events that favors β-catenin-Tcf/Lef-dependent transcription.

PGE2 was found to prevent DMAT-induced decreases in survivin and COX-2 expression in most cells tested here. Whether PGE2-dependent activation of EP2 receptor leads to up-regulation of β-catenin activity in a manner independent of CK2/Akt phosphorylation is unknown. Further experiments are required to clarify such issues. In ZR-75 breast cancer cells, the loss of cell viability following DMAT treatment was much higher and the protective effect of PGE2 was almost undetectable. Thus, in ZR-75 cells COX-2 expression appears to be highly dependent of CK2 activity. Conversely, this was not the case for HT29-US cells where

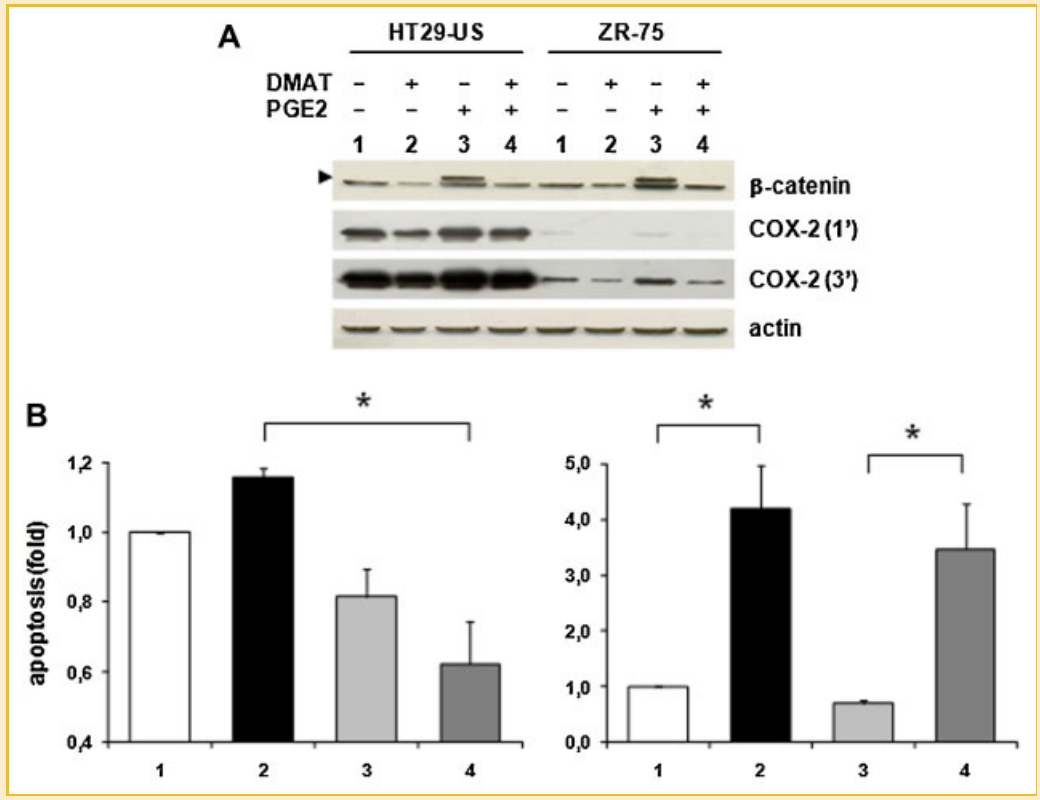

Fig. 6. PGE2 prevented the loss of viability in colon and breast cancer cells treated with DMAT. A: HT29-US colon and ZR-75 breast cancer cells were grown for 24 h in the absence (−) or presence (+) of 40 μM DMAT, as well as in the absence (−) or presence (+) of 10 μM PGE2. Then, 30 μg of lysates were analyzed for the presence of β-catenin and COX-2 by Western blotting using specific antibodies. Note that a non-specific band detected with the anti-β-catenin antibody is indicated with an arrowhead. Also, COX-2 bands are shown after two different exposure times (1 or 3 min) to facilitate the comparison between both HT29-US and ZR-75 cells. B: HT29-US colon (left) and ZR-75 breast (right) cancer cells were treated as in A (see numbering) and apoptosis was measured using the Caspase Glo® assay. Statistically significant differences were calculated by the Student's method (mean ± SEM; $^{*}P < 0.05$).

CK2-independent mechanism(s) that permit over-expression of COX-2 appear to prevail.

In summary, our results demonstrate that CK2 enhances cell viability and link these events to up-regulation of COX-2 expression via the Wnt/β-catenin pathway in human colon and breast cancer, as well as embryonic cells. Decreases in CK2 activity, achieved either by pharmacological inhibition or expression of a dominant negative variant, significantly reduced cell viability and this effect was linked to reduced expression of COX-2. Additionally, PGE2 supplementation prevented decreases in cell viability following CK2 inhibition. Therefore, these data provide important new insights to the role of CK2 as an oncogene by identifying COX-2/PGE2 as key components downstream of this kinase. Given the relevance of CK2 activity for tumor cell viability, these findings raise the interesting possibility of improving available cancer therapies by selectively inhibiting CK2.

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REFERENCES

Ahmed K, Gerber DA, Cochet C. 2002. Joining the cell survival squad: An emerging role for protein kinase CK2. Trends Cell Biol 12:226–230.

Altieri DC. 2004. Molecular circuits of apoptosis regulation and cell division control: The survivin paradigm. J Cell Biochem 92:656–663.

Araki Y, Okamura S, Hussain SP, Nagashima M, He P, Shiseki M, Miura K, Harris CC. 2003. Regulation of cyclooxygenase-2 expression by the Wnt and ras pathways. Cancer Res 63:728–734.

Beachy PA, Karhadkar SS, Berman DM. 2004. Tissue repair and stem cell renewal in carcinogenesis. Nature 432:324–331.

Belguise K, Guo S, Yang S, Rogers AE, Seldin DC, Sherr DH, Sonenshein GE. 2007. Green tea polyphenols reverse cooperation between c-Rel and CK2 that induces the aryl hydrocarbon receptor, slug, and an invasive phenotype. Cancer Res 67:11742–11750.

Blomme EA, Chinn KS, Hardy MM, Casler JJ, Kim SH, Opsahl AC, Hall WA, Trajkovic D, Khan KN, Tripp CS. 2003. Selective cyclooxygenase-2 inhibition

does not affect the healing of cutaneous full-thickness incisional wounds in SKH-1 mice. Br J Dermatol 148:211–223.

Castellone MD, Teramoto H, Williams BO, Druey KM, Gutkind JS. 2005. Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. Science 310:1504–1510.

Castellone MD, Teramoto H, Gutkind JS. 2006. Cyclooxygenase-2 and colorectal cancer chemoprevention: The beta-catenin connection. Cancer Res 66:11085–11088.

Di Maira G, Salvi M, Arrigoni G, Marin O, Sarno S, Brustolon F, Pinna LA, Ruzzene M. 2005. Protein kinase CK2 phosphorylates and upregulates Akt/PKB. Cell Death Differ 12:668–677.

DuBois RN, Radhika A, Reddy BS, Entingh AJ. 1996. Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. Gastroenterology 110:1259–1262.

Frolov RV, Ignatova II, Singh S. 2010. Mechanism of K(v)2.1 channel inhibition by a selective COX-2 inhibitor SC-791-modification of gating. Brain Res 1359:67–74.

Gao Y, Wang HY. 2006. Casein kinase 2 is activated and essential for wnt/beta-catenin signaling. J Biol Chem 281:18394–18400.

Guerra B, Issinger OG. 1999. Protein kinase CK2 and its role in cellular proliferation, development and pathology. Electrophoresis 20:391–408.

Gupta RA, Dubois RN. 2001. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer 1:11–21.

Hardy MM, Blomme EA, Lisowski A, Chinn KS, Jones A, Harmon JM, Opsahl A, Ornberg RL, Tripp CS. 2003. Selective cyclooxygenase-2 inhibition does not alter keratinocyte wound responses in the mouse epidermis after abrasion. J Pharmacol Exp Ther 304:959–967.

Heeg-Truesdell E, LaBonne C. 2006. Wnt signaling: A shaggy dogma tale. Curr Biol 16:R62–R64.

Hla T, Bishop-Bailey D, Liu CH, Schaefers HJ, Trifan OC. 1999. Cyclooxygenase-1 and -2 isoenzymes. Int J Biochem Cell Biol 31:551–557.

Kanaoka S, Takai T, Yoshida K. 2007. Cyclooxygenase-2 and tumor biology. Adv Clin Chem 43:59–78.

Kerbel RS. 2008. Tumor angiogenesis. N Engl J Med 358:2039–2049.

Kim SJ, Im DS, Kim SH, Ryu JH, Hwang SG, Seong JK, Chun CH, Chun JS. 2002. Beta-catenin regulates expression of cyclooxygenase-2 in articular chondrocytes. Biochem Biophys Res Commun 296:221–226.

Kimelman D, Xu W. 2006. Beta-catenin destruction complex: Insights and questions from a structural perspective. Oncogene 25:7482–7491.

Kobayashi T, Nakatani Y, Tanioka T, Tsujimoto M, Nakajo S, Nakaya K, Murakami M, Kudo I. 2004. Regulation of cytosolic prostaglandin E synthase by phosphorylation. Biochem J 381:59–69.

Krysan K, Dalwadi H, Sharma S, Pold M, Dubinett S. 2004a. Cyclooxygenase 2-dependent expression of survivin is critical for apoptosis resistance in non-small cell lung cancer. Cancer Res 64:6359–6362.

Krysan K, Merchant FH, Zhu L, Dohadwala M, Luo J, Lin Y, Heuze-Vourc'h N, Pold M, Seligson D, Chia D, Goodglick L, Wang H, Strieter R, Sharma S, Dubinett S. 2004b. COX-2-dependent stabilization of survivin in non-small cell lung cancer. FASEB J 18:206–208.

Kutchera W, Jones DA, Matsunami N, Groden J, McIntyre TM, Zimmerman GA, White RL, Prescott SM. 1996. Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: Evidence for a transcriptional effect. Proc Natl Acad Sci USA 93:4816–4820.

Landesman-Bollag E, Romieu-Mourez R, Song DH, Sonenshein GE, Cardiff RD, Seldin DC. 2001a. Protein kinase CK2 in mammary gland tumorigenesis. Oncogene 20:3247–3257.

Landesman-Bollag E, Song DH, Romieu-Mourez R, Sussman DJ, Cardiff RD, Sonenshein GE, Seldin DC. 2001b. Protein kinase CK2: Signaling and tumorigenesis in the mammary gland. Mol Cell Biochem 227:153–165.

Litchfield DW. 2003. Protein kinase CK2: Structure, regulation and role in cellular decisions of life and death. Biochem J 369:1–15.

Liu CH, Chang SH, Narko K, Trifan OC, Wu MT, Smith E, Haudenschild C, Lane TF, Hla T. 2001. Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J Biol Chem 276:18563–18569.

Meggio F, Pinna LA. 2003. One-thousand-and-one substrates of protein kinase CK2? FASEB J 17:349–368.

Nagy JA, Dvorak AM, Dvorak HF. 2007. VEGF-A and the induction of pathological angiogenesis. Annu Rev Pathol 2:251–275.

Pinna LA. 2002. Protein kinase CK2: A challenge to canons. J Cell Sci 115: 3873–3878.

Ponce DP, Maturana JL, Cabello P, Yefi R, Niechi I, Silva E, Armisen R, Galindo M, Antonelli M, Tapia JC. 2011. Phosphorylation of AKT/PKB by CK2 is necessary for the AKT-dependent up-regulation of beta-catenin transcriptional activity. J Cell Physiol 226:1953–1959.

Rodriguez DA, Tapia JC, Fernandez JG, Torres VA, Munoz N, Galleguillos D, Leyton L, Quest AF. 2009. Caveolin-1-mediated suppression of cyclooxygenase-2 via a {beta}-catenin-Tcf/Lef-dependent transcriptional mechanism reduced prostaglandin E2 production and survivin expression. Mol Biol Cell 20:2297–2310.

Segditsas S, Tomlinson I. 2006. Colorectal cancer and genetic alterations in the Wnt pathway. Oncogene 25:7531–7537.

Seldin DC, Landesman-Bollag E, Farago M, Currier N, Lou D, Dominguez I. 2005. CK2 as a positive regulator of Wnt signalling and tumourigenesis. Mol Cell Biochem 274:63–67.

Shao J, Lee SB, Guo H, Evers BM, Sheng H. 2003. Prostaglandin E2 stimulates the growth of colon cancer cells via induction of amphiregulin. Cancer Res 63:5218–5223.

Shao J, Jung C, Liu C, Sheng H. 2005. Prostaglandin E2 Stimulates the beta-catenin/T cell factor-dependent transcription in colon cancer. J Biol Chem 280:26565–26572.

Tapia J, Jacob G, Allende CC, Allende JE. 2002. Role of the carboxyl terminus on the catalytic activity of protein kinase CK2alpha subunit. FEBS Lett 531:363–368.

Tapia JC, Bolanos-Garcia VM, Sayed M, Allende CC, Allende JE. 2004. Cell cycle regulatory protein p27KIP1 is a substrate and interacts with the protein kinase CK2. J Cell Biochem 91:865–879.

Tapia JC, Torres VA, Rodriguez DA, Leyton L, Quest AF. 2006. Casein kinase 2 (CK2) increases survivin expression via enhanced beta-catenin-T cell factor/lymphoid enhancer binding factor-dependent transcription. Proc Natl Acad Sci USA 103:15079–15084.

Tawfic S, Yu S, Wang H, Faust R, Davis A, Ahmed K. 2001. Protein kinase CK2 signal in neoplasia. Histol Histopathol 16:573–582.

Telliez A, Furman C, Pommery N, Henichart JP. 2006. Mechanisms leading to COX-2 expression and COX-2 induced tumorigenesis: Topical therapeutic strategies targeting COX-2 expression and activity. Anticancer Agents Med Chem 6:187–208.

Torres VA, Tapia JC, Rodriguez DA, Parraga M, Lisboa P, Montoya M, Leyton L, Quest AF. 2006. Caveolin-1 controls cell proliferation and cell death by suppressing expression of the inhibitor of apoptosis protein survivin. J Cell Sci 119:1812–1823.

Torres VA, Tapia JC, Rodriguez DA, Lladser A, Arredondo C, Leyton L, Quest AF. 2007. E-cadherin is required for caveolin-1-mediated down-regulation of the inhibitor of apoptosis protein survivin via reduced beta-catenin-Tcf/Lef-dependent transcription. Mol Cell Biol 27:7703–7717.

Tsujii M, Kawano S, DuBois RN. 1997. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA 94:3336–3340.

Wang MT, Honn KV, Nie D. 2007. Cyclooxygenases, prostanoids, and tumor progression. Cancer Metastasis Rev 26:525–534.

Zhang T, Otevrel T, Gao Z, Ehrlich SM, Fields JZ, Boman BM. 2001. Evidence that APC regulates survivin expression: A possible mechanism contributing to the stem cell origin of colon cancer. Cancer Res 61:8664–8667.