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CK2 functionally interacts with AKT/PKB to promote the β-catenin-dependent expression of survivin and enhance cell survival

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Abstract β-Catenin is crucial in the canonical Wnt signaling pathway. This pathway is up-regulated by CK2 which is associated with an enhanced expression of the antiapoptotic protein survivin, although the underlying molecular mechanism is unknown. AKT/PKB kinase phosphorylates and promotes β-catenin transcriptional activity, whereas CK2 hyperactivates AKT by phosphorylation at Ser129; however, the role of this phosphorylation on β-catenin transcriptional activity and cell survival is unclear. We studied in HEK-293T cells, the effect of CK2-dependent hyperactivation of AKT on cell viability, as well as analyzed β-catenin subcellular localization and transcriptional activity and survivin expression. CK2α overexpression led to an augmented β-catenin-dependent transcription and protein levels of survivin, and consequently an enhanced resistance to apoptosis. However, CK2α-enhancing effects were reversed when an AKT mutant deficient in Ser129 phosphorylation by CK2 was co-expressed. Therefore, our results strongly suggest that CK2α-specific enhancement of β-catenin transcriptional activity as well as cell survival may depend on AKT hyperactivation by CK2.

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Abbreviations

CK2	Acronym for the former name casein kinase II
CK2α	CK2's catalytic subunit α
Tcf/Lef	T-cell factor/lymphoid enhancer binding factor
DMAT	2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole
TBB	4,5,6,7-tetrabromobenzotriazole
IAP	Inhibitor of apoptosis protein

Introduction

Wnt/β-catenin signaling is associated with the development and progression of multiple cancers [1]. Activation of this pathway is linked to accumulation of β-catenin in the nucleus followed by increased expression of genes, including *cyclin D1*, *vegf*, and *survivin*, all of which are the known contributors to cancer progression [2–5].

Protein kinase CK2 regulates essential cellular processes, such as proliferation and death. This enzyme has been shown to interact with and phosphorylate β-catenin, regulating its stability and transcriptional activity [6, 7]. Similarly, AKT/PKB is known to regulate cell progression and viability [8, 9]; however, recently it has been reported that AKT phosphorylates β-catenin at Ser552 which leads to its dissociation from cell–cell contacts, nuclear import and, consequently, increases β-catenin transcriptional activity [10, 11].

The putative role of AKT in regulating β-catenin has emerged as a relevant issue since CK2 interacts with, phosphorylates and hyperactivates AKT [12, 13]. Indeed, it

has been shown that the residue Ser129 in AKT is phosphorylated by CK2, and mutation of this residue to alanine causes a marked decrease in AKT activity in vivo and in vitro and, interestingly, a reduced phosphorylation of Thr308, which is known as a canonical residue linked to its activation [12]. This suggests that phosphorylation of Ser129 by CK2 may lead to enhanced activity of AKT. Nevertheless, whether activation of AKT by CK2 does involve increased β-catenin activity both in the normal and cancer cells is still unsolved.

The data presented here indicate that the CK2α-dependent up-regulation of β-catenin transcriptional activity is a process involving the kinase AKT. When the role of CK2α on β-catenin-Tcf/Lef signaling was analyzed upon co-expression of an AKT mutant deficient in Ser129 phosphorylation by CK2, namely AKT-S129A, a decreased β-catenin transcriptional activity was observed which correlated with a diminished expression of the antiapoptotic protein survivin and, subsequently, decreased survival in HEK-293T cells. Therefore, a functional interaction between CK2 and AKT may be occurring in the Wnt/β-catenin pathway-dependent cancer progression.

Materials and methods

Cell culture and transfection

Cells were cultured at 37°C and 5% CO_2 in DMEM (Invitrogen, Paisley, Scotland, UK) supplemented with 10% FBS (HyClone, Logan, Utah, USA) and antibiotics (10,000 U/ml penicillin, 10 µg/ml streptomycin). Superfect® was used for transfections according to the instructions provided by the manufacturer (Qiagen, Valencia, CA, USA).

Plasmids

Plasmid for CK2α has been previously described [14, 15]. Plasmid pEGFP-β-catenin encodes GFP-tagged β-catenin wild-type [16], and pCMV6-HA-myr-AKT1-S129A encodes an AKT mutant deficient in phosphorylation by CK2 in Ser129 [12]. Reporter plasmids pLuc-1710 (containing the survivin promoter with Tcf/Lef binding sites) and pLuc-420-3M (mutated Tcf/Lef binding sites) have already been described [17].

Reporter assay

Cells were transfected with different plasmids (15 µg total DNA) as indicated in each figure. Cells were lysed in 100 mM KH_2PO_4 (pH 7.9)/1 mM DTT/0.5% Triton X-100 and supernatants used to measure luciferase activity. Values were used for calculating the 1710/420-3M ratios.

Microscopy

Cells were grown on glass coverslips and transfected as indicated. After rinsing with PBS, cells were fixed at 4°C in PBS/4% paraformaldehyde and mounted onto slides with 10% Mowiol/2.5% DABCO. Fluorescence was visualized in an inverted epifluorescence microscope Eclipse E400 (Nikon) equipped with a CCD camera DS-RI1 (Nikon).

Results

CK2-dependent phosphorylation of AKT is necessary to enhance cell viability

We initially evaluated the effect of overexpressing the catalytic CK2α subunit and the AKT mutant deficient in Ser129 phosphorylation by CK2, namely AKT-S129A [12], on viability of HEK-293T cells. As observed in Fig. 1, overexpression of CK2α enhanced a 26% cell viability whereas no effect was observed with the AKT-S129A mutant. Interestingly, when AKT-S129A was co-expressed together with CK2α, this mutant almost completely abolished the CK2α-dependent enhancement in cell viability. These data suggested that CK2-mediated phosphorylation of AKT is important to promote cell viability in HEK-293T cells.

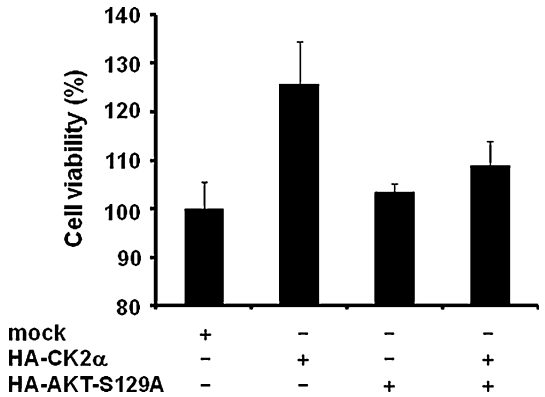

Fig. 1 CK2α-dependent enhance of cell viability was impaired by the AKT-S129A mutant. HEK-293T cells were co-transfected with plasmids encoding HA-CK2α and/or HA-AKT-S129A mutant. Eight hours post-transfection, cells were trypsinized and seeded by triplicate in a 96-well plate and grown for 16–18 h. Viability was determined using the MTS® assay according to the instructions provided by the manufacturer (Promega Corp, Madison, WI). Data depicted (mean±SE) were calculated considering the mock-transfected cells as 100%

CK2-dependent phosphorylation of AKT promotes nuclear accumulation of β-catenin

Wnt/β-catenin activation is linked to nuclear migration followed by an augmented target gene expression. Therefore, overexpression of an AKT form deficient in Ser129 phosphorylation by CK2 can lead to a decreased nuclear accumulation of β-catenin. Thus, we analyzed the effect of AKT-S129A on β-catenin subcellular localization using the fusion protein GFP-β-catenin. We observed a change in GFP localization from a broadly cell-distributed (Fig. 2a) to a more nuclear-restricted in cells co-expressing HA-CK2α (Fig. 2b). Rather than observing a change in subcellular localization, GFP-β-catenin fluorescence seemed to be decreasing in the nucleus of cells overexpressing AKT-S129A (Fig. 2c), whereas nuclear as well as cytoplasmic fluorescence appeared to be diminished in the cells co-expressing CK2α and AKT-S129A (Fig. 2d). Noteworthy, we observed a change in morphology when CK2α and AKT-S129A were co-expressed, which was highly suggestive of apoptosis in a way similar to when CK2 was inhibited by the specific inhibitor DMAT, which correlates to a reduced expression of the antiapoptotic protein survivin [14].

Impairment of AKT phosphorylation by CK2 inhibits both CK2α-dependent up-regulation of survivin and resistance to apoptosis

The change in cell morphology after co-expression of CK2α and AKT-S129A prompted us to evaluate the expression of survivin, and subsequently the resistance to apoptosis. We analyzed the transcription of survivin gene using a specific survivin's promoter-dependent reporter assay. As expected, reporter activity was significantly reduced when the AKT-S129A mutant was overexpressed alone (Fig. 3a). In addition, AKT-S129A co-expressed together with CK2α led to a complete blockage of the CK2α-dependent up-regulation of survivin, which was reminiscent of results using both TOP/FOP-Flash (data not shown) and microscopy (Fig. 2). Likewise, protein levels of survivin under the same conditions paralleled to data observed on reporter assay (Fig. 3b). Finally, we evaluated the roles of CK2α and AKT-S129A overexpression on resistance to apoptosis of cells grown in the presence of the specific CK2 inhibitor DMAT (50 μM). CK2α overexpression had a minor effect, whereas AKT-S129A promoted a significant increase of apoptosis (Fig. 3c). Notably, when AKT-S129A was co-expressed

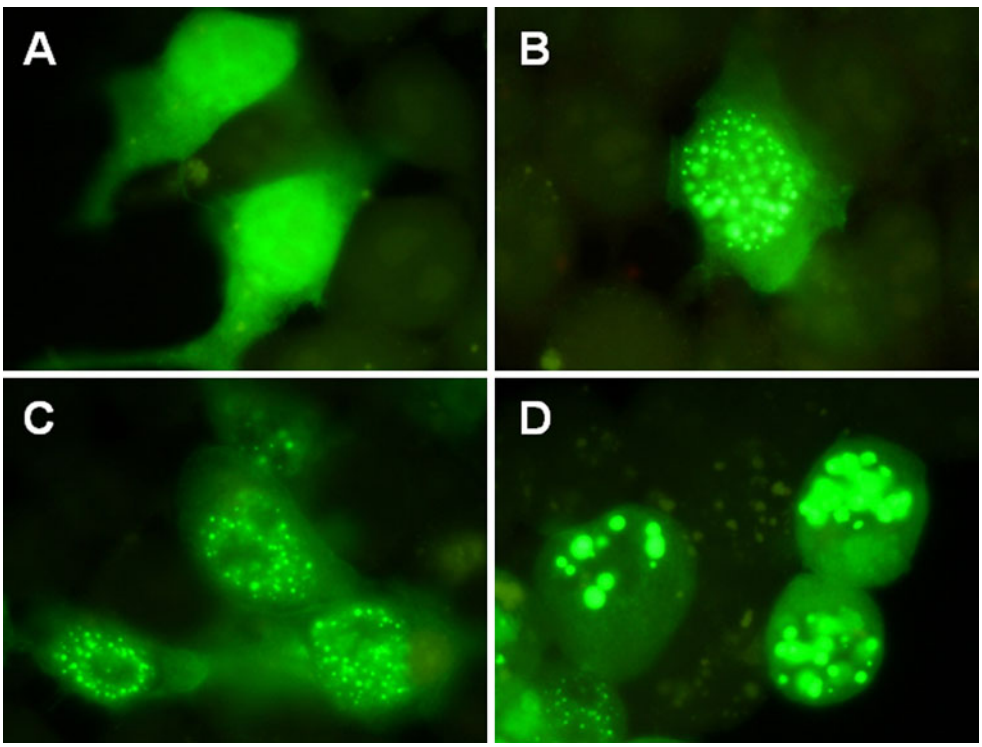

Fig. 2 Nuclear localization of β-catenin was modulated by phosphorylation of AKT by CK2. HEK-293T cells were grown on glass coverslips and transfected with plasmids encoding GFP-β-catenin alone (**a**) or together with HA-CK2α (**b**), HA-AKT-S129A (**c**), or HA-CK2α + HA-AKT-S129A (**d**). Cells were cultured for 16 h, fixed in paraformaldehyde, mounted onto slides with mowiol, and analyzed for GFP by epifluorescence microscopy

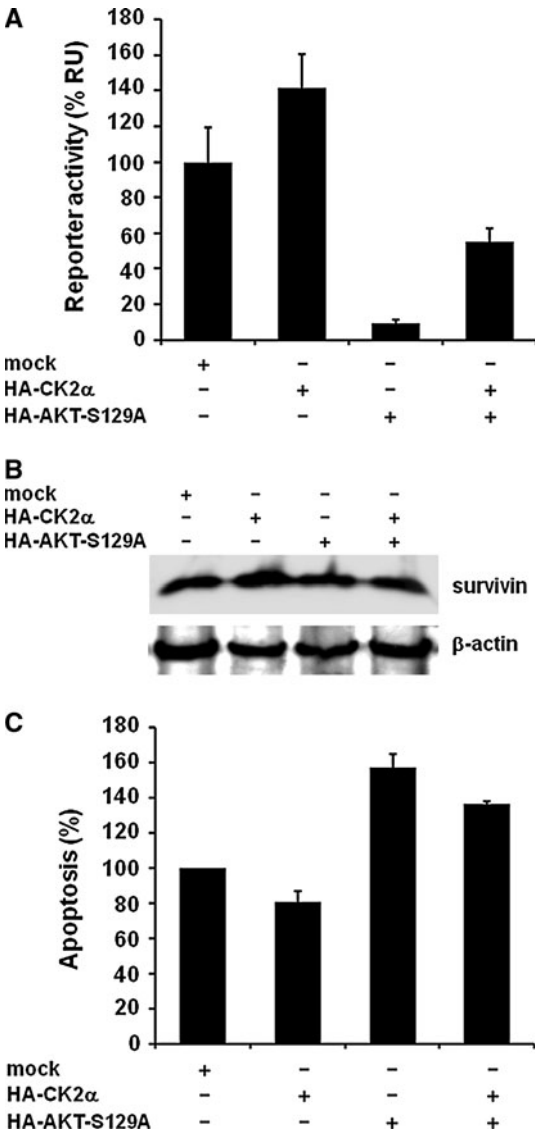

Fig. 3 CK2α-dependent expression of survivin and resistance to apoptosis were abolished by the AKT-S129A mutant. **a** HEK-293T cells were co-transfected with combinations of the survivin-specific reporter vector pLuc-1710 (survivin promoter containing wild-type Tcf/Lef-binding sites) or pLuc-420-3 M (mutated Tcf/Lef-binding sites), and plasmids encoding HA-CK2α wild-type and/or HA-AKT-S129A mutant. Values for luciferase activity were used for calculating the 1710/420-3M ratios (mean ± SE). **b** Cell lysates of conditions as in A were used to determine survivin protein levels by Western blot using a specific polyclonal antibody (R&D Systems, Minneapolis, MN, USA). **c** Cells transfected as in A were incubated in the presence of 50 μM DMAT for 12–16 h, and apoptosis was measured by the Caspase Glo® assay according to the instructions provided by the manufacturer (Promega Corp, Madison, WI)

together with CK2α, this mutant almost completely abolished the CK2α-dependent effect in resistance to apoptosis, correlating with the above-mentioned effects in cell viability (Fig. 1).

Discussion

Protein kinase CK2 has been suggested to be part of a protein complex that positively regulates the Wnt/β-catenin signaling pathway by interacting and phosphorylating β-catenin, which result in its increased transcriptional activity [6, 7]. In addition, data indicate that phosphorylation of β-catenin at Ser552 by protein kinase AKT/PKB leads to its increased transcriptional activity [10]. Likewise, we have observed that overexpressions of both a constitutively active and a dominant negative form of AKT resemble to those with wild-type and dominant negative forms of CK2α, respectively [18], supporting the role of AKT in promoting the transcriptional activity of β-catenin as well as the role of CK2α in those events.

Interestingly, it has been demonstrated that CK2 hyperactivates AKT by phosphorylation at Ser129 while mutation of this residue to alanine decreases the activity of AKT both in vitro and in vivo [12]. Indeed, overexpression of an AKT deficient in Ser129 phosphorylation by CK2, namely AKT-S129A, had a negative effect on β-catenin-dependent activity in HEK-293T cells, which is reminiscent of that observed with CK2α dominant negative [18]. These data suggested that phosphorylation of AKT by CK2 is important for regulating the transcriptional activity of β-catenin.

In addition, nuclear levels of β-catenin appeared decreased when the AKT-S129A mutant was overexpressed either alone or together with CK2α, indicating that phosphorylation of β-catenin by AKT would bypass the Axin/APC/GSK3β complex and, consequently, increase the nuclear localization and the transcriptional activity of β-catenin.

The morphological changes observed in HEK-293T cells co-transfected with CK2α and AKT-S129A, were indicative of the occurrence of apoptosis. We have already demonstrated that CK2α overexpression or specific inhibitors modulate the β-catenin-dependent expression of the IAP member survivin, leading to changes in proliferation and resistance to apoptosis in HEK-293T cells [14]. Likewise, we observed a marked reduction in mRNA and protein levels of survivin in colon and breast cancer cell lines upon treatment with the CK2-specific inhibitor TBB, which significantly reduced viability, augmented apoptosis, and altered the cell cycle in these cells [14]. In agreement with our results, decreased expressions of survivin and other IAP members have been observed upon treatment with TNFα or TRAIL in prostate cancer cells [19]. As expected in this study, low mRNA and protein levels of survivin were indeed indicative of the decreased nuclear localization and transcriptional activity of β-catenin, when CK2α and AKT-S129A were co-expressed, leading to both decreased viability and increased apoptosis in human embryonic kidney cells.

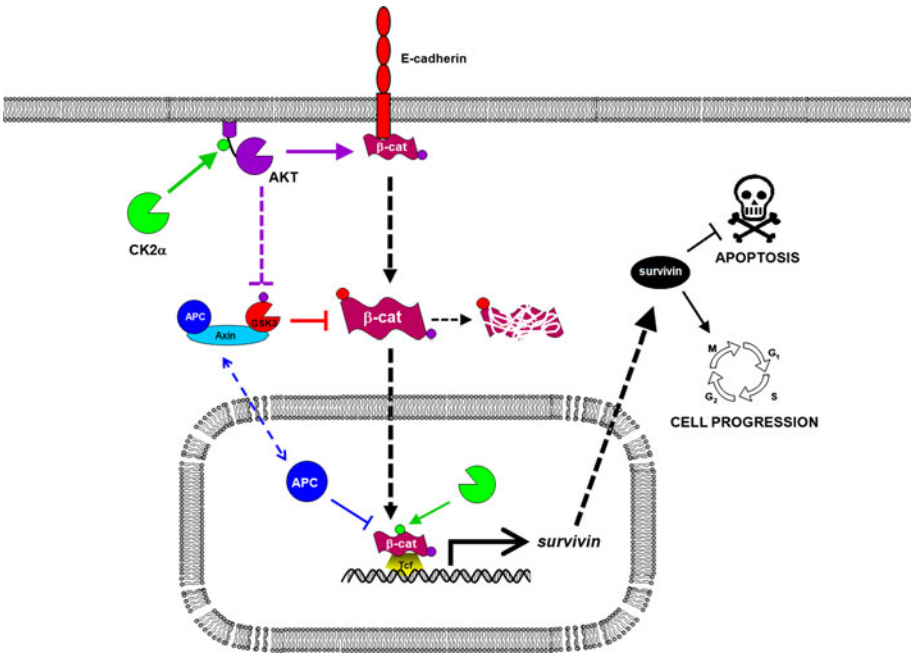

Fig. 4 Mechanism model for up-regulation of β-catenin activity by CK2. β-Catenin can be found in normal epithelial cells at the plasma membrane (bound to E-cadherin), cytosolic (N-terminal phosphorylated and targeted for proteasomal degradation), and nuclear (transactivating the expression of its target genes). In colon cancer cells, an aberrant activation of this pathway may occur through CK2, by which β-catenin escapes from binding to E-cadherin, and proteasomal degradation no longer occurs. This mechanism takes into consideration of not only the phosphorylation of β-catenin at Thr393 by CK2 [6, 7] but also the phosphorylation of AKT at Ser129 [12, 13], which subsequently would phosphorylate β-catenin at Ser552 [10]. As a consequence, β-catenin migrates to the nucleus, enhancing the expression of cancer-related genes such as survivin [14]. In cancer cells, survivin should promote increased proliferation and resistance to apoptosis, as well as the ability for invading distant tissues

In summary, our data support a mechanism of CK2α-dependent regulation of β-catenin transcriptional activity by bypassing the complex Axin/APC/GSK3β but running through the CK2-mediated phosphorylation of AKT at Ser129 (see model in Fig. 4). This phosphorylation results in hyperactivation of AKT which subsequently may phosphorylate β-catenin at Ser552, probably promoting the binding of protein(s) to β-catenin and enhancing its nuclear import and transcriptional activity on cancer-related genes [10, 11, 14]. Despite promoting the β-catenin transcriptional activity, phosphorylation of AKT by CK2 does not affect the cytosolic stability of β-catenin [18], but the protein levels of survivin are augmented, which enhances cell viability and resistance to apoptosis. Taken together, these data suggest a novel functional interaction of CK2 with AKT in the Wnt/β-catenin signaling-dependent cancer progression.

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References

1. Beachy PA, Karhadkar SS, Berman DM (2004) Tissue repair and stem cell renewal in carcinogenesis. Nature 432(7015):324–331
2. Altieri DC (2004) Molecular circuits of apoptosis regulation and cell division control: the survivin paradigm. J Cell Biochem 92(4):656–663
3. Kerbel RS (2008) Tumor angiogenesis. N Engl J Med 358(19): 2039–2049
4. Nagy JA, Dvorak AM, Dvorak HF (2007) VEGF-A and the induction of pathological angiogenesis. Annu Rev Pathol 2: 251–275
5. Neri D, Bicknell R (2005) Tumour vascular targeting. Nat Rev Cancer 5(6):436–446
6. Song DH, Dominguez I, Mizuno J, Kaut M, Mohr SC, Seldin DC (2003) CK2 phosphorylation of the armadillo repeat region of beta-catenin potentiates Wnt signaling. J Biol Chem 278(26): 24018–24025
7. Song DH, Sussman DJ, Seldin DC (2000) Endogenous protein kinase CK2 participates in Wnt signaling in mammary epithelial cells. J Biol Chem 275(31):23790–23797
8. Hanada M, Feng J, Hemmings BA (2004) Structure, regulation and function of PKB/AKT–a major therapeutic target. Biochim Biophys Acta 1697(1–2):3–16
9. Nicholson KM, Anderson NG (2002) The protein kinase B/Akt signalling pathway in human malignancy. Cell Signal 14(5): 381–395
10. Fang D, Hawke D, Zheng Y, Xia Y, Meisenhelder J, Nika H, Mills GB, Kobayashi R, Hunter T, Lu Z (2007) Phosphorylation

of beta-catenin by AKT promotes beta-catenin transcriptional activity. J Biol Chem 282(15):11221–11229
11. Tian Q, Feetham MC, Tao WA, He XC, Li L, Aebersold R, Hood L (2004) Proteomic analysis identifies that 14–3-3zeta interacts with beta-catenin and facilitates its activation by Akt. Proc Natl Acad Sci USA 101(43):15370–15375
12. 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(6):668–677
13. Guerra B (2006) Protein kinase CK2 subunits are positive regulators of AKT kinase. Int J Oncol 28(3):685–693
14. 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(41): 15079–15084
15. 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(2):363–368
16. Votin V, Nelson WJ, Barth AI (2005) Neurite outgrowth involves adenomatous polyposis coli protein and beta-catenin. J Cell Sci 118(Pt 24):5699–5708
17. 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(8):2297–2310
18. Ponce D, 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(7):1953–1959
19. Wang G, Ahmad KA, Harris NH, Ahmed K (2008) Impact of protein kinase CK2 on inhibitor of apoptosis proteins in prostate cancer cells. Mol Cell Biochem 316:91–97