Casein kinase II inhibition induces apoptosis in pancreatic cancer cells

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Abstract. Pancreatic cancer is one of the most common causes of cancer death in western civilization. The five-year survival rate is below 1% and of the 10% of patients with resectable disease only around one-fifth survives 5 years. Survival rates have not changed much during the last 20 years, demonstrating the inefficacy of current available therapies. To improve the prognosis of pancreatic cancer, there is the need to develop effective non-surgical treatment for this disease. The protein kinase casein kinase II (CK2) is a ubiquitously expressed serine-threonine kinase and its activity is enhanced in all human tumors examined so far. The contribution of CK2 to the tumor maintenance of pancreatic cancer has not been investigated. To investigate the function of CK2 in pancreatic cancer cells we used the CK2 specific inhibitors 5,6-Dichloro-1-ß-D-ribofuranosylbenzimidazole and Apigenin. Furthermore, we interfered with CK2 expression using CK2 specific siRNAs. Interfering with CK2 function led to a reduction of pancreatic cancer cell viability, which was due to caspase-dependent apoptosis. The induction of apoptosis correlated with a reduced NF-κB-dependent transcriptional activity. This study validates CK2 as a molecular drug target in a preclinical *in vitro* model of pancreatic cancer.

Introduction

More than 30000 people develop pancreatic adenocarcinoma each year in the United States, and almost all are expected to die from the disease (1). Five-year survival rates stay below 5% and have not changed much during the last 20 years demonstrating the insufficiency of current therapeutic strategies. Hence, there is the urgent need to characterize new molecular therapeutic strategies in preclinical settings.

Carcinogenesis of ductal pancreatic cancer is mediated by mutations in oncogenes such as *K-RAS* and tumor suppressor genes such as $p16^{INK4A}$, *TP53* and *MADH4/DPC4/SMAD4* (2). Oncogenes, such as *K-RAS*, activate intrinsic tumor suppressive pathways, such as oncogene-induced apoptosis and oncogene-induced premature senescence. Therefore a cancer cell has to adopt the ability to conquer intrinsic surveillance programs (3). One consequence is resistance towards apoptosis, an important factor for the survival and drug resistance of cancer cells (4,5). The apoptosis sensing, inducing and executing machinery is regulated at multiple levels. Each level is targeted by apoptosis-resistance mechanisms in pancreatic cancer cells (6). Although pancreatic cancer cells express death receptors such as TNF-R, TRAIL-R and CD95, most resist death-receptor ligand induced apoptosis (7,8). At the mitochondrial level the balance between pro-death and pro-survival members of the BCL-2 protein family decides over resistance towards apoptosis (4). The family member $BCL\text{-}_{XL}$ is overexpressed in pancreatic cancer, suggesting an important role of this pro-survival factor in mediating apoptosis and chemotherapy resistance (9-11).

Casein kinase II (CK2) is a highly conserved serine/threonine kinase, which is constitutively active and ubiquitously expressed in mammalian cells. Two catalytic subunits CK2α/CK2α' and two regulatory subunits CK2ß form a tetrameric holoenzyme (12). CK2 phosphorylates more than 300 different substrates and is involved in many aspects of cell biology, such as cell cycle, cell survival or apoptosis (12,13). All human cancers examined so far show an enhanced activity of CK2 (14,15). There is multiple evidence linking CK2 to apoptosis. Thus, phosporylation by CK2 protects proteins from caspase-mediated cleavage. CK2 phosphorylated BID, a pro-apoptotic member of the BCL-2 family, restrains caspase-8-mediated cleavage, preventing crosstalk between intrinsic and extrinsic apoptotic signaling (16). CK2 has also direct anti-apoptotic function by phosphorylating and activating the caspase-8 inhibitory protein ARC (17). Furthermore, the pivotal anti-apoptotic transcription factor NF-κB is activated by CK2, leading to changes in the expression of pro- and anti-apoptotic genes (18-21). In addition, CK2 increases survivin expression in a ß-catenin-dependent manner (22). Together, this may explain the sensitization towards drug and death-receptor induced apoptosis observed after inhibition of CK2 (23-27).

In this study, we show that the inhibition of CK2 by the specific inhibitors Apigenin and 5,6-Dichloro-1-ß-D-ribofuranosylbenzimidazole (DRB) induces dose-dependent reduction of viability of pancreatic cancer cells, which is due

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to caspase-dependent apoptosis. The specificity of the pharmacological inhibitors was demonstrated with functional genomics using RNA interference. siRNAs directed against CK2α as well as against CK2α' reduce viability and induce apoptosis. These observations suggest that inhibition of CK2 activity may provide a new strategy for the treatment of pancreatic cancer.

Materials and methods

Reagents. 5,6-Dichloro-1-ß-D-ribofuranosylbenzimidazole (DRB) (EMD Biosciences, San Diego, CA, USA), Apigenin (EMD Biosciences) and zVAD-fmk (Biomol, Hamburg, Germany) were dissolved in DMSO and stored at -20˚C.

Cell culture, transfection, siRNAs, plasmid. MiaPaCa2 cells were cultivated in DMEM and DanG cells were cultivated in RPMI supplemented with 10% fetal calf serum and 1% (w/v) penicillin/streptomycin. The pGL3-3xκB-Luc reporter gene construct was recently described (28). Transfections of the reporter gene (500 ng/well) were performed using FuGene6 (Roche Applied Science, Mannheim, Germany) according to the manufacturer's protocol in 12-well plates. After 24 h cells were treated with the indicated inhibitors for 6 h. Afterwards cells were incubated in lysis buffer (Promega, Mannheim, Germany) for 15 min, harvested, and cleared by centrifugation for 15 min. Lysates were normalized for protein content. Luciferase activity was determined in an LB 9501 luminometer (Berthold, Bad Wildbad, Germany) using a luciferase assay system (Promega). At least three independent transfection experiments were performed in triplicate. Data are presented as mean and standard error of the mean (SEM). Double-stranded siRNAs were transfected in a concentration of 200 nM using oligofectamine (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. Fifty thousand cells/well were transfected in a 6-well plate, 2000 cells/well were transfected in a 96-well plate. The sequence for the control siRNA was 5'-AACAGUCGCGUUUGCGACUGG-3', the target sequences of the CK2 specific siRNAs (sense strand) were CK2α + 411 5'-AAGCAGGGCCAGAGUUUACAC-3'; CK2α' + 49 5'-AACAGUCUGAGGAGCCGCGAG-3'. siRNAs were purchased from Ambion, Austin, TX, USA and were stored in a 20-μM stock at -80˚C.

Preparation of total cell lysates. Whole-cell lysates were prepared by incubating cell pellets for 30 min at 4˚C in immunoprecipitation buffer (50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 10% glycerol, 1 mM dithiothreitol, 1 mM phenylmethysulfonylfluoride and 5 mM NaF). Insoluble material was removed by centrifugation and lysates were aliquoted and stored at -80˚C.

Viability assay. Viability of the cells was measured using MTT-assay according to the manufacturer's protocol (Roche Applied Science). Five thousands cells/well were grown for the indicated time periods under the indicated treatment conditions. After the incubation period MTT was added to a final concentration of 0.5 mg/ml. After 4 h the formed formazan crystals were solubilized with the solubilization solution over night. The spectrophotometrical absorbance was measured using an ELISA reader (Anthos 2001, Anthos Microsystems, Cologne, Germany) at a wavelength of 600 nM. As a reference wavelength 650 nM was used. All experiments were performed three times as a triplicate and presented as mean and standard error of the mean (SEM).

Western blot analysis. Extracts were normalized for protein and heated at 95˚C for 5 min in Lämmli buffer. Proteins were resolved on 10% SDS-polyacrylamide gels and electrophoretically transferred to polyvinylidene difluoride membranes (Millipore, MA, USA) in a semi-dry blotting system. Membranes were blocked in phosphate-buffered saline (PBS) supplemented with 5% skim-milk and 0.1% NP-40, and incubated with antibodies against CK2, PARP (BD Pharmingen, San Diego, CA, USA), BCL-$_{XL}$ (Cell Signal Technology, Beverly, MA, USA) and ß-actin (Sigma-Aldrich, Munich, Germany) for 1 h at room temperature. Proteins recognized by the antibodies were detected by the Odyssey Infrared Imaging System (Licor, Bad Homburg, Germany) using Alexa680-coupled (Molecular Probes, Leiden, Netherland) or IRDeye800-coupled (Rockland, Gilbertsville, PA, USA) secondary antibodies.

Apoptosis-stain. Chromatin was stained with Hoechst 33342 (4 μM) and typical apoptotic morphological changes were visualized using fluorescence microscopy. The apoptotic fractions of 300 cells were counted per individual experiment. All experiments were performed three times as triplicate and presented as mean and standard error of the mean (SEM).

Statistical methods. All data were obtained from at least three independent experiments performed in duplicate, and the results are presented as mean and standard error of the mean (SEM). To demonstrate statistical significance Student's t-test was used. P-values are indicated in the figure legends.

Results

The CK2 specific inhibitors 5,6-Dichloro-1-ß-D-ribofuranosylbenzimidazole (DRB) and Apigenin reduce viability of pancreatic cancer cells. To investigate the influence of CK2 on tumor maintenance of pancreatic cancer cells, we treated MiaPaCa2 and DanG cells with the specific chemical CK2 inhibitors 5,6-Dichloro-1-ß-D-ribofuranosylbenzimidazole (DRB) and Apigenin. Viability of cells was measured after treatment with increasing doses of the inhibitors over a time course of 48 h using MTT assays. In MiaPaCa2 cells, the viability was dose-dependently decreased to 51 and 31% of controls after 24 and 48 h of treatment with the highest concentration DRB (200 μM) used (Fig. 1A, upper graph). With Apigenin (150 μM) at the highest concentration the viability of MiaPaCa2 cells was reduced to 45 and 33% of controls after 24 and 48 h of treatment (Fig. 1A, lower graph). In DanG cells the viability was dose-dependently reduced to 48% of controls after 24 h and 22% of controls after 48 h of treatment with the highest concentration DRB (200 μM) used (Fig. 1B, upper graph). Apigenin (150 μM) reduced the viability of DanG cells to 55% of controls after 24 h and to 27% of controls after 48 h of treatment (Fig. 1B, lower graph). These data suggest that CK2 maintains viability of pancreatic cancer cells.

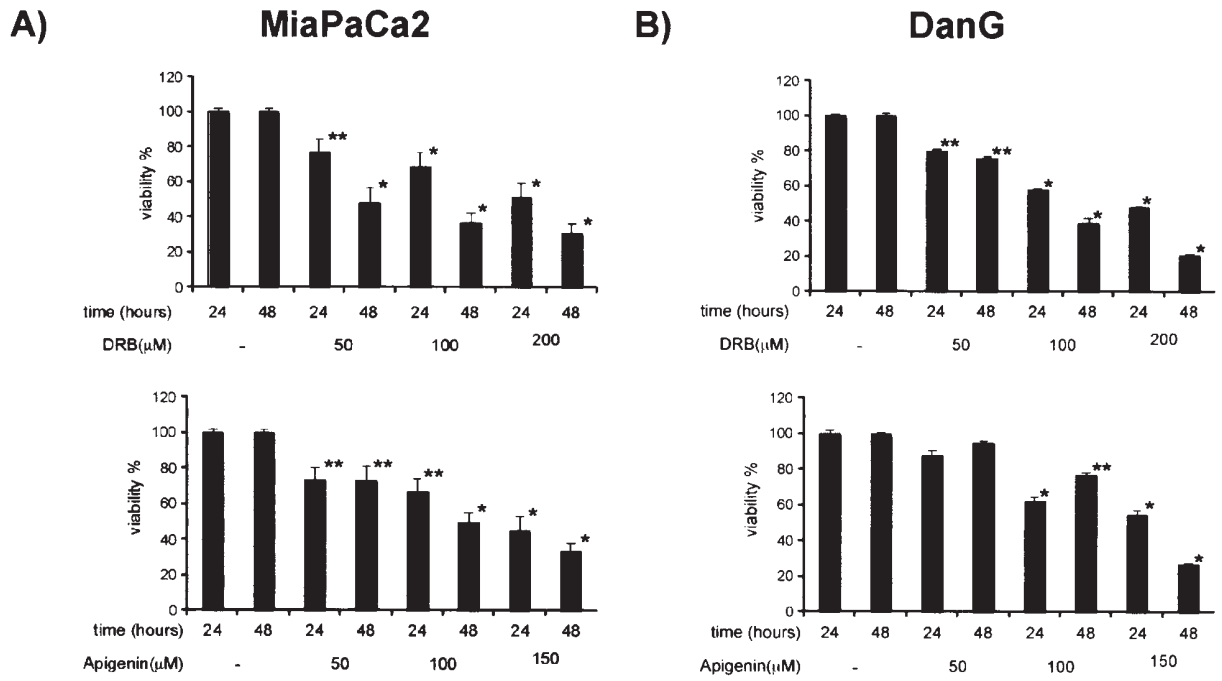

Figure 1. CK2 inhibition reduces viability of pancreatic cancer cells. (A) MiaPaCa2 cells were treated with 50, 100 and 200 μM DRB (upper graph) or with 50, 100 and 150 μM Apigenin (lower graph). After 24 and 48 h MTT assays were performed (Student's t-test, $^{*}p<0.001$ versus controls; $^{**}p<0.05$ versus control). (B) DanG cells were treated with 50, 100 and 200 μM DRB (upper graph) or with 50, 100 and 150 μM Apigenin (lower graph). After 24 and 48 h MTT assays were performed (Student's t-test, $^{*}p<0.001$ versus controls; $^{**}p<0.05$ versus control).

Reduced viability after the inhibition of CK2 is due to caspase-dependent apoptosis in pancreatic cancer cells. To investigate the cause of the reduced viability of pancreatic cancer cells we looked for apoptotic changes after the inhibition of CK2. As shown in Fig. 2A, chromatin condensation, a hallmark of apoptosis, was detected in DRB (Fig. 2A, upper row, middle) and Apigenin (Fig. 2A, lower row, left) treated MiaPaCa2 cells after staining the chromatin with Hoechst dye, which was absent in cells treated with vehicle alone (Fig. 2A, upper row, left). DRB-induced apoptotic changes as well as Apigenin-induced apoptotic changes were prevented by the pan-caspase inhibitor zVAD-fmk (Fig. 2A, upper row, right and lower row, right). Next we quantified the apoptotic changes of DRB- or Apigenin-treated MiaPaCa2 cells. DRB and Apigenin induced a dose-dependent increase of the apoptotic fraction. Thirty-five percent of the cells were apoptotic after 24 h of treatment with DRB (200 μM) (Fig. 2B, left upper graph) and 55% of the cells were apoptotic after 48 h of treatment with DRB (Fig. 2B, left lower graph). Simultaneous treatment with DRB and zVAD-fmk (3.6% after 24 h and 5.4% after 48 h) reduced the apoptotic fraction to control levels (1.7% after 24 h and 3.5% after 48 h). Apigenin also induced apoptosis of MiaPaCa2 cells in a dose-dependent fashion. After 24 h of treatment with 150 μM Apigenin, 48% of the cells were apoptotic reaching 71% after 48 h (Fig. 2B, right upper and lower graph). Again, the apoptotic fraction after the treatment with Apigenin was significantly reduced by zVAD-fmk co-treatment to 12% after 24 h and 18% after 48 h (Fig. 2B). Also, we found a dose-dependent increase in PARP cleavage 24 h after the treatment of MiaPaCa2 with DRB and Apigenin (Fig. 2C). DRB- as well as Apigenin-induced PARP cleavage was completely inhibited by zVAD-fmk (Fig. 2C).

These data demonstrate that inhibition of CK2 by DRB and Apigenin induced caspase-dependent apoptosis in MiaPaCa2 cells.

Kinetics of apoptosis after CK2 inhibition. To investigate the kinetics of apoptosis induction after treatment with CK2 inhibitors in more detail, we determined PARP cleavage over time. In DRB-treated MiaPaCa2 cells, the cleavage product of PARP was detected 6 h after the treatment with 200 μM DRB further increasing over time (Fig. 3A). Similarly, Apigenin-induced PARP cleavage was seen 6-8 h after treatment and further increased over the investigated time period (Fig. 3B). These data suggest an early onset of apoptosis induction by CK2 inhibition. One major anti-apoptotic principle activated by CK2 is the transcription factor NF-κB (18-20). To determine the contribution of NF-κB to the observed apoptotic phenotype we measured NF-κB-dependent transcriptional activity using luciferase reporter gene assays in MiaPaCa2 cells 6 h after treatment. At this time-point we detected a dose-dependent definite reduction of NF-κB-dependent transcriptional activity after treatment with DRB (Fig. 3C) and Apigenin (Fig. 3D). With the highest concentration used the activity was reduced to 33% (DRB) and 9% (Apigenin) of controls, respectively. These data suggest an early-onset apoptosis induction by CK2 inhibition. Furthermore, the activity of the anti-apoptotic transcription factor NF-κB was reduced by CK2 inhibition.

CK2 inhibitors induce apoptosis independent of BCL-$_{XL}$ regulation. It was shown that DRB and Apigenin reduce the expression of the anti-apoptotic BCL-2 family member BCL-$_{XL}$ (24). To investigate the contribution of BCL-$_{XL}$ to the observed apoptotic phenotype after CK2 inhibition in MiaPaCa2 cells

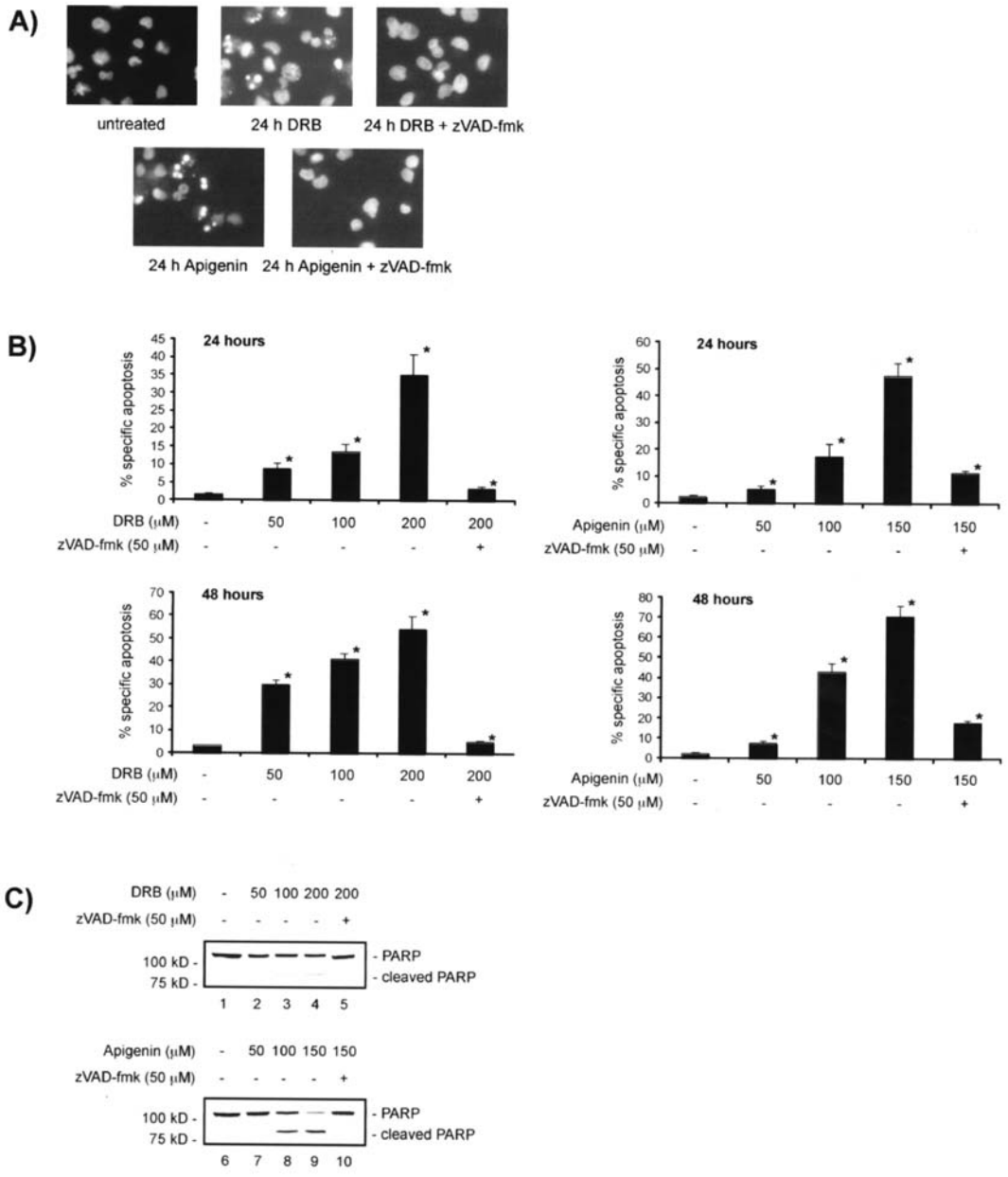

Figure 2. CK2 inhibition induces apoptosis of pancreatic cancer cells. (A) Photomicrographs (original magnification, x40) of MiaPaCa2 cells after the staining of the nuclei with Hoechst. Cells were treated for 24 h with vehicle (upper row, left), 200 μM DRB (upper row, middle), 200 μM DRB and 50 μM zVAD-fmk (upper row, right), 150 μM Apigenin (lower row, left) or 150 μM Apigenin and 50 μM zVAD-fmk (lower row, right). (B) Quantification of apoptotic cells 24 h (upper graphs) and 48 h (lower graphs) by fluorescence microscopy after Hoechst stain. MiaPaCa2 cells were treated with indicated doses of DRB (left graphs) or Apigenin (right graphs). To demonstrate caspase dependency cells were co-treated with 50 μM zVAD-fmk at the highest concentration DRB and Apigenin used (Student's t-test, $^*p<0.001$ versus controls). (C) PARP Western blot of DRB (upper Western blot) or Apigenin (lower Western blot) treated MiaPaCa2 cells. Increasing concentration of DRB and Apigenin were used as indicated. In lane 5 and 10 MiaPaCa2 cells were co-treated with 50 μM zVAD-fmk.

we analyzed BCL-$_{XL}$ expression by Western blotting. As demonstrated in Fig. 4A, a significant reduction of BCL-$_{XL}$ protein abundance was not observed over a time period of 24 h after the treatment with DRB. Also, no definite reduction of BCL-$_{XL}$ protein expression was detected after the treatment with Apigenin over a time period of 24 h in MiaPaCa2 cells (Fig. 4B). These data argue for a BCL-$_{XL}$-independent pathway of apoptosis after the inhibition of CK2 in pancreatic cancer cells.

Knock-down of the catalytical CK2 subunits reduces viability and induces apoptosis of MiaPaCa2 cells. To control the specificity of the effects observed after the treatment with the chemical inhibitors of CK2, we used RNA interference technology. Therefore we transfected MiaPaCa2 cells with siRNAs directed against CK2α' and CK2α. As demonstrated in Fig. 5A, the transfection of the siRNAs against CK2α' led to a reduction of CK2α' protein abundance below 10% compared to control siRNA transfected or untransfected MiaPaCa2 cells 48 h after transfection. Furthermore, CK2α expression was not changed in CK2α' siRNA transfected cells. Transfection of the CK2α siRNA led to reduction of CK2α expression to 22% compared to untransfected or control siRNA transfected cells (Fig. 5A). Again, no change of the CK2α' expression was detected after the transfection of the CK2α siRNA. Next we investigated the effects after the knock-down of the catalytical CK2 subunits

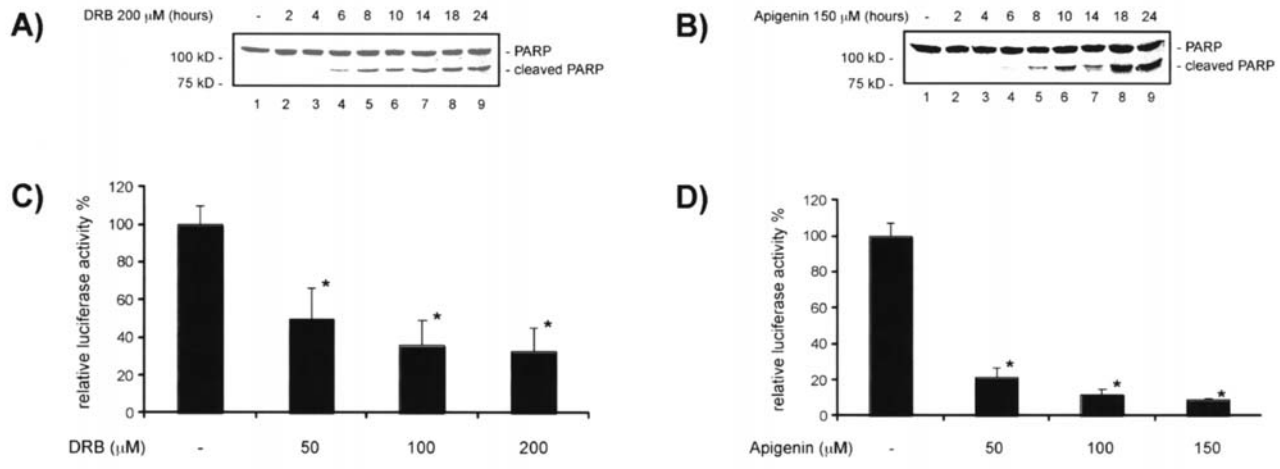

Figure 3. Kinetics of apoptosis after CK2 inhibition and NF-κB reporter gene assay. (A) MiaPaCa2 cells were treated with 200 μM DRB over time and onset of apoptosis was determined by PARP Western blotting. The PARP cleavage product is indicated. (B) MiaPaCa2 cells were treated with 150 μM Apigenin over time and onset of apoptosis was determined by PARP Western blotting. The PARP cleavage product is indicated. (C) MiaPaCa2 cells were transfected with 500 ng pGL3-3xκB-Luc luciferase reporter gene construct. Twenty-four hours after the transfection the cells were treated with DRB as indicated. Six hours after the treatment NF-κB transcriptional activity was measured (Student's t-test, $^*p<0.001$ versus controls). (D) MiaPaCa2 cells were transfected with 500 ng pGL3-3xκB-Luc luciferase reporter gene construct. Twenty-four hours after the transfection the cells were treated with Apigenin as indicated. Six hours after the treatment NF-κB transcriptional activity was measured (Student's t-test, $^*p<0.001$ versus controls).

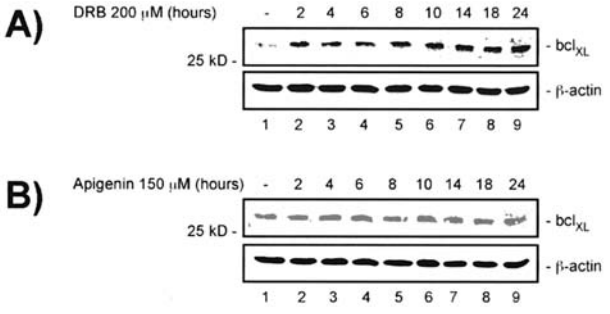

Figure 4. Apoptosis after the inhibition of CK2 is independent of BCL-$_{XL}$ protein expression. (A) MiaPaCa2 cells were treated with 200 μM DRB and BCL-$_{XL}$ protein expression was monitored over a time course of 24 h using Western blotting. (B) MiaPaCa2 cells were treated with 150 μM Apigenin and BCL-$_{XL}$ protein expression was monitored over a time course of 24 h using Western blotting.

over time on MiaPaCa2 cell viability. In control siRNA transfected MiaPaCa2 cells no significant change of viability was detected (Fig. 5B). Transfection of the CK2α' siRNA reduced the viability of MiaPaCa2 cells to 73, 63 and 45% of controls 24, 48 and 96 h after the transfection, respectively (Fig. 5B). The knock-down of the CK2α subunit also reduced viability of MiaPaCa2 cells. Here, a reduction of viability to 90, 68 and 51% compared to untransfected controls 48, 72 and 96 h after the transfection was detected.

To investigate whether the observed reduction of viability after the knock-down of the catalytical CK2 subunits is due to apoptosis we counted the apoptotic fraction after staining the cells with Hoechst. Whereas the fraction of apoptotic cells remained below 5% in control siRNA and untransfected MiaPaCa2 cells over a time period of 96 h, definite apoptosis was induced by the CK2 knock-down (Fig. 5C). After the transfection of the CK2α' siRNA 10, 25 and 28% apoptotic cells were detected 48, 72 and 96 h after the transfection, respectively (Fig. 5C). In CK2α siRNA transfected cells 10, 18 and 31% apoptotic cells were detected 48, 72 and 96 h after the transfection, respectively (Fig. 5C). In accordance, we found PARP cleavage after the transfection of the siRNAs against the catalytical CK2 subunits 96 h after the transfection (Fig. 5D).

The reduced viability after the knock-down of the catalytical CK2 subunits and the detection of apoptosis validates the results obtained with the chemical inhibitors and further supports the notion that CK2 contributes to the survival of pancreatic cancer cells.

Discussion

CK2 is essential for survival in many cellular systems (23,29-31). This prompted us to investigate the influence of CK2 towards the viability of pancreatic cancer cells. Our studies showed that the viability of pancreatic cancer cells depends on CK2 activity, since the pharmacological inhibition, as well as RNA interference approach led to a definite reduction of viable cells. Furthermore, we demonstrated that apoptosis is a major mechanism leading to the observed reduction of viability after the inhibition of CK2. In addition, the apoptotic phenotype correlated with a reduced activity of the transcription factor NF-κB. Therefore, our pre-clinical model defines CK2 as a potential drug target for future therapies of pancreatic cancer. This is in line with a recent observation, finding CK2 in a siRNA screen for survival kinases in pancreatic cancer cells (32).

Interfering with CK2 activity induces apoptosis or confers susceptibility towards chemotherapeutic or death-receptor mediated apoptosis (24-27,33-35). Furthermore, inhibition of CK2 by antisense oligonucleotide strategies induced apoptosis in a xenograft model of prostate cancer (36). Even though, apoptosis after the inhibition of CK2 is a common cellular response, the molecular mechanisms are not entirely clear. In the mammary cancer cell line Hs578 the anti-apoptotic BCL-2 family member BCL-$_{XL}$ has been shown to be regulated by

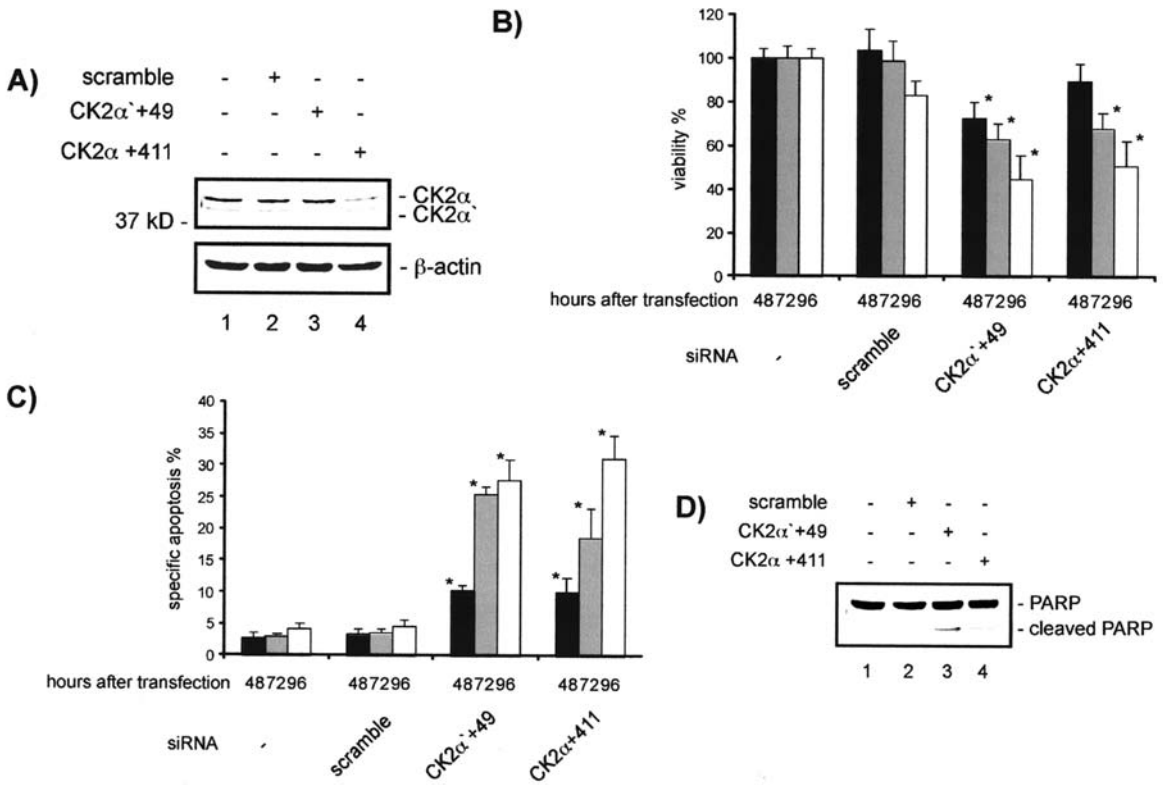

Figure 5. Knock-down of the catalytical CK2 subunits CK2α' and CK2α reduces the viability and induces apoptosis of MiaPaCa2 cells. (A) Western blot analysis of CK2α and CK2α' 48 h after the transfection of MiaPaCa2 cells with a scramble control siRNA (lane 2), a CK2α' siRNA (lane 3) and a CK2α siRNA (lane 4) compared to untransfected control cells (lane 1). ß-actin controls equal protein loading. (B) MTT assay of MiaPaCa2 cells 48, 72 and 96 h after the transfection of a scramble control siRNA, a CK2α' siRNA and a CK2α siRNA compared to untransfected control cells (Student's t-test, $^{*}p<0.001$ versus controls). (C) Quantification of apoptotic cells 48, 72 and 96 h after the transfection of a scramble control siRNA, CK2α' and CK2α siRNAs compared to untransfected control cells by fluorescence microscopy after Hoechst stain (Student's t-test, $^{*}p<0.001$ versus controls). (D) PARP Western blot of CK2α and CK2α' transfected MiaPaCa2 cells. MiaPaCa2 cells were transfected with the indicated siRNAs. Ninety-six hours after the transfection extracts were prepared and PARP cleavage was detected by Western blots.

CK2 (24). Although we found a rapid and distinct decrease of NF-κB transcriptional activity after the treatment of MiaPaCa2 cells with the CK2 inhibitors used and BCL-$_{XL}$ is an NF-κB target gene, we detected no decrease of BCL-$_{XL}$ protein abundance, suggesting BCL-$_{XL}$-independent apoptosis. Since it was demonstrated that in pancreatic cancer cell lines inhibition of NF-κB activity enhances the sensitivity of cells towards chemotherapeutic agents as well as death receptor-mediated apoptosis and since the observed inhibition of NF-κB activity after the treatment with CK2 inhibitors parallels the onset of apoptosis, NF-κB seems to lower the apoptotic threshold in our cellular model (37,40). The molecular mechanism of NF-κB regulation observed after the inhibition of CK2 is not clear at the moment. The NF-κB inhibitor IκBα was shown to be destabilized by CK2-dependent phosphorylation in its C-terminal PEST domain (19,21). We observed a reduction of IκBα expression after the inhibition of CK2 in MiaPaCa2 cells, suggesting an IκBα-independent pathway of NF-κB inhibition in our model (data not shown). Whether a direct phosphorylation of RelA/p65 at Ser-529 by CK2, shown to contribute to NF-κB activation after TNF-α signaling contributes to NF-κB activity in pancreatic cancer cells awaits further investigation (26,41).

The molecular mechanism of apoptosis initiation after inhibition of CK2 in pancreatic cancer cells is unclear. Interestingly, a recent report demonstrated phosphorylation of pro-caspase-2 at Ser-157 by CK2. Dephosphorylated pro-caspase-2 after CK2 inhibition is activated by dimerization and cleaves pro-caspase-8 (42). In accordance, we found cleavage of pro-caspase-8 after the inhibition of CK2 in MiaPaCa2 cells (data not shown). Therefore, CK2 might act in two ways to induce apoptosis in pancreatic cancer cells. On the one hand, CK2 inhibition lowers the anti-apoptotic threshold via NF-κB. On the other hand, the caspase-2/8 pathway activated after CK2 inhibition leads to BID cleavage, whereas BID is more susceptible to caspase-8 cleavage after lack of CK2 phosphorylation.

In summary, our studies demonstrate the requirement of CK2 for survival of pancreatic cancer cells. Interfering with CK2 activity might be a novel approach for pancreatic cancer therapies.

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