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Role of polyamines in determining the cellular response to chemotherapeutic agents: modulation of protein kinase CK2 expression and activity

Jan N. Kreutzer · Birgitte B. Olsen · Karolina Lech · Olaf-Georg Issinger · Barbara Guerra

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Abstract Numerous studies have shown that platinum compounds stimulate the expression of the polyamine catabolic enzyme spermidine/spermine N^1-acetyltransferase resulting in anti-proliferative activity and apoptosis. As many cancer cell types including pancreatic cancer cells express high levels of polyamines, the possibility to develop anti-tumor strategies to deplete polyamine pools has drawn considerable attention in recent years. This has been effectively accomplished by treating cells with platinum drugs in combination with polyamine analogs such as N^1,N^{11}-diethylnorspermine (DENSPM). The present study, examined the cytotoxic effects of oxaliplatin in combination with stimulators of polyamine catabolism in human pancreatic cancer cells, that are notoriously resistant to chemotherapeutic treatment, and colorectal cancer cells. Additionally, as protein kinase CK2 has been shown to be an anti-apoptotic and pro-survival enzyme regulated by the intracellular polyamine pools, we aimed to investigate the effect of combined DENSPM and oxaliplatin treatment on CK2-mRNA and -protein levels. Results reported here show that treatment with oxaliplatin and DENSPM in combination impairs cell viability particularly in the case of colorectal cancer cells. The analysis of CK2 expression and activity indicates that the response to a specific treatment may depend on the impact that individual compounds exert on pro-survival and pro-death proteins at the transcription and translation levels that should be carefully evaluated in view of subsequent clinical studies.

Keywords Protein kinase CK2 · Oxaliplatin · Polyamine analogs · Spermidine/spermine N^1-acetyltransferase

Introduction

Polyamines are amino acid-derived organic molecules that are synthesized in all organisms [1]. Proteins involved in the synthesis of polyamines, e.g., ornithine decarboxylase (ODC) which is the rate-limiting enzyme, are expressed in all tissues [1]. Polyamines are essential molecules for many physiological processes such as growth, development and tissue repair. In the late 1960s the association of increased polyamines synthesis and high levels of ODC activity with cell growth and cancer arising from epithelial tissues such as skin, prostate and colon was reported [1]. Despite the fact that a link between cancer and polyamines deregulation has been reported more than 30 years ago, it is only recently that evidence emerged about a causative rather than an associative effect of polyamines in tumor development. Studies conducted on colon cancer have revealed that ODC is negatively modulated by the adenomatous polyposis coli tumor suppressor gene (*APC*). Loss of APC function leads to increased expression of Myc transcription factor which, in turn, stimulates the synthesis of ODC [2, 3]. Oncogenic mutations in *KRAS,* result in aberrant activation of the enzyme, suppresses the polyamine catabolism by inhibiting the expression of the peroxisome-proliferator-activated receptor-γ (PPARγ) which stimulates the transcription of the spermidine/spermine N^1-acetyltransferase (*SSAT*) gene by binding to its promoter [4, 5]. Polyamines contribute to the regulation of numerous

Jan N. Kreutzer and Birgitte B. Olsen contributed equally to the study.

J. N. Kreutzer · B. B. Olsen · K. Lech · O.-G. Issinger · B. Guerra (✉)
Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, 5230 Odense, Denmark
e-mail: bag@bmb.sdu.dk

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cancer-related functions such as cellular proliferation and blood-vessel development in growing tumor tissues [6]. Increasing evidence indicates that polyamine metabolic pathways are frequently deregulated in cancer. Hence, it is not surprising that the development of polyamine metabolic inhibitors has gained interest in recent years leading to the synthesis of selective inhibitors of ODC such as 2-difluoromethylornithine (DFMO, reviewed in [7]). The cytotoxicity of DFMO observed with various cancer cell types encouraged clinical investigations in which the activity of DFMO as single agent or in combination with other chemotherapeutic agents, was tested against several tumor models ([8] and for a review see [9]). As the target inhibition of enzymes involved in the active synthesis of polyamines did not seem to confer a significant clinical success in the treatment of malignancies, subsequent strategies have focused on the suppression of polyamines by polyamine analogs such as N^1,N^{11}-diethylnorspermine (DENSPM) and N^1,N^{14}-diethylhomospermine (DEHSPM) [10]. Numerous studies have shown that cells respond to exposure to polyamine analogs with a large increase in SSAT expression in multiple tumor cell types such as melanoma, pancreatic, bladder and breast cancers (reviewed in [9]). Interestingly, treatment of colorectal cancer cells with DENSPM in combination with oxaliplatin used as standard chemotherapeutic agent, led to a significant increase in SSAT mRNA levels as compared to oxaliplatin and DENSPM single treatments, respectively, indicating a synergistic effect with respect to the expression of SSAT [11, 12].

Protein kinase CK2 is a pleiotropic and constitutive active serine/threonine enzyme expressed in all eukaryotic organisms. It is composed of two catalytic α- and/or α'-subunits and two regulatory β-subunits. The regulatory subunits have been shown to confer stability, modulate substrate specificity as well as the activity of the holoenzyme [13]. CK2 appears distributed in tetrameric complexes inside the cells but significant evidence indicates that the individual subunits also exist as free proteins. CK2 phosphorylates and/or interacts with many intracellular proteins involved in a variety of biological processes including gene expression, cell cycle regulation, transformation, apoptosis, DNA sensing and repair (for reviews see [14–16]). Although CK2 is considered a constitutively active enzyme, evidence indicates that certain structural conditions, intracellular distribution or effector molecules such as polyamines can modulate the activity of CK2 (for a recent review see [16]). CK2 has been shown to be activated in vitro by polyamines by directly binding to the β-subunits while in vivo studies have revealed that increased polyamine levels result in enhanced CK2 activity through mechanisms distinct from the direct interaction with the regulatory subunits observed earlier [17–19].

In this study, as pancreatic cancer cells are notoriously resistant to treatment with chemotherapeutic agents such as gemcitabine and platinum analogs used in various combinations (reviewed in [20]), we aimed to determine whether the incubation of cells with oxaliplatin and other compounds that modulate polyamine metabolism may have synergistic effects in inducing significant cytotoxicity as reported in the case of colorectal cancer cells [11, 21]. In addition, as CK2 expression and activity are elevated in highly proliferating normal and cancer cells and stimulated by increased cellular polyamine levels, we evaluated the effects on CK2 at the mRNA and protein levels following treatment with the aforementioned compounds used alone or in combination. The data presented here, indicate that cell treatment with compounds regulating polyamine metabolism does not affect the viability and proliferation of pancreatic cancer cells to the same extent as reported in colorectal cancer cells. Moreover, the analysis of CK2 mRNA levels, protein expression and activity suggests that the outcome of a specific combination treatment largely depends on the effect exerted by the individual compounds on the transcriptional and translational regulation of the expression of key survival proteins.

Materials and methods

Cell culture and treatments

The human pancreatic cancer PANC-1 and colorectal cancer HCT116 cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Taastrup, Denmark) supplemented with 10% fetal bovine serum and maintained in a humidified atmosphere containing 5% CO_2 at 37°C. Cells were treated with DENSPM (Tocris Bioscience, Bristol, United Kingdom) and oxaliplatin (Sigma, MO, USA) as indicated in the figure legends.

Viability and proliferation assays

Cell viability was determined by the WST-1 assay (Roche, Penzberg, Germany) in 96-well plates. Twenty-four hours after seeding, cells were treated with various concentrations of oxaliplatin and DENSPM for 72 h as indicated in the figure legends. The viability assay was performed according to the manufacturer's recommendations. In brief, cells were incubated with the WST-1 reagent for 2 h at 37°C before absorbance was measured with a microtiter plate reader (VERSAmax, Molecular Devices, Sunnyvale, CA, USA) at wavelengths indicated in the figure legends. Similarly to the WST-1 test, the cell proliferation assay

was performed in 96-well plates. Twenty-four hours after seeding, cells were treated with various compounds as described in the figure legends. Afterward, cells were labeled with 5-bromo-2′deoxyuridine (BrdU) for 3 h (Calbiochem, Nottingham, United Kingdom). Cells were then fixed, the DNA was denatured and cells were incubated with a peroxidase-conjugated anti-BrdU antibody according to the manufacturer's instructions. The immune complexes were revealed in a microtiter plate reader as indicated in the figure legends.

Cell cycle analysis

The distribution of cells in the various phases of the cell cycle was estimated by flow cytometry. Cells were fixed in 70% ethanol at −20°C overnight and subsequently incubated with 20 µg/ml propidium iodide (Sigma) and 40 µg/ml RNase A (Roche) in PBS for 30 min in the dark. Cells were then analyzed on a FACS-Calibur flow cytometer (Becton–Dickinson, CA, USA). The acquired data were analyzed by CellQuest Pro Analysis software (Becton–Dickinson). For each measurement, 10,000 cells were analyzed.

Western blot analysis and densitometric analysis

Whole cell extracts were prepared as described in [22]. For protein detection on western blot membranes, the following antibodies have been used: mouse monoclonal anti-CK2α and -β were from Calbiochem, mouse monoclonal anti-β-actin was from Sigma, rabbit polyclonal anti-CK2α' was obtained by immunizing rabbits with a specific peptide (SQPCADNAVLSSGTAAR), rabbit polyclonal anti-SSAT (H-77) was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Densitometric analysis of protein bands on western blot was performed with the UVIsoft analysis software (UVItec, Cambridge, UK).

In vitro protein kinase assay

The activity of native protein kinase CK2 present in whole extracts from HCT116 and PANC-1 cells was determined as described in [23].

Quantitative real-time PCR

Total RNA from cells treated as indicated in the figure legends was isolated using TRIzol reagent (Invitrogen). 300 ng of total RNA was used for reverse transcription using the GeneAmp® RNA PCR Core Kit (Applied Biosystems by Life Technology, Carlsbad, CA, USA). The obtained cDNAs were then used as a template for the subsequent PCR. PCR reactions were performed in a 20 µl volume consisting of 30 ng template, 1x SYBR® Green JumpStart™ Taq ReadyMix™ (Sigma), forward and reverse primers relative to the cDNA of the analyzed proteins each at 200 nM final concentration. All samples were prepared in triplicates. The reactions consisted of a 10 min initial denaturation (95°C) followed by 40 cycles of denaturation (95°C, 15 s) and annealing/extension (60°C, 1 min). Measurement of SSAT, β-actin, CK2α, -α' and -β gene expression levels was carried out according to the quantification method of the StepOnePlus real-time PCR System (Applied Biosytems). All mRNA expression values are ratios relative to β-actin. Fold changes in samples derived from drug-treated cells were determined relative to untreated (control) samples. Primer pairs against the human SSAT1, β-actin, CK2α, -α' and -β mRNAs were as follows: for SSAT-1, forward primer 5′-TCTAAGCCAGGTTGCAATGAGGTGT-3′ and reverse primer 5′-ACAGTCTCCAACCCTCTTCACTGGAC-3′; β-actin, forward primer 5′-GACAGGATGCAGAAGGAGATTACT-3′ and reverse primer 5′-TGATCCACATCTGCTGGAAGGT-3′; CK2α, forward primer 5′-ACGAGTCACATGTGGTGGAA-3′ and reverse primer 5′-TTTACCTCGGCCTAATTTTCG-3′; CK2α', forward primer 5′-TCAAGGTACTTCAAGGGACCAG-3′ and reverse primer 5′-TCCACATGTCCAAGCTATAATCA-3′; CK2β, forward primer 5′-ACCTGCCAACCAGTTTGTG-3′ and reverse primer 5′-GACTGGGCTCTTGAAGTTGC-3′.

Statistical analysis

The statistical significance of differences between the mean of two sets of data was evaluated by the two-tailed-*t*-test (Student's *t*-test) and the level of significance is indicated in the figure legends.

Results

The combination of oxaliplatin and the spermine analog DENSPM results in synergistic effects on pancreatic and colorectal cancer cell lines

Changes in SSAT-mRNA and -protein expression levels were investigated in the human pancreatic adenocarcinoma PANC-1 cell line following treatment with oxalipatin and DENSPM as single agents or in combination. By comparison, the evaluation included also the analysis of the human colorectal cancer HCT116 cell line previously reported to respond to the aforementioned treatment by a marked increase in SSAT mRNA levels [21]. As shown in Fig. 1a, treatment of both cell lines with combined oxaliplatin and DENSPM markedly increased the SSAT mRNA level up to 48 h upon drug treatment. The increase was more pronounced in the case of HCT116 cells, where a

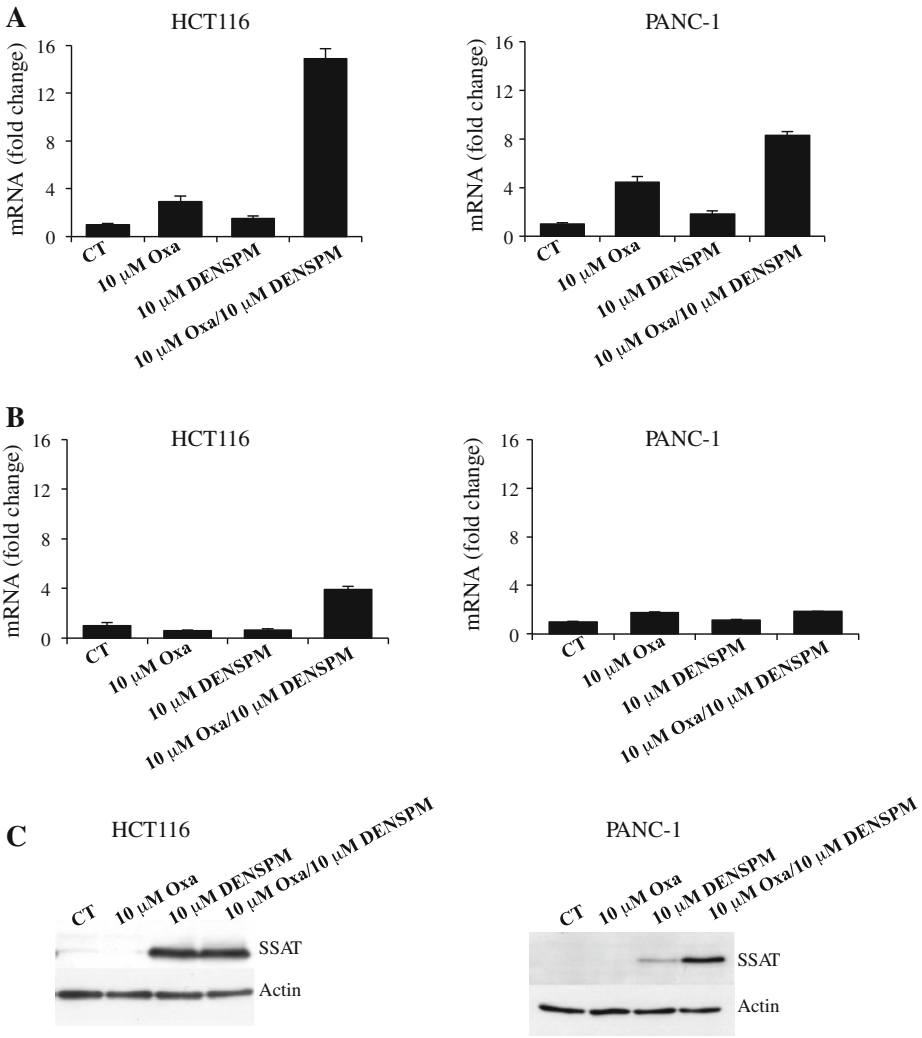

Fig. 1 Effect on SSAT mRNA and protein levels following cellular treatment with oxalipatin and DENSPM. **a** Human HCT116 and PANC-1 cells were incubated singly with oxaliplatin (Oxa) and DENSPM or in combination for 24 h followed by 24 h incubation in drug-free medium. SSAT mRNA was quantified (triplicate measurements) relative to β-actin by quantitative RT-PCR. Fold changes are relative to control experiments (CT). **b** Experiments were performed as in (**a**) except that cells were harvested after 48 h incubation in drug-free medium. **c** Whole protein extracts from cells treated as in (**b**) were analyzed by SDS-PAGE followed by Western blot analysis. The expression of SSAT was verified by probing the membrane with a rabbit polyclonal anti-SSAT antibody. Detection of β-actin protein was used as a loading control. Three separate experiments were performed obtaining similar results. Data from one representative assay are shown. Bars indicate the average of three independent measurements ± standard deviation (SD)

~15-fold increase in the expression with respect to control experiment was observed. In PANC-1 cells, the combination treatment led to a ~8-fold increase in SSAT mRNA level in comparison to control cells. The level of SSAT mRNA returned almost to control values by 72 h after drug treatment in both cell lines (Fig. 1b). Oxaliplatin slightly increased the amount of SSAT mRNA transcripts as compared to control experiments (~3- and ~4-fold in HCT116 and PANC-1 cells, respectively, Fig. 1a). Next, the expression of SSAT protein was analyzed in both cell lines 72 h after adding the drugs to the culture. The combination of oxaliplatin and DENSPM significantly induced SSAT protein expression (Fig. 1c). DENSPM alone slightly induced SSAT expression in PANC-1 while the protein expression level was higher in HCT116 cells. Although oxaliplatin treatment resulted in enhanced SSAT mRNA levels in both cell lines, a concomitant increase in SSAT protein expression under the indicated experimental conditions was not observed. As induction of SSAT has been linked to the anti-proliferative effects of DENSPM [24, 25], dose–response experiments were performed by measuring the metabolic activity of cells treated with oxaliplatin and DENSPM as single agents or in combination for 72 h (Fig. 2). Oxaliplatin at 0.25 μM concentration in combination with 2.5 μM DENSPM led to severe cytotoxicity of HCT116 cells resulting in ~34% viable cells with respect to untreated cells. Incubation at higher concentration of oxaliplatin (i.e. 0.5 μM) dropped the survival to almost 0%. PANC-1 were more resistant as ~61% cells were still viable in comparison to HCT116 cells. Although the combination treatment showed a synergistic effect on cell viability, a higher concentration of oxaliplatin (i.e. 0.5 μM) did not further increase the cytotoxicity of PANC-1 cells as observed in the case of HCT116 cells. The anti-proliferative effects of oxaliplatin and DENSPM were also investigated. 0.25 μM oxaliplatin in combination with 2.5 μM DENSPM for 72 h exerted a major effect on HCT116 cells as their proliferation decreased by ~75% with respect to control experiments while the proliferation of PANC-1 cells decreased about 38% under the same

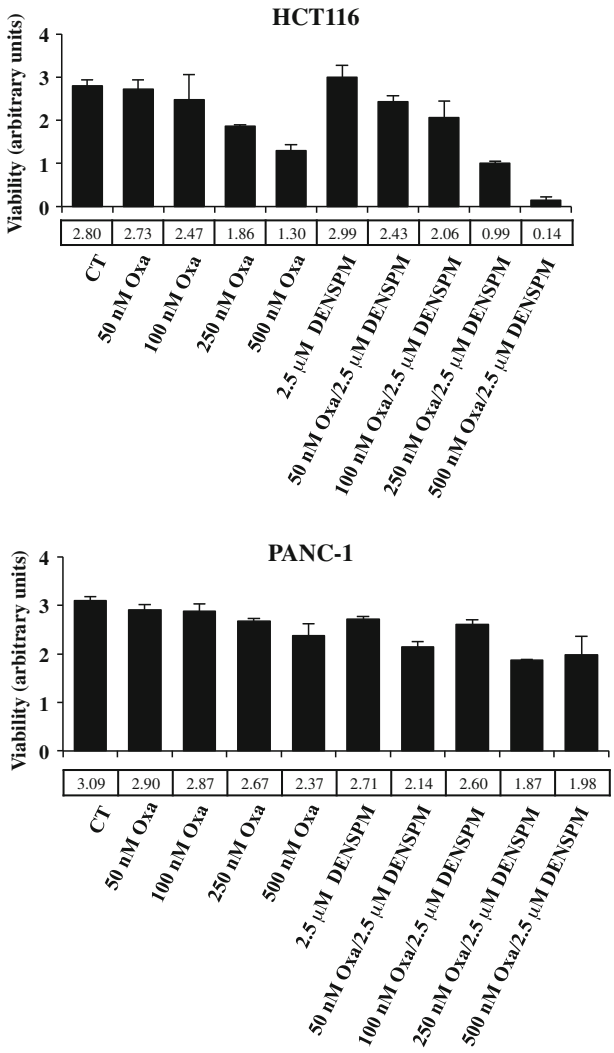

Fig. 2 Effect of oxaliplatin and DENSPM on the viability of human colorectal HCT116 and pancreatic adenocarcinoma PANC-1 cell lines. Cells were treated with the indicated concentrations of oxaliplatin and DENSPM for 72 h. Proportions of viable cells determined by the WST-1 assay are shown in arbitrary units as the difference in absorbance measured at 450 and 690 (reference) nm wavelengths, respectively. Each bar indicates the average of six independent measurements ± SD. Experiments were repeated three times obtaining similar results and data from one representative experiment are shown

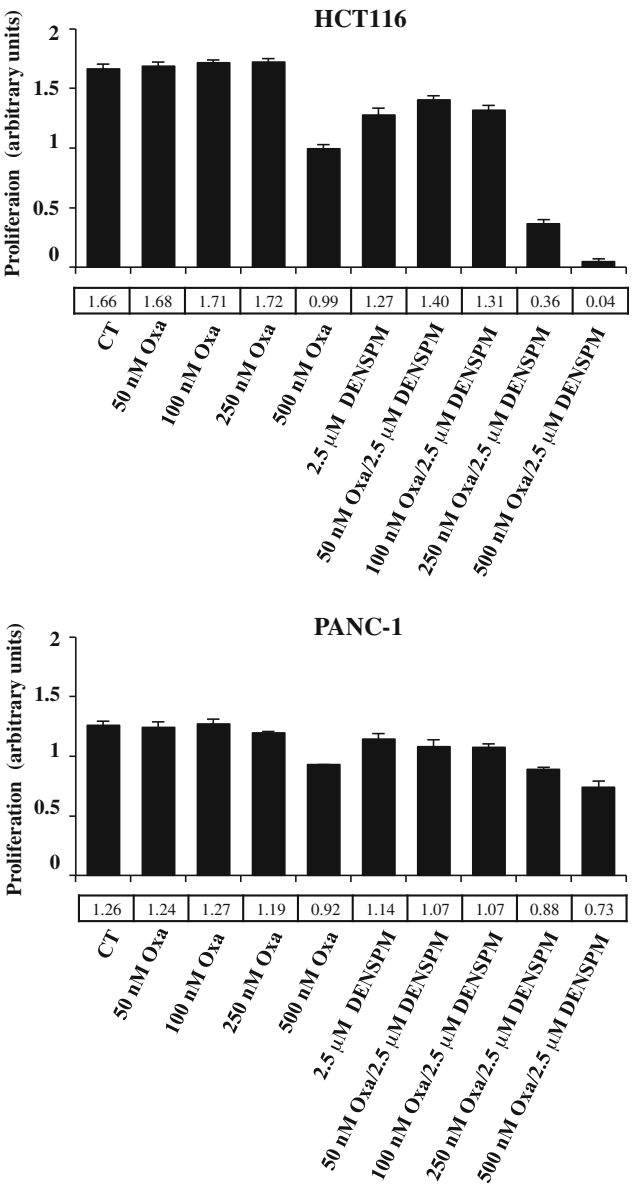

Fig. 3 Effect of oxaliplatin and DENSPM on cell proliferation. Cell proliferation for the indicated cell lines treated as described in Fig. 2, was determined by BrdU incorporation into genomic DNA. Results are expressed in arbitrary units as the difference in absorbance measured at 450 and 540 (reference) nm wavelengths, respectively. Experiments were repeated three times obtaining similar results and data from one representative experiment (bars indicate means ± SD of three measurements) are shown

treatment conditions (Fig. 3). Experiments described above, revealed dose–response effects of oxaliplatin and DENSPM alone, respectively, but also synergistic effects when compounds were used in combination.

Subsequent experiments were conducted with PANC-1 cells by applying conditions described in the legend to Fig. 1 as they led to the observations reported in the subsequent figures. In parallel, HCT116 cells were also employed confirming data from previous studies on the induction of SSAT-gene and -protein expression levels in these cells [21]. The analysis of cell death by flow cytometry induced by 10 μM oxaliplatin incubation of HCT116 cells for 24 h followed by 48 h in drug-free medium, led to ~15% cell death. This percentage further increased to ~40% in the oxaliplatin/DENSPM combination treatment (Fig. 4). In the case of PANC-1 cells, oxaliplatin alone induced a 15% increase in cell death with respect to control experiment while the addition of DENSPM did not lead to a further change in the percentage of cells with reduced DNA content observed with the sole oxaliplatin treatment.

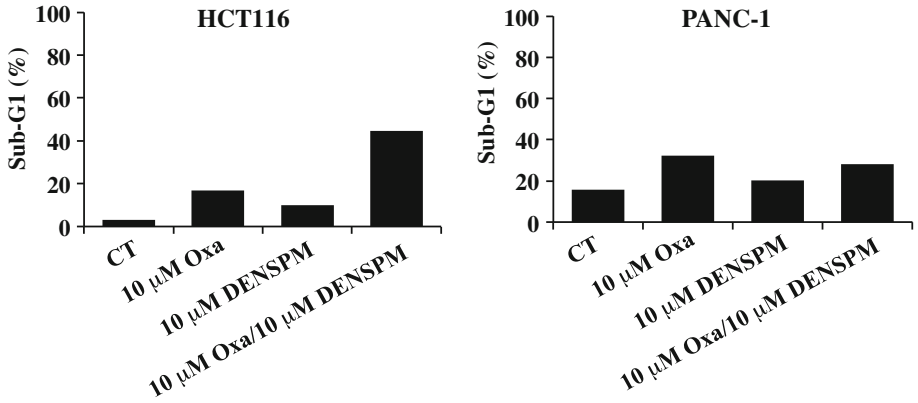

Fig. 4 Effect on cell death in cells treated with oxaliplatin and DENSPM. Cells treated singly with oxaliplatin and DENSPM or in combination for 24 h were subsequently incubated in drug-free medium for additional 48 h prior to harvesting. Fixed cells were subjected to flow cytometry analysis following propidium iodide staining. The fraction of cells in sub-G1 (i.e. with reduced level of DNA indicative of late apoptosis and/or necrosis) is reported in percentage and calculated with respect to the total number of cells. Experiments were performed three times obtaining similar results. Data from one representative experiment is shown

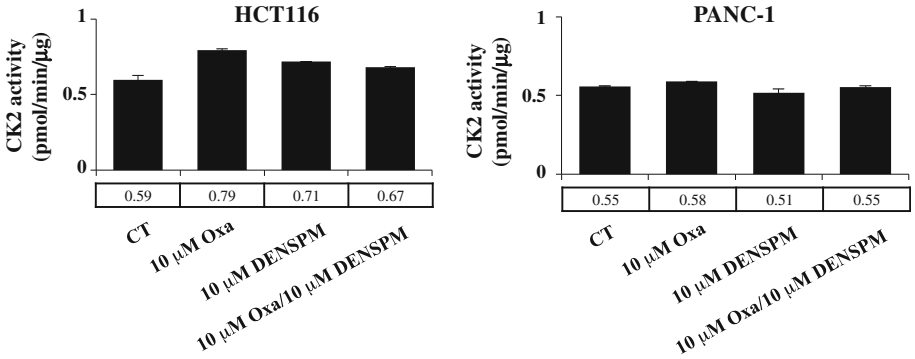

Fig. 5 Determination of protein kinase CK2 activity following incubation of cells with oxalipatin and DENSPM. Cells were treated as indicated in Fig. 4. Total lysates (20 μg) were subjected to a radioactive CK2 kinase assay with a specific substrate peptide as indicated in the materials and methods section. The average ± SD of three independent experiments is shown

Cellular treatment with oxaliplatin and DENSPM affects the CK2-mRNA and -protein expression levels

Previously, it has been reported that the activity of protein kinase CK2 is enhanced as a result of polyamines binding to the regulatory β-subunit in vitro [17, 18]. However, later studies showed that overexpression of ODC resulted in a 3-fold increase in CK2 mRNA molecules suggesting that the regulation of endogenous CK2 may occur through mechanisms distinct from the direct stimulation by polyamines as reported earlier [19]. Because of the role of CK2 as anti-apoptotic and pro-survival protein in cancer cells and the effects mediated by polyamine levels, we first investigated whether the simultaneous treatment of cells with oxaliplatin and DENSPM affected the kinase activity of CK2. In Fig. 5, treatment of HCT116 cells with oxaliplatin and DENSPM resulted in a slightly higher activity of CK2 with respect to control experiment. This effect was observed also in PANC-1 cells at longer incubation time (i.e. up to 144 h, results not shown).

To test whether oxaliplatin and DENSPM treatments used as individual agents or in combination had an effect on *CK2*-gene transcription, real-time PCR analysis was performed to quantify the transcript levels in both cell lines. As shown in Fig. 6a, oxaliplatin alone produced a slight increase of the individual CK2 subunit mRNA levels in HCT116 cells while the induction was much more significant in PANC-1 cells compared to control experiment. DENSPM itself did not cause any effect while the combination treatment did not result in a higher increase in mRNA, with respect to oxaliplatin alone, in both cell lines indicating that oxaliplatin is responsible for the marked induction of *CK2*-gene transcription. Unexpectedly, increased expression of the individual CK2 subunits transcripts was not accompanied by higher protein expression levels (Fig. 6b) as anticipated from the results shown in Fig. 6a. The indicated treatments caused decreased expression of the individual CK2 subunits, with respect to control cells, in both cell lines which was significant in the case of CK2α with respect to the

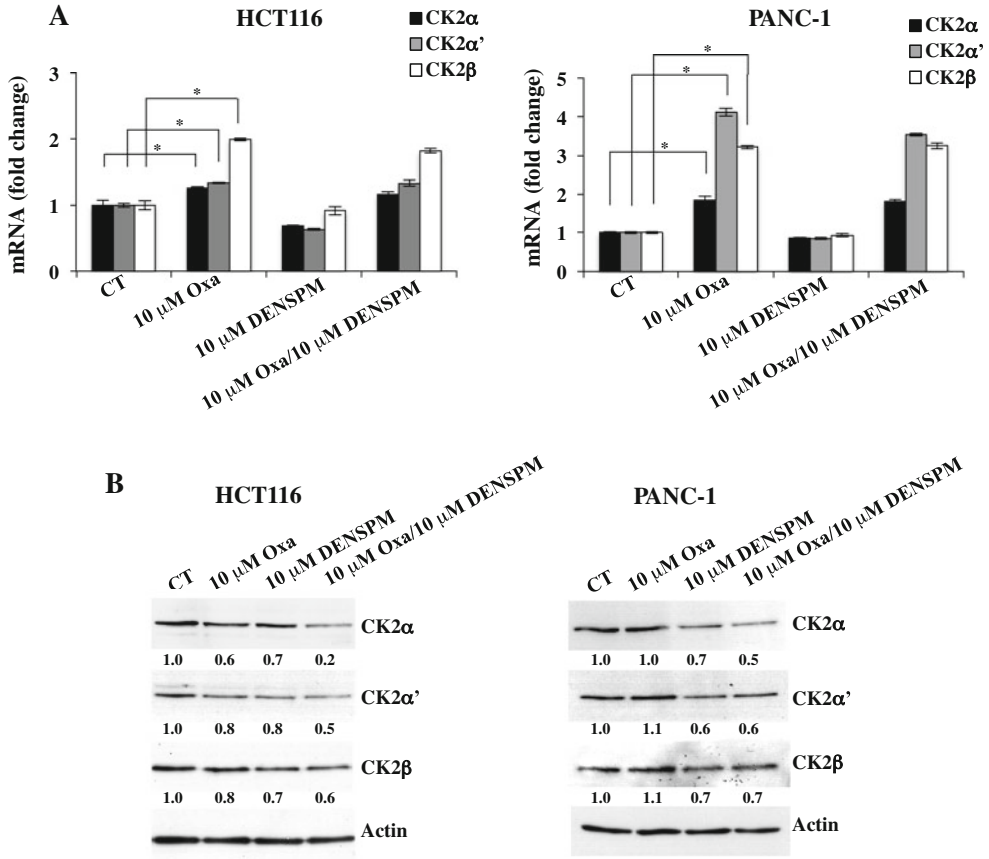

Fig. 6 Effect of oxaliplatin and DENSPM alone and in combination on CK2 mRNA (**a**) and protein levels (**b**) in HCT116 and PANC-1 cells. Cells were exposed to 10 μM oxaliplatin, 10 μM DENSPM singly or in combination for 24 h followed by 48 h incubation in drug-free medium. Cells were harvested and subsequently processed for either quantitative RT-PCR or Western blot analysis. In (**a**), CK2 mRNA was quantified (triplicate measurements) relative to *β*-actin. Fold-changes are relative to untreated controls. In the two graphs, the vertical axis have different scales to better highlight differences in gene expression levels between control and treated cells in the two cell lines. Statistical significance of the differences between control and oxaliplatin-treated groups, respectively, was determined by the Student's *t*-test which resulted in $*P < 0.005$ (statistically significant) for all the determinations. In (**b**), protein expression levels were quantified by densitometric analysis from a representative Western blot deriving from one out of three separate experiments. Fold changes were calculated with respect to protein levels in untreated cells. *β*-actin was used as loading control

combination treatment as also indicated by the densitometric analysis of protein bands.

Discussion

Previous studies reported that incubation of colorectal cancer cells with platinum drugs such as oxaliplatin results in a slight induction of SSAT mRNA which significantly increases when cells are treated with the drug in combination with the polyamine analog DENSPM. In the present work, we observed a similar effect in the pancreatic cancer cell line PANC-1 although not as pronounced as in the case of HCT116 cells. The analysis of SSAT protein expression levels in both type of cells revealed that induction of SSAT mRNA by oxaliplatin apparently did not result in a concomitant expression of the protein unless DENSPM was present. Moreover, the combination of DENSPM with oxaliplatin led to a significant increase in SSAT protein level compared to the treatment with the single agents, only in PANC-1 cells. In HCT116 cells there was no difference in the expression of SSAT induced by DENSPM alone or in combination with oxaliplatin. While it is unclear what the underlying mechanisms are, it is likely that differences observed with the two cell lines might be related to their biology and that DENSPM appears to facilitate the translation of SSAT mRNA. The cytotoxic and cell proliferation effects of oxaliplatin and DENSPM used as single agents or in combination were extensively studied in both cell lines at various concentrations. Our results indicate that the effects were synergistic at the concentrations employed in the study although marked cytotoxicity and reduced cell proliferation were observed only in HCT116 cells. Results obtained with PANC-1 cells suggest that these cells might

be not highly dependent on the intracellular level of polyamines although numerous studies have shown that pancreatic cancer cell lines have significantly higher levels of polyamines than normal pancreatic tissue. In this respect, Black and colleague demonstrated a positive correlation between the levels of ODC, protein content and chemoresistance of the various pancreatic cancer cell lines analyzed in their studies [26, 27]. However, despite the enhanced expression of SSAT protein in PANC-1 cells, one cannot exclude the possibility that the polyamine levels might still be elevated, thus, counteracting the effects induced by the oxaliplatin/DENSPM combination treatment. Moreover, despite the higher expression, SSAT enzyme activity might be comparable to basal values. In this respect, studies on human lung carcinoma cell lines sensitive and resistant to the effects of DENSPM, respectively, have demonstrated a correlation between SSAT activity and cytotoxicity induced by the polyamine analog [28].

Numerous studies have shown that protein kinase CK2 is an important pro-survival enzyme since its overexpression protects against drug-induced apoptosis and chemical inhibition or siRNA-mediated downregulation can sensitize cancer cells toward cell death (reviewed in [29]). In our study, treatment with oxaliplatin alone stimulates transcription of the *CK2*-gene and the transcripts are not translated into proteins in both cell lines (Fig. 6). Instead, DENSPM seems to negatively contribute to the regulation of CK2 protein expression and the lowest expression of the individual CK2 subunits is observed following incubation of cells with DENSPM in combination with oxaliplatin. In this respect, it would be interesting to investigate the role of DENSPM with respect to the modulation of CK2 protein synthesis and degradation. In HCT116 cells, a significant percentage of cell death is observed with oxaliplatin and DENSPM co-treatment (Fig. 4). This effect is accompanied by marked down-regulation of CK2 subunits expression supporting the notion that the kinase plays an important pro-survival role in colorectal cancer cells. Decreased CK2 expression was also observed in PANC-1 cells but this was not accompanied by a concomitant increase in cell death under the same experimental conditions (Fig. 6). The present data suggest that apparently CK2 does not seem to have any influence on the survival of pancreatic cancer cells as reported earlier (Fig. 4, [30] and reviewed in [31]). However, data obtained from experiments where PANC-1 cells were exposed to lower concentrations of the aforementioned compounds for an

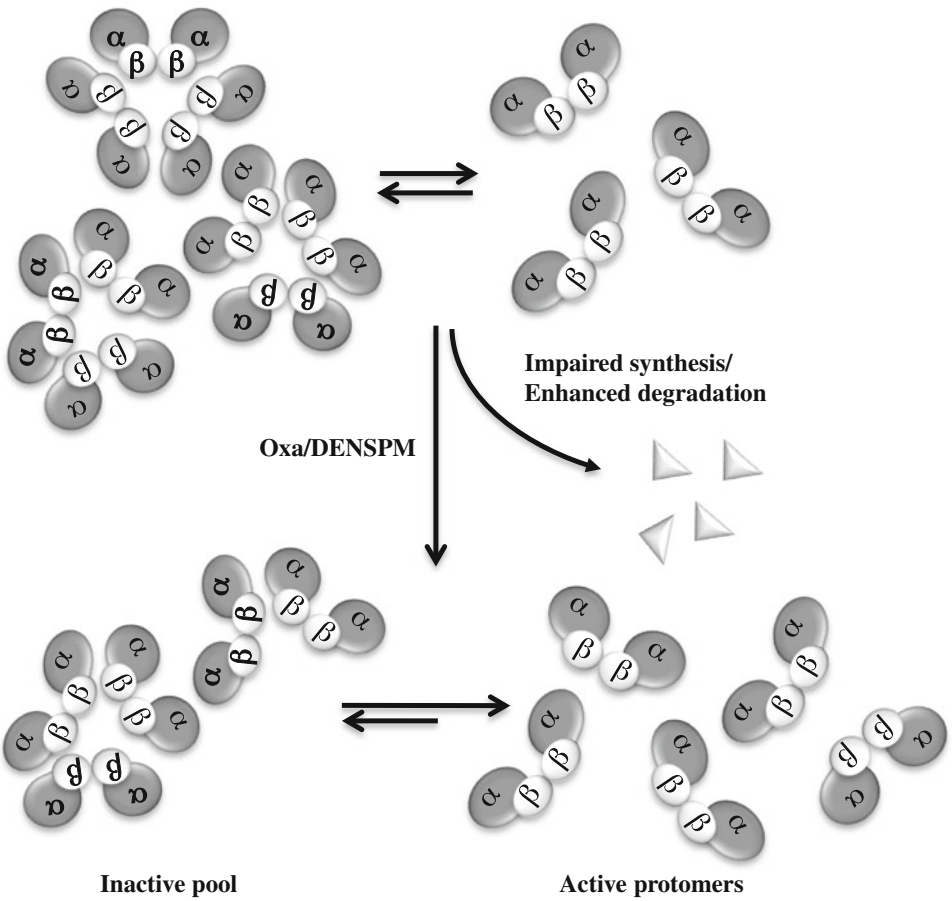

Fig. 7 Model of the proposed effects on the CK2 holoenzyme molecules following the simultaneous exposure to oxalipatin and DENSPM. Equilibrium exists inside cells between CK2 molecules present in the form of catalytically active protomers (i.e. the holoenzyme) and inactive oligomeric aggregates. Simultaneous exposure to oxaliplatin and DENSPM negatively affects the expression of the free tetrameric forms causing the release of active protomers. Only this latter fraction accounts for the kinase activity measured in vitro while both pools (i.e. oligomeric aggregates and protomers) contribute to the expression level of CK2 detected by Western blot

extensive amount of time (i.e. up to 144 h) led to significant cell death also in this cell line (data not shown).

As mentioned above, HCT116 cells showed enhanced cell death in the combination treatment and this was accompanied by down-regulation of protein kinase CK2 expression levels. However, slight increased CK2 activity was observed under the same experimental conditions (Fig. 5). It is conceivable that the expression of the individual CK2 subunits might contribute to the regulation of cell survival through the interaction with specific intracellular proteins. However, in cell-fate decision one cannot exclude that there might be a threshold beyond which the activity of CK2 does not play a decisive role with respect to survival. The mechanism by which drug combination leads to slightly higher kinase activity remains to be elucidated. However, as previously suggested by biochemical and structural studies [32–35] it is conceivable that the cellular regulation of CK2 catalytic activity might be based on the presence of an inactive pool of high molecular mass aggregates of CK2 molecules in equilibrium with the active tetrameric form. Treatment of cells with oxaliplatin and DENSPM in combination would negatively affect the expression of the individual CK2 holoenzyme molecules causing the release of tetramers from the supra-molecular aggregates (Fig. 7).

In summary, our study shows that the combination of oxaliplatin and DENSPM provides an effective treatment by inducing high cytotoxicity primarily in colon cancer cells through modulation of the polyamine pathway. Moreover, the analysis of protein kinase CK2 at the mRNA and protein expression levels indicates that the outcome of a specific treatment strategy is the result of molecular changes at both transcriptional and translational levels that should be carefully analyzed and interpreted.

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