Cancer Science
The official journal of the Japanese Cancer Association

Epicatechin gallate and catechin gallate are superior to epigallocatechin gallate in growth suppression and anti-inflammatory activities in pancreatic tumor cells

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(Received August 17, 2010/Revised December 15, 2010/Accepted December 27, 2010/Accepted manuscript online January 17, 2011/Article first published online February 24, 2011)

Green tea catechins are considered as possible cancer preventive agents for several cancer types but little is known regarding their effects on pancreatic cancer cells. The best studied catechin and the major polyphenol present in green tea is epigallocatechin gallate (EGCG). In the present study, we investigated the *in vitro* anti-tumoral properties of EGCG on human pancreatic ductal adenocarcinoma (PDAC) cells PancTu-I, Panc1, Panc89 and BxPC3 in comparison with the effects of two minor components of green tea catechins, catechin gallate (CG) and epicatechin gallate (ECG). We found that all three catechins inhibited proliferation of PDAC cells in a dose- and time-dependent manner. Interestingly, CG and ECG exerted much stronger anti-proliferative effects than EGCG. Western blot analyses performed with PancTu-I cells revealed catechin-mediated modulation of cell cycle regulatory proteins (cyclins, cyclin-dependent kinases [CDK], CDK inhibitors). Again, these effects were clearly more pronounced in CG or ECG than in EGCG-treated cells. Importantly, catechins, in particular ECG, inhibited TNFα-induced activation of NF-κB and consequently secretion of pro-inflammatory and invasion promoting proteins like IL-8 and uPA. Overall, our data show that green tea catechins ECG and CG exhibit potent and much stronger anti-proliferative and anti-inflammatory activities on PDAC cells than the most studied catechin EGCG. (*Cancer Sci* 2011; 102: 728–734)

Pancreatic ductal adenocarcinoma (PDAC) is the 10th most commonly diagnosed cancer, but it has the fourth highest mortality rate among all cancer-related deaths in the US. Since 1975 long-term survival rates have improved only marginally and are currently around 5%.[1] The poor prognosis of PDAC is attributed to an aggressive cancer progression with a high invasive and metastatic potential. Conventional chemotherapy and radiotherapy are still largely ineffective in improving pancreatic cancer and surgical resection has only limited benefits because of a high rate of recurrent disease.[2] Recently, we have shown in a murine orthotopic xenotransplantation model that tumor recurrence and metastasis after surgical resection of PDAC is substantially driven by tumor cell-derived, tumor necrosis factor α (TNFα).[3] Furthermore, we showed a significant contribution of endogenous TNFα to the growth and invasiveness of primary PDAC tumors. We proposed that TNF-inhibiting drugs, infliximab and etanercept, which are broadly used for the treatment of chronic inflammatory diseases, will also be beneficial in PDAC treatment, especially after subtotal pancreaticoduodenectomy.

Plant phytochemicals like quercetin, resveratrol and catechins are some examples of alternative chemotherapeutics being extensively tested for their anti-tumoral and anti-inflammatory potential.[4,5]

Catechins are polyphenols present in green and black tea, red wine and chocolate. Catechins, which mainly occur in green tea, comprise epigallocatechin gallate (EGCG), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), catechin gallate (CG), epicatechin gallate (ECG), gallocatechin gallate (GCG) and catechin (C).[6] One gram of dried green tea leaves contains more than 200 mg catechins.[7] The best studied catechin is EGCG, the major polyphenol in green tea.[8]

Epigallocatechin gallate has been shown to inhibit the proliferation of many cancer cells by inducing apoptosis and cell cycle arrest.[9–14] Beyond the suppression of tumor cell proliferation, EGCG may inhibit inflammation, invasion and metastasis of carcinoma cells.[15–17] There is some evidence that other catechins, including CG and ECG, may also act as anti-proliferative agents.[18–21] Babich *et al.*[22] reported that CG and its epimer ECG, more potently than EGCG, inhibit proliferation of human cancer cells of the oral cavity. The effect of green tea catechins other than EGCG on pancreatic cancer cells is currently unknown.

Thus, in the present study we analyzed the potential anticancer properties of CG and ECG in comparison with EGCG on pancreatic cancer cell line PancTu-I, which has been extensively characterized by us *in vitro* as well as in an appropriate PDAC model system.[3,23] We examined the effects of catechins on proliferation and the TNFα-mediated inflammatory response, and the uncovered mechanisms underlying this activity.

Materials and Methods

Cell culture. Pancreatic ductal adenocarcinoma cells PancTu-I, Panc1, Panc89 and BxPC3 were cultivated as previously described.[3] Catechin gallate, ECG and EGCG (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were freshly dissolved in H_2O and the catechin-containing medium was replaced every 24 h.

To determine the effect of catechins on cell proliferation, cells were seeded on six-well plates, allowed to attach for 24 h, treated with different concentrations (20–80 μM) of catechins for 24–72 h and counted with the automatic cell counter Casy System (INNOVATIS AG, Reutlingen, Germany).

Immunofluorescence and Hoechst staining. Cells grown on cover slips were fixed with ice-cold methanol for 10 min, washed with phosphate-buffered saline (PBS), blocked with

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doi: 10.1111/j.1349-7006.2011.01870.x

0.5% BSA in PBS for 15 min at RT, and incubated with anti-α-tubulin antibody (Sigma) diluted in 0.5% BSA/PBS for 45 min at room temperature (RT). After washing three times with 0.5% BSA/PBS, the cells were incubated with secondary Alexa fluor 488 anti-mouse IgG (Invitrogen GmbH, Karlsruhe, Germany) and Hoechst 33258 (Sigma) for 45 min in the dark. After two washes with 0.5% BSA/PBS and one with H_2O, the cover slips were mounted on a microscope slide by IS Mounting Medium (Dianova, Hamburg, Germany). The staining was visualized by fluorescence microscopy (Carl Zeiss Jena, Jena, Germany). In parallel, phase contrast images were monitored under the inverted light function of the same microscope.

Cell cycle analysis with flow cytometry. After catechin treatment, the cells were detached using Accutase (PAA Laboratories GmbH, Pasching, Austria), washed twice with PBS/0.5 mM EDTA and fixed with ethanol for 30 min at RT. Cells were then pelleted, resuspended in PBS/0.5 mM EDTA buffer containing RNAse A (20 μg/probe) and incubated for 30 min at RT. Finally, cells were incubated with propidium iodide (10 μg/mL in PBS/0.5% EDTA) for 1 h at 4°C. Flow cytometry was performed with FACScan (Becton Dickinson, Heidelberg, Germany) and analyzed with the Cell Quest program. For each probe, 10 000 events were analyzed.

Western blot analysis. Cells were lysed in RIPA-buffer as described.[24] Whole cell lysates were separated on SDS–PAGE, blotted onto PVDF membrane and incubated with the appropriate primary antibody followed by incubation with the HRP-conjugated secondary antibody (Cell Signaling, Frankfurt, Germany). Antigen visualization was performed by enhanced chemiluminescence on Hyperfilms (Amersham, Munich, Germany). Primary antibodies used were purchased from: Cell Signaling (anti-p15, anti-p21, anti-p27, anti-cyclin D1, anti-cyclin D3, anti-cyclin E, anti-CDK4, anti-CDK6, anti-phospho-Rb [Ser795], anti-Rb, anti-cyclin B1, anti-phospho-cdc2 [Tyr15], anti-cdc2, anti-survivin), BD Pharmingen, Heidelberg, Germany (anti-TRAF2) and Sigma (anti-β-actin). Densitometric analysis was performed with AlphaEase FC Software 4.0 (San Leandro, CA, USA).

IL-8 and uPA ELISA. The levels of IL-8 und urokinase plasminogen activator (UPA) in cell culture supernatants were determined by IL-8 (R&D Systems, Wiesbaden-Nordenstadt, Germany) or uPA (American Diagnostica, Pfungstadt, Germany) ELISA according to the manufacturer's instruction. Concentrations of both proteins were normalized to the cell numbers determined in parallel.

Electrophoretic mobility shift assay (EMSA). To detect NF-κB DNA-binding activity, nuclear protein extracts were prepared as previously described.[25] Nuclear extracts (5 μg) were analyzed by EMSA with Gelshift NF-κB (Carcinoma) kit according to the manufacturer's protocol (Active Motif, Rixensart, Belgium) and the probes were separated on native 6% polyacrylamide gels in 0.25× Tris–boric/EDTA buffer. The gels were dried and exposed to film by autoradiography.

For the supershift assay, 0.4 μg of anti-p50 or anti-p65 antibodies (both from Santa-Cruz Biotechnology, Heidelberg, Germany) were added to the sample (10 μg of nuclear extract of untreated PancTu-I cells) and incubated for an additional 1 h at 4°C. For competition assays, a 50-fold excess of either unlabeled specific NF-κB oligonucleotides or mutated NF-κB oligonucleotides (both provided in the Gelshift NF-κB Carcinoma kit) was added to cell extracts 10 min prior to incubation with the labeled NF-κB-binding oligonucleotides.

Statistical analysis. Data are given as mean ± standard deviation and were statistically analyzed by one-way analysis of variance (ANOVA) followed by a Bonferroni multiple comparison test. When inhomogenity of variance was evident, the Dunnett-T3 test was performed. Results were considered significant at $P < 0.05$.

Results

Catechins suppress cell proliferation and change PancTu-I cell morphology. To study the effects of different catechins on the proliferation of pancreatic tumor cells, PancTu-I cells were treated with various concentrations of CG, ECG or EGCG and cells were counted 24, 48 and 72 h following treatment. As shown in Figure 1a, cell proliferation was inhibited by all catechins in a dose- and time-dependent manner. A significant reduction of cell proliferation was observed for all catechins starting at 40 μM. At a lower concentration (20 μM) only CG showed a significant suppressive effect. The inhibitory effect of the catechins tested could be observed already after 24 h but became much more pronounced after 48 and 72 h. Overall, the anti-proliferative action of CG and ECG was stronger than those of EGCG. Additionally, catechin treatment changed the morphology of PancTu-I cells, as visualized by microscopy after 48 h (Fig. 1b). Isolated clusters of cells and elongated spindle-like structures were observed in catechin-treated cells. Furthermore, ECG-treated cells displayed relatively huge cytoplasmatic areas. An inhibition of cell proliferation was also evident in other pancreatic cell lines including Panc1, Panc89 and BxPC3 cells (Fig. 1c). All three catechins significantly inhibited cell proliferation at 80 μM after 48 h catechin treatment in these cell lines.

Staining of PancTu-I cells with Hoechst (Fig. S1) showed no fragmented nuclei, indicating that cells did not undergo apoptosis after treatment with CG, ECG and EGCG for 48 h. Interestingly, almost no dividing cells were detected in CG- (Fig. S1b) or ECG-treated (Fig. S1c) PancTu-I cells in contrast to untreated (Fig. S1a) or EGCG-treated (Fig. S1d) cells. In support of this observation, α-tubulin immunostaining (Fig. S1) revealed no mitotic spindle formation in cells treated with CG and ECG. Summing up, these data indicate that the anti-proliferative effect of catechins resulted from an inhibition of proliferation and not from induction of apoptosis. Moreover, the catechin treatment caused a disorganised network of microtubule filaments, whereas in untreated cells a uniform pattern of filaments were observed.

Because CG and ECG strongly affected cell proliferation, we studied whether the effects could be reversed by removing catechins from the cell culture medium. Therefore, PancTu-I cells were treated with daily changes of catechin-containing medium and after 7 days of treatment the cells were washed with PBS and cultured for an additional 7 days in medium without catechins. Long-term treatment with CG and ECG led to even more pronounced effects than those observed after 48 h (Fig. S2a). The proliferation was strongly inhibited, no mitotic spindles could be detected and cells grew as isolated small islands of multilayered cells with a perturbed α-tubulin network. The morphology of the nuclei was also changed and sporadic apoptotic nuclei were visible. Seven days after removal of catechins the cells recommenced cell proliferation, the morphology of the nuclei became normal and the α-tubulin network resembled that of healthy untreated cells (Fig. S2b). However, the proliferation of cells was still slower compared with untreated cells and many giant multi-nuclei cells and cells with multipolar spindles could be observed, especially in cells treated with ECG.

Catechin treatment leads only to minor changes in cell cycle profile. To further investigate the mechanism by which treatment with catechins affects cell proliferation, we analyzed the cell cycle by flow cytometry after treatment of PancTu-I cells with 80 μM CG, ECG or EGCG for 48 h (Fig. 2). When treated with CG and ECG, the percentage of PancTu-I cells in the G1 phase significantly decreased (CG 46.3%, ECG 44.9% *vs* untreated 55.3%) and a slight increase in S phase cells (CG 20.9%, ECG 23.0% *vs* untreated 17.0%) could be detected. After EGCG treatment the percentage of cells in the G1 phase decreased more compared with CG and ECG (EGCG 38.5% *vs*

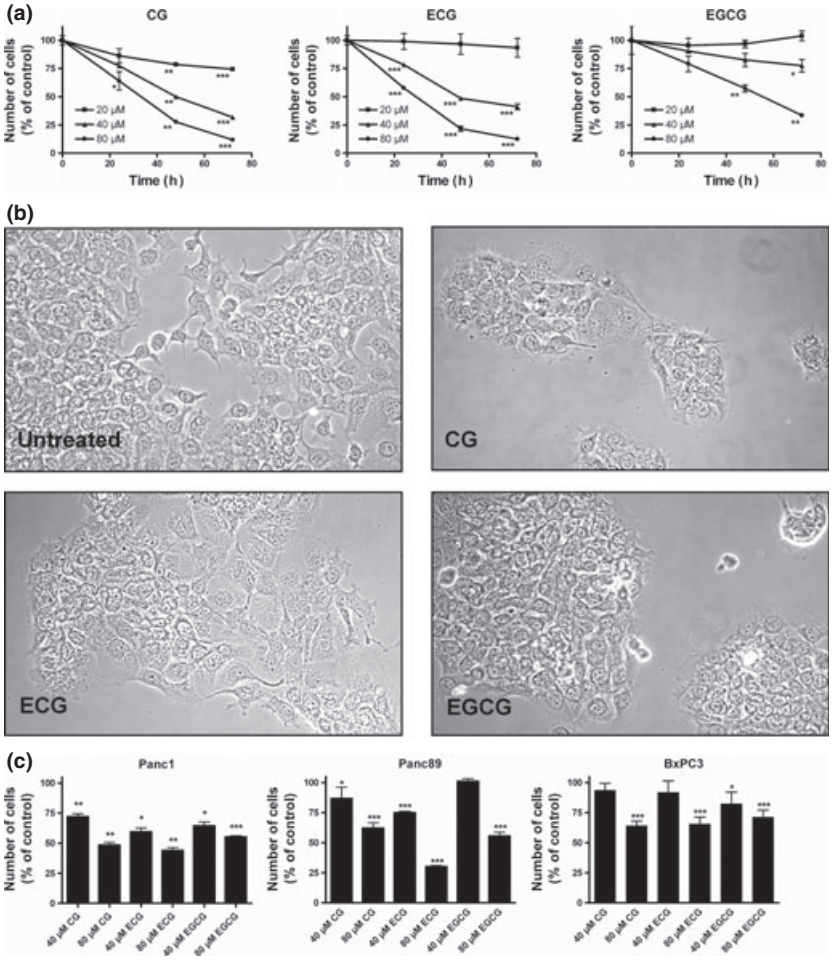

Fig. 1. Effects of catechins on proliferation and morphology of pancreatic ductal adenocarcinoma (PDAC) cells. (a) Inhibition of PancTu-I cell proliferation was assessed by counting the cells after treatment with 0, 20, 40 or 80 μM of catechins for 0, 24, 48 and 72 h. Representative results from two independent experiments performed in triplicate are shown. The influence of catechin concentration on cell proliferation was statistically significant between controls and treated cells as indicated by asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). (b) Morphology of PancTu-I cells after treatment with catechins (48 h, 80 μM) was analyzed by phase contrast images. Cells were visualized at ×200 magnification. (c) Panc1, Panc89 and BxPC3 cells were treated with 40 or 80 μM of catechins for 48 h and cell proliferation was assessed by cell counting. Cell culture and statistics were performed as mentioned above. CG, catechin gallate; ECG, epicatechin gallate; EGCG, epigallocatechin gallate.

untreated 55.3%) with a significant shift of cells to the sub-G1 phase (EGCG 12.7% *vs* untreated 5.2%) indicating apoptosis. Surprisingly, no arrest of cells in a particular cell cycle phase that could explain the observed suppression of cell proliferation (Fig. 1a) could be detected for any treatment.

Treatment with catechins alters expression of cell cycle regulatory proteins. Because cell cycle analysis reveals no information about the underlying mechanism by which green tea catechins may suppress cell proliferation, we also studied the protein levels of cell cycle regulators by western blotting. As shown in Figure 3, treatment of PancTu-I cells with different concentrations of CG, ECG and EGCG (20, 40 and 80 μM) for 48 h affected the levels of many proteins, classified in functional groups such as cyclins, cyclin-dependent kinases (CDK), CDK inhibitors, retinoblastoma and survivin.

CDK inhibitors p21 and p27 were upregulated after treatment with CG and ECG (40 and 80 μM), whereas treatment with EGCG increased the protein level of p21 and decreased the p27 protein level. P15 was upregulated in ECG and EGCG-treated cells, whereas CG-treated cells showed reduced p15 levels. P16 and p53 were not analyzed, because PancTu-I cells possess mutations in these two genes.[26]

Catechins also reduced the protein levels of different cyclins like cyclin D1, cyclin D3 and cyclin B1. Epicatechin gallate caused the most potent dose-dependent inhibition of cyclins followed by CG and EGCG. Interestingly, the cyclin E level was dose-dependently upregulated by all catechins. Additionally, CG, ECG and EGCG treatment caused a reduction of cyclin-dependent kinases CDK4, CDK6 and cdc2 protein levels with an almost undetectable level of phospho-cdc2 phosphorylated at Thr15 in CG- and ECG-treated cells. Epicatechin gallate showed, except for CHK6, the most potent effect.

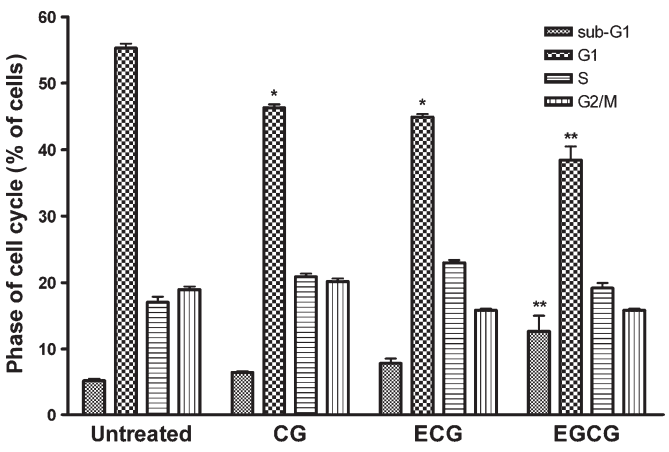

Fig. 2. Analysis of cell cycle profile by flow cytometry after treatment of PancTu-I cells with 80 μM catechins for 48 h. Cells were trypsynized and stained with propidium iodide. The percentage of apoptotic cells (sub-G1) and cells in the G1-, S- and G2/M-phase were determined by flow cytometry. The mean ± SD from three samples is shown and the significant difference between the control and the catechin-treated group is indicated with asterisks (*$P < 0.05$, **$P < 0.01$). CG, catechin gallate; ECG, epicatechin gallate; EGCG, epigallocatechin gallate.

doi: 10.1111/j.1349-7006.2011.01870.x

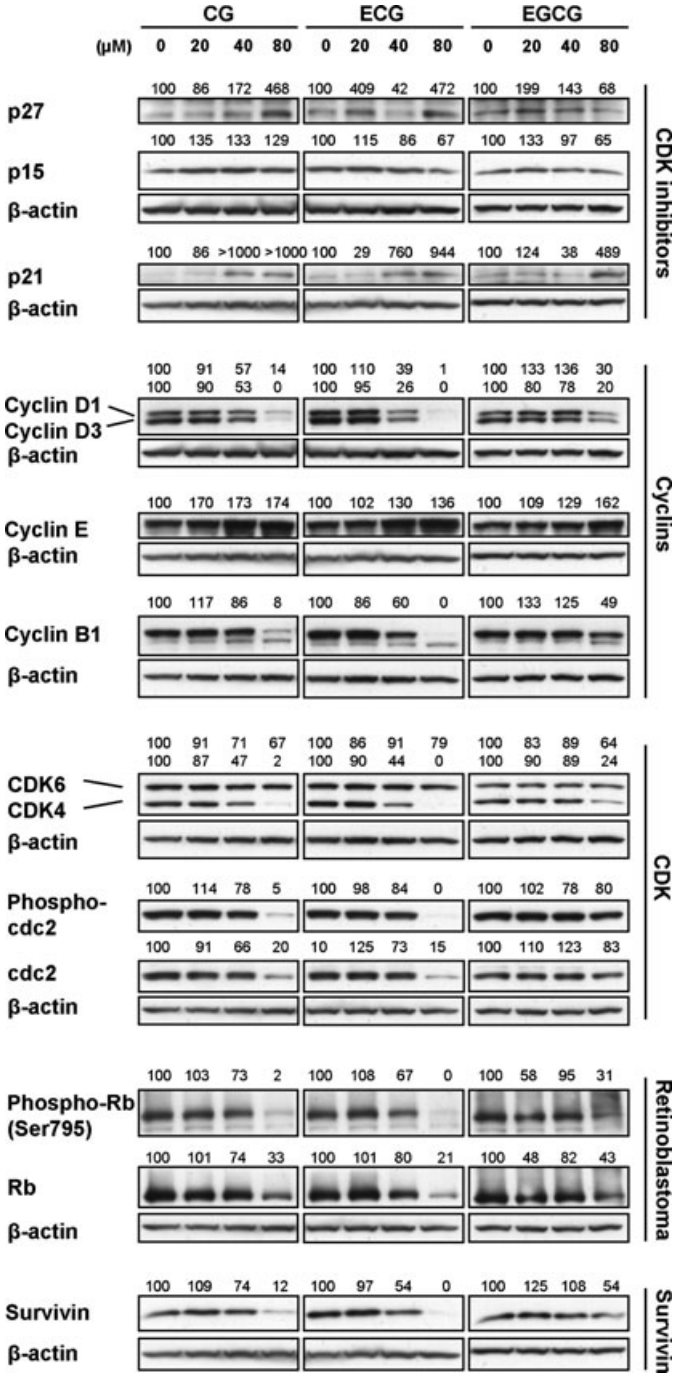

Fig. 3. Influence of catechins on the expression of cell cycle regulators in PancTu-I cells. Cells were treated for 48 h with different concentrations (20, 40 and 80 μM) of catechins. Whole cell lysates were analyzed by western blot using specific antibodies or β-actin as gel loading control. Protein bands were densitometricly analyzed, normalized to β-actin and displayed as a percentage of the control. Representative results of two independent experiments are shown. CDK, cyclin dependent kinase; CG, catechin gallate; ECG, epicatechin gallate; EGCG, epigallocatechin gallate.

The major substrate of CDK4 and CDK6 is retinoblastoma protein (Rb), which inhibits in its hypophosphorylated form the activity of transcription factor E2F, arresting the cell cycle in the G1 phase.[27] Catechin-treated cells showed a downregulation of total Rb protein level and its phosphorylated form at the Ser795 position.

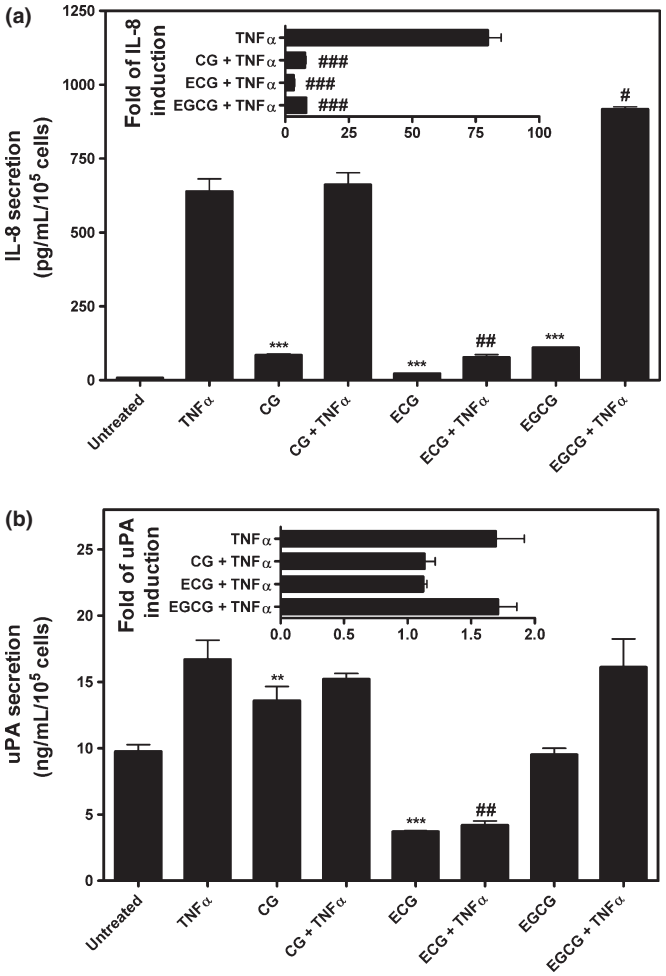

Fig. 4. Influence of catechins on TNFα-mediated secretion of IL-8 (a) and uPA (b) in PancTu-I cells. Cells were treated with catechins (80 μM) for 48 h and subsequently exposed to TNFα (50 ng/mL) that was added to the medium for an additional 8 h. Cell culture supernatants were collected and IL-8 and uPA secretion was measured by ELISA. The concentrations were normalized to the cell numbers determined in parallel. Data show the amount of secreted proteins and fold of induction (insert) as means ± SD of three independent experiments. *Significant difference between the control and catechin-treated group (**$P < 0.01$, ***$P < 0.001$); #significant difference between the TNFα control and TNFα-stimulated catechin-treated groups (#$P < 0.05$, ##$P < 0.01$). CG, catechin gallate; ECG, epicatechin gallate; EGCG, epigallocatechin gallate.

Finally, we analyzed the protein level of the cell cycle regulator survivin, associated with the spindle checkpoint.[28] Again, CG and ECG dose-dependently suppressed the survivin level, whereas EGCG showed less potent activity compared with CG and ECG.

Summing up, catechin treatment resulted in an increase of CDK inhibitors and a decrease of proteins that positively regulate cell cycle progression through the G1, G2 and M phases. Again, stronger effects were observed for CG and ECG than for EGCG.

Catechins inhibit the TNFα-mediated pro-inflammatory and pro-invasive response. Recently, we have shown that TNFα increases invasion and metastasis of pancreatic cancer cells *in vitro* and *in vivo*.[3] To find out whether catechins could influence TNFα-induced activation of pro-inflammatory and invasion-promoting pathways we first cultured PancTu-I cells with catechins for 48 h and subsequently exposed them for an additional 8 h to recombinant TNFα. Cell culture supernatants

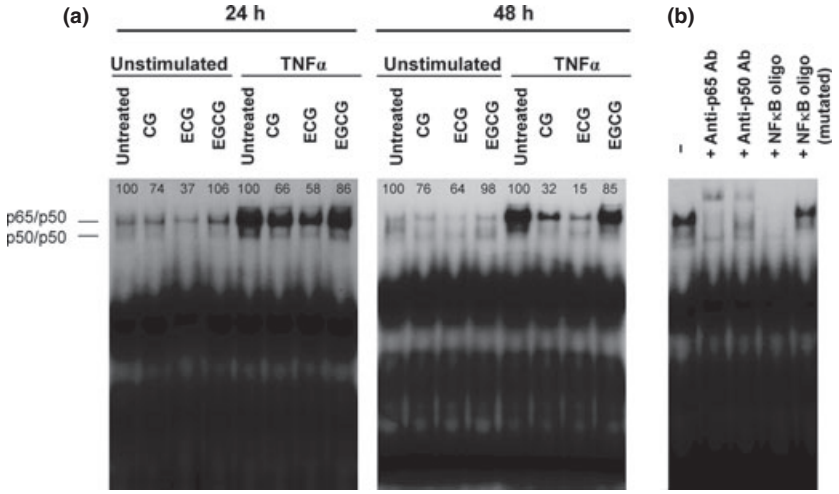

Fig. 5. Effect of catechins on TNFα-stimulated NF-κB DNA binding activity. (a) PancTu-I cells were treated for 24 or 48 h with catechins (80 μM) and stimulated with TNFα (50 ng/mL) for an additional 30 min. NF-κB activity was analyzed in nuclear extracts by electrophoretic mobility shift assay (see Materials and Methods). Densitometric values were calculated and given as a percentage of unstimulated or TNFα-stimulated control, respectively. Data represent one of two independent experiments. (b) 2.5×10^6 PancTu-I cells were seeded in a six-well plate and allowed to grow for 24 h. Nuclear extracts were prepared and the NF-κB binding activity as well as the composition of NFκB complexes were analyzed by supershift, as described in the Materials and Methods. CG, catechin gallate; ECG, epicatechin gallate; EGCG, epigallocatechin gallate.

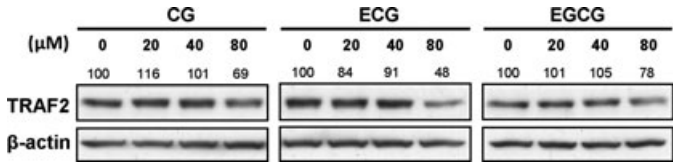

Fig. 6. Effect of catechins on TRAF2 protein level in PancTu-I cells. Cells were treated with different concentrations of catechins for 48 h. Whole cell lysates (20 μg) were separated in 4–20% SDS–PAGE and analyzed by western blotting for the expression of TRAF2. As a gel loading control, the level of β-actin was determined in parallel. Densitometric values are normalized to β-actin and presented as a percentage of the control. CG, catechin gallate; ECG, epicatechin gallate; EGCG, epigallocatechin gallate.

were collected and IL-8 and uPA release were quantified with IL-8 and uPA ELISA, respectively.

Stimulation of cells with TNFα resulted in dramatic induction of IL-8 secretion (79.8-fold, Fig. 4a). This response was strongly diminished by CG (7.8-fold), ECG (3.3-fold) and EGCG (8.3-fold). Similarly, TNFα induced the secretion of uPA (1.7-fold, Fig. 4b). This effect was also reduced by CG (1.1-fold) and ECG (1.1-fold) but not by EGCG (1.7-fold). Thus, under the conditions investigated, ECG was the most potent catechin in decreasing TNFα-mediated secretion of IL-8 und uPA. Because IL-8 and uPA are both NF-κB target genes and TNFα treatment leads to activation of NF-κB, we next studied the effects of catechins on NF-κB DNA-binding activity. After pre-incubation with 80 μM catechins for 24 or 48 h, PancTu-I cells were stimulated for 30 min with 50 ng/mL TNFα and nuclear extracts were analyzed by EMSA. As shown in Figure 5, TNFα dramatically induced NF-κB in cells cultured without catechins. This cellular response could only marginally be reduced by pre-treatment of cells with EGCG for 24 or 48 h. In contrast, CG and ECG effects were much more pronounced and pre-incubation of cells with these catechins for 48 h dramatically reduced (CG) and almost completely blocked (ECG) TNFα-induced NF-κB activity. Because decreased NF-κB activity could be caused by downregulation of proteins involved in TNF receptor (TNFR) signal transduction, we further analyzed the expression of the adaptor protein TRAF2 that plays an important role in activation of NF-κB.[24] As shown in Figure 6, we observed a decrease in the TRAF2 protein level after treatment with CG, ECG and EGCG for 48 h. However, treatment with ECG revealed the strongest decrease of the TRAF2 protein level.

Discussion

Epidemiological studies have failed to identify a definite and consistent association between the consumption of green tea and the risk of pancreatic cancer.[29–31] On the other hand, some cell culture[32–34] and animal studies[35,36] demonstrated beneficial effects of green tea catechins on pancreatic cancer cells *in vitro* and *in vivo*. Several studies on cancer cells of other origin strengthen the evidence for potential anticancer activity of both EGCG and catechin mixtures.[37,38] To extend these findings, we investigated *in vitro* the potential anticancer action of three different catechins in PancTu-I cells. We chose this human PDAC cell line due to its aggressive phenotype characterised by a resistance to death receptor-induced apoptosis, as well as its high potential for invasion and metastasis.[3,39] Cancer preventive agents that modulate this phenotype are in demand for therapies of pancreatic cancer.

Our results demonstrate differences in anticancer activity between CG, ECG and EGCG. All catechins tested in this study possess a polyphenolic structure including a gallate group at position three of the C-ring, two hydroxyl groups at the A-ring and two or three hydroxyl groups at the B-ring. In contrast to CG and ECG, EGCG contains an additional 5′-OH group in the B-ring. Epicatechin gallate represents the epimer of CG showing a differential steric configuration of the B-ring. The typical chemical structure of CG, ECG and EGCG is responsible for their free radical scavenging activity.[40] Among green tea catechins, EGCG exhibits the most potent free radical scavenging activity *in vitro*.[41,42] Thus, differences in the anticancer potential of catechins in pancreatic cancer cells seem to be not mainly related to free radical scavenging activity.

It is known that catechins are susceptible to autoxidation under cell culture conditions. Auto-oxidized catechins accumulate in catechin dimers and H_2O_2 and may act as pro-oxidants, thereby influencing apoptosis, cell growth and several other cellular signalling.[43,44] Recent experimental data demonstrate that the extent of autoxidation differs between the different catechins as determined by the production of H_2O_2. Catechin gallate and ECG generated much less H_2O_2 in tissue culture medium than EGCG.[45] The clearly weaker effects of EGCG compared with CG and ECG regarding the inhibition of cell proliferation and inflammation may therefore be caused by increased degradation of EGCG due to autoxidation.

Interestingly, although catechins markedly inhibited cell proliferation we did not observe any specific cell cycle arrest. However, we found strong modulation of cell cycle regulators

doi: 10.1111/j.1349-7006.2011.01870.x

of the G1/S phase (cyclin D1 and D3, CDK4, p21) and the G2/M phase (cdc2, cyclin B1). These results are in line with previous studies in which catechins modulated G1-regulatory proteins like cyclin D1, cyclin E, p21, CDK4 and CDK6,[9,11,12,14,46] but in contrast to our results the authors observed clear G1-arrest under catechin treatment. It is known that catechins, generally EGCG, are incorporated into the phospholipid bilayer of membranes and inhibit the binding of ligands to the cell membrane-surface receptors.[47] Consequently, multiple signaling pathways, including membrane-associated receptor tyrosine kinases (EGFR, Her2),[48,49] MAP kinases, Akt[48] as well as AP-1 and NF-κB[48,50,51] have been determined as putative targets for catechins. Due to the inhibition of these and other still unknown pathways, many signaling molecules, for example the above mentioned G1-S- and G2-phase driving proteins and survivin, are modulated in parallel. Consequently, catechins most likely induce cell cycle arrest in all cell cycle phases and therefore FACS analyses of unsynchronized growing tumor cells revealed no specific cell cycle arrest.

Interestingly, we found that catechins, especially ECG, almost completely blocked TNFα-induced NF-κB activity and consequently strongly diminished the secretion of IL-8 and uPA following TNFα treatment. Both IL-8 and uPA are proteins overexpressed in pancreatic cancer cells and linked to invasion, angiogenesis and metastasis.[24,52–54] Thus, we selected IL-8 and uPA as molecular biomarkers of inflammation. It is known that tumor cells are exposed to TNFα by infiltrated immune cells or tumor cells themselves.[3,55] Blocking TNFα with TNF-neutralising drugs (infliximab, etanercept) inhibited IL-8 and uPA secretion and NF-κB activity and resulted in decreased invasion. Inhibition of TNFα has an even stronger effect *in vivo* where it reduced tumor growth and metastasis.[3] These data indicate that catechins, especially ECG, are also able to inhibit TNFα-mediated effects *in vitro*. Therefore, it is possible that catechin treatment could diminish the aggressive phenotype of pancreatic cancer supporting its potential use in anticancer therapy.

One possible mechanism for the catechin-mediated inhibition of TNFα-induced NF-κB activity could be the downregulation of TRAF2 expression, which correlated with the extent of inhibitory power for the particular catechin. However, because catechins can alter the plasma membrane structure,[47] it is also possible that they change the localization/function of TNFR1 and thereby diminish the TNF-mediated response. Further studies will be needed to clarify this issue.

In this study we used catechin concentrations comparable with other studies in pancreatic cancer cells in the range of 25–200 μM.[32–34] However, all three catechins showed anticancer activity at concentrations (≥40 μM) that are much higher than physiologically achievable in blood after green tea catechin consumption.[56] Peak plasma levels in healthy volunteers receiving 687.5 mg EGCG or 663.5 mg ECG reached 1.3 μM/L and 3.1 μM/L, respectively.[57] As CG is only a minor constituent in green tea catechins, there is no information about its level in blood plasma. It is therefore most likely that an anticancer therapy of pancreatic cancer with catechins in general and ECG in particular may be only effective when green tea catechins are administered at pharmacological concentrations.

In summary, our results provide evidence that catechins show an anti-proliferative and, especially ECG, an anti-inflammatory potential that could possibly increase the efficacy of classical anticancer therapies. The presented *in vitro* mechanisms of catechins need to be evaluated in animal models of pancreatic cancer in future *in vivo* studies.

Acknowledgments

This work was supported by the DFG grant SFB 415/A3 given to Holger Kalthoff.

Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Catechins reduce mitotic spindle formation and influence the microtubule network.

Fig. S2. Catechin gallate- and epicatechin gallate-treated cells partly reconstitute after removing catechins.

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doi: 10.1111/j.1349-7006.2011.01870.x