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γ-Glutamyltransferase is upregulated after oxidative stress through the Ras signal transduction pathway in rat colon carcinoma cells

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Abstract

γ-Glutamyltransferase (GGT) plays a central role in the homeostasis of the antioxidant glutathione (GSH). The expression of GGT has been shown to be upregulated after oxidative stress, but the signalling pathways implicated remain poorly characterized. The results here show that acute exposure of CC531 cells to oxidative stress resulted in activation of Ras and augmented GGT enzyme activity, both at the transcriptional and at the translation level. Moreover, an involvement of the GGT promoter II was detected after RT-PCR and transient transfection studies. Ectopic expression of activated Ras, but not dominant negative Ras, also resulted in increased GGT promoter II transcriptional activity, an effect that was attenuated by over-expression of dominant negative mutants of Akt, p38 MAPK and MEK1. Addition of specific inhibitors of these kinases during oxidative stress diminished the activation of GGT. In conclusion, oxidative stress-induced activation of GGT involves Ras and several downstream signalling pathways.

Keywords: *γ-Glutamyltransferase, glutathione, oxidative stress, antioxidant, colon carcinoma cells, Ras signal transduction pathways.*

Abbreviations: *ERK, extracellular signal-regulated kinase; GGT, γ-Glutamyltransferase; GSH, glutathione; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/ERK kinase; PI-3K, phosphoinositide-3-kinase; JNK/SAPK, Jun N-terminal kinase/stress-activated protein kinase; ROS, reactive oxygen species.*

Introduction

The tripeptide glutathione (GSH; γ-glutamyl-cysteinyl-glycine) participates in maintaining the intracellular redox balance and acts as an antioxidant protecting cells against oxidative stress. In cancer cells, GSH also plays important roles in regulation of carcinogenic processes, cell proliferation and in development of multi-drug and radiation resistance [1,2]. Tumour cells with elevated GSH or ability to resynthesize GSH possess a higher invasive potential and it has particularly been shown that GSH protects tumour cells against oxidative stress in liver microvasculature [3–6]. Intracellular GSH will therefore contribute to tumour cell survival during tumour progression as well as during treatment.

The intracellular synthesis of GSH is controlled by the activity of γ-glutamyl-cysteine synthetase, which catalyses the first step in GSH synthesis, and the availability of cysteine. Sufficient cysteine may be critical in oxidative stress situations as most cells lack uptake mechanisms or transporters of intact GSH [7]. Degradation of extracellular GSH is initiated by

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the enzyme γ-glutamyltransferase (GGT) that hydrolyses the γ-glu-cys bond. The remaining part, cys-gly, is then hydrolysed by peptidases. The action of GGT and the uptake of the resulting amino acids will provide cells with the monomers needed for GSH biosynthesis [8–10]. GGT may therefore play an important role in GSH salvage and homeostasis and, hence, also in tumour malignancy [11,12].

The importance of the GGT enzyme in cell survival is evident at low cyst(e)ine availability. GSH levels are significantly reduced in cells grown in cysteine-depleted medium and these cells experienced oxidative stress and apoptosis. Supplementing the medium with GSH restored the intracellular GSH level due to the activity of GGT. These studies showed that GGT acts as a glutathionase and can provide cells with cysteine and confirmed that GGT activity in this way protects the cells from GSH depletion and oxidation-induced cell death [7,9,10]. In a recent article on melanoma cells using a perfusion system that mimics *in vivo* conditions, GSH depletion and GGT inhibition significantly increased tumour cell cytotoxicity when challenged with oxidative stress during adhesion to hepatic sinusoidal endothelium [12].

The rat GGT enzyme is encoded by a single-copy gene, but five distinct promoters may control the transcription of the gene. Transcription from these different promoters generates distinct mRNAs (types I–V) that differ in their 5'-untranslated regions [13–15]. Each promoter seems to be activated in a cell-specific manner, which results in cell-specific transcription of GGT mRNAs. This may be obtained through the combination of several regulatory elements which bind cell-specific transcription factors (for a recent review, see [16]).

Oxidative stress may lead to apoptosis not only through direct damage to macromolecules but also by multiple effects on signal transduction pathways [17,18]. The Ras/mitogen-activated protein kinase (MAPK) pathways are involved in cellular response to oxidative stress and the activity of several of the enzymes in these pathways is regulated by the red-ox state of the cell [19–21]. Signalling from Ras is important in resistance to oxidative stress and pathways downstream of Ras have been found to participate, particularly the proliferative pathway Raf > Mitogen-activated or extracellular signal-regulated protein kinase (MEK) > extracellular signal-regulated kinase (ERK), the survival pathways phosphatidylinositol 3-kinase (PI3-K) > Akt > NF-κB and the stress response pathways involving Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and the mitogen-activated protein kinase p38 (p38 MAPK) [22–26]. Studies on Ras mediated transformation have revealed that Ras-signalling pathways increase the threshold of ROS tolerance by upregulating the overall antioxidant capacity of cells [18,25,26].

Tumour cells frequently display enhanced GGT activity compared to normal cells and increased GGT activity has been used as a marker of experimental carcinogenesis [27]. In studies on metastatic growth of melanoma cells *in vivo*, elevated activity was found to increase invasive growth [11]. Melanoma cells transfected with GGT possessed higher growth and resistance to cisplatin treatment [28]. Several studies have shown that GGT is upregulated in different cells after acute exposure to oxidative stress [29–32]. This could be of significance to enhance the resistance of tumour cells to oxidative stress, as elevated GGT activity may increase the availability of the cells for cysteine and, thus, facilitate GSH homeostasis. In an earlier report we described a model of an acute oxidative stress situation obtained by exposing CC531 colon carcinoma cells to a high concentration of the superoxide producer menadione for 15 min. After this exposure, the intracellular GSH level was reduced by 25% and the ROS level was nearly doubled [32]. In the present study, we elaborated the regulation of the enzyme after such an oxidative stress situation on the same cells. Because the Ras pathway plays an important role in redox regulation and cell proliferation, we investigated whether the regulation of GGT is linked to the Ras pathway. Our results suggest that oxidative stress-induced GGT activation can be mediated through Ras and its downstream effectors, the PI3-K/Akt, p38 MAPK and JNK signalling pathways.

Materials and methods

Cell line and culture conditions

The colorectal carcinoma cell line studied, CC531, is a moderately differentiated adenocarcinoma cell line originally developed in rats after chemical carcinogenesis [33]. The cells were cultured in growth medium without serum (Quantum 263 complete medium for tumour cells, PAA Labs GmbH, Pasching, Austria) in a humid atmosphere with 5% CO_2 at 37°C. The cells were replated by trypsinization each 4^{th} to 6^{th} day.

Cell treatment and harvesting and enzyme measurements

Cells were harvested by trypsinization, washed in normal growth medium and counted. Viable cells were seeded in 3.5 cm dishes (0.35×10^6) and allowed to proliferate in 2 ml medium overnight. After exposure to acute oxidative stress for 15 min with 50 μm menadione (2-methyl-1,4-naphthoquinone, vitamin K3, Sigma-Aldrich Norway AS, Oslo, Norway) in the growth medium, the cells were washed and further cultivated without menadione as described in the Results section. Actinomycin D (Sigma-Aldrich) or cycloheximide (Schuchard, München, Germany) was added at concentrations

of 0.25 μg/ml or 10 μg/ml, respectively, for 40 min before menadione treatments. One hour before exposing cells to menadione, the inhibitors of protein kinases p38 MAPK (SB203580; Alexis Biochemical Corp, Lausanne, Switzerland), MEK1/2 (U0126; Alexis Biochemical Corp) and LY294002 (PI3-K; Calbiochem, Merck Eurolab, Darmstadt, Germany) were administered at 10 μM. Cells were incubated with these inhibitors until harvesting.

For GGT activity measurements, the cells were harvested by trypsinization and solubilized at a concentration of 2×10^6 cells/ml in phosphate buffered saline (PBS) with 1% Triton X-100 by gentle mixing for 30 min at room temperature. The supernatant was collected after a brief centrifugation (5 min at 5000 x g) for GGT activity measurement, being performed at 37°C using a commercial reagent kit (Roche Diagnostics, Boehringer Mannheim Lab Diagnostics, Germany).

Western blots

Cells were seeded in 3.5 cm dishes (0.3×10^6) and cultured for 24 h prior to treatment and then harvested by scraping in 90 μl SDS sample buffer (NuPage LDS sample buffer, Invitrogen Norway AS, Oslo) and reducing agent. After sonication and boiling for 5 min, the samples were run on 4–12% gradient NuPage BisTris gel (Invitrogen) and then blotted onto PVDF membranes (Amersham Biosciences Europe GmbH, Freiburg, Germany) using XCell II Blot Module (Invitrogen), as described by the manufacturer. The primary antibodies used were a rabbit anti-GGT (specific to C-terminal human GGT heavy chain and generously supplied by professor Aldo Paolicchi, University of Pisa), rabbit anti-actin (Sigma-Aldrich), anti-phospho-Akt (Ser 473), anti-p38 and anti-phospho-p38, polyclonal antibody and mouse anti-phosphop44/42 MAPK (Thr 202/Tyr 204) all from Cell Signaling Technology (Beverly, MA). Secondary antibodies were swine anti-rabbit or swine anti-mouse IgG, alkaline phosphatase conjugated (DAKO Norway AS, Oslo). Protein bands were detected using CDP-Star chemiluminiscence substrate and visualized on LumiImager F1 station with LumiAnalyst 3.0 Software (Boehringer Mannheim, GmbH, Germany).

RNA isolation and GGT mRNA quantitation

Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen), according to the recommendations from the manufacturer. For RT-PCR, 1 μg of RNA was used together with RNAsine (Promega, Madison, WI), oligodT (Invitrogen) and Superscript TM reverse transcriptase (Invitrogen). For PCR, the cDNA was amplified with Dynazyme (Finnzyme Oy, Espo, Finland) and gene-specific primers for GGT mRNA [15] and GAPDH [34]. The PCR products were visualized by ethidium bromide staining after electrophoresis and the gels were scanned using BioRad Multianalyst TM/PC (BioRad Laboratories, Oslo, Norway).

Transient transfection studies

The luciferase reporter plasmid pGL3-II-Luc and the expression plasmids for activated Ras (RasL61) and dominant negative mutants of Ras (RasN17), p38, Akt and MEK have been previously described [31,35]. For transient transfections, 0.3×10^6 CC531 cells were seeded per well in six well cell culture plates. The cells were cultivated for 24 h, which resulted in 60–70% confluency. Then the cells were transfected with 1.5–2 μg luciferase reporter plasmid(s) using Lipofectamine 2000 (Invitrogen) as described by the manufacturer. The total amount of DNA in each transfection was kept constant by adjusting with empty vector plasmid DNA. The transfected cells were incubated for 24 h at 37°C before stimulation with menadione. The luciferase assays were performed as previously described [36].

Activated Ras analysis

Ras activation after oxidative stress was monitored using the Small GTPase Chemi ELISA kit from Active Motif (Rixenhart, Belgium). CC531 cells were seeded in 6 cm dishes (0.2×10^6), cultivated overnight and then treated with 50 μM menadione for 15 min. The cells were cultivated in growth medium for a period up to 4 h and harvested in lysis buffer with protease inhibitors from the kit. The ELISA was performed as recommended by the manufacturer. The microtiter plate was read and scanned using a Fuji Film LAS3000 luminescent image analyser.

Statistical analysis

Statistical data were obtained by comparison of mean values using Student *t*-test. Differences with $p < 0.05$ were considered significant.

Results

GGT activity in rat colon carcinoma cells is transcriptionally upregulated after acute oxidative stress

Earlier studies have shown that GGT is upregulated after acute exposure to oxidative stress [29,31,37]. Correspondingly, the GGT activity in rat CC531 colon carcinoma cells was doubled 24 h after a 15 min incubation period with 50 μM menadione. The induction was attenuated by actinomycin D, an inhibitor of transcription, as well as by the protein synthesis inhibitor cycloheximide (Figure 1). These findings indicate that menadione-induced GGT activity is regulated both at mRNA and protein level. Western blot analysis of extracts of cells exposed to

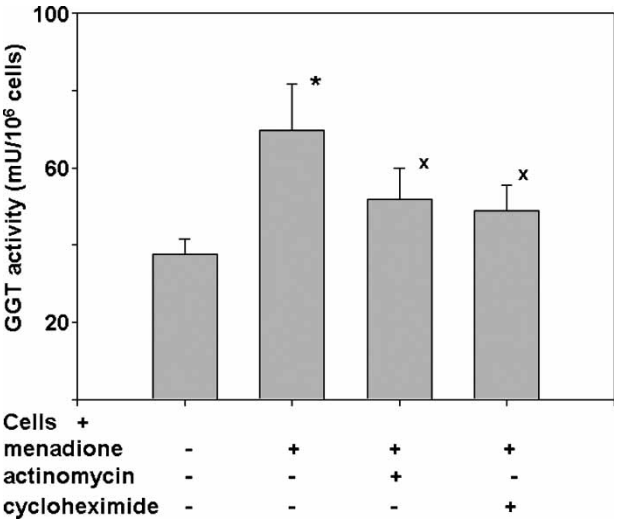

Figure 1. Increase in GGT activity after acute oxidative stress in rat colon carcinoma cells. CC531 cells were exposed to 50 μM menadione for 15 min and GGT activity was measured in cell homogenates 24 h afterwards. In some experiments, actinomycin A (0.25 μg/ml) or cycloheximide (0.2 mM) was added to the cell cultures 1 h prior to and following the oxidative stress period. Data are means (+ SD) of at least four independent experiments. The asterix * indicates a significant difference ($p < 0.05$) in mean value compared to the level of control cells and ^{x} compared to the menadione (alone) treated cells.

menadione displayed increased GGT protein levels compared to untreated cells (left panel, Figure 2). As suggested by the actinomycin D studies, RT-PCR of RNA isolated from treated cells also revealed higher levels of both total GGT mRNA and type II mRNA in menadione-treated cells (right panel, Figure 2). The GGT mRNA type II was used in this experiment and in the following transfection studies, as an increase in mRNA II and IV was indicated in preliminary experiments with menadione treatment.

Menadione exposure of the CC531 cells results in activation of Ras

Ras is a small protein with GTPase activity, which binds GTP in its active state. Inactivation of Ras is accompanied by hydrolysis of GTP bound to Ras into GDP [38]. Oxidative stress has been shown to involve activation of Ras [19,39]. To investigate whether Ras was activated in the CC531 cells after acute oxidative stress, the GTPase activation ELISA (Active Motif) was used to quantify activated H-Ras in untreated and menadione-treated cells. The results showed a clear elevation of GTP-bound Ras 2–4 h after the acute menadione exposure of the CC531 cells (Figure 3).

Activated H-Ras stimulates GGT promoter II activity

We have shown that menadione-induced GGT activation was inhibited by actinomycin D and GGT transcript levels increased in cells exposed to oxidative stress (Figures 1 and 2). Moreover, menadione stimulated Ras activity (Figure 3). To explore whether Ras is involved in menadione-induced transcription of GGT, transient transfection studies with a luciferase reporter plasmid driven by the GGT II promoter in the presence of an activated Ras or a dominant negative Ras mutant was performed. The GGT promoter II was chosen because transcripts derived from this promoter increased after acute oxidative stress (Figure 2). While dominant negative Ras (RasN17) was unable to stimulate the GGT II promoter, an ~4-fold induction was observed in the presence of activated Ras (RasL61) (Figure 4). These results suggest that the Ras is implicated in the induction of GGT type II transcripts in menadione-treated cells.

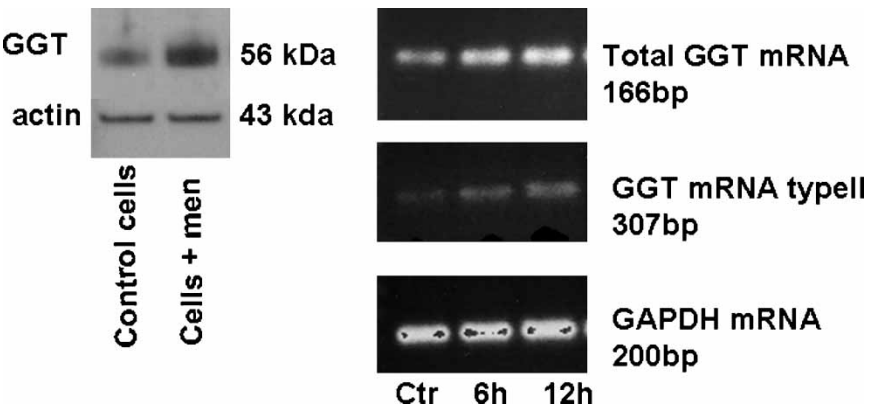

Figure 2. Increase in GGT protein and GGT mRNA levels after acute oxidative stress in rat colon carcinoma cells. (A) CC531 cells were exposed to menadione as described in Figure 1 and harvested after 24 h. Proteins were subjected to Western blotting. The blots represent a result of the amounts of GGT and actin. Similar results were obtained in two other independent experiments. (B) CC531 cells were exposed to menadione as described in Figure 1 and harvested after 6 and 12 h. Total RNA was isolated and reversed transcribed to cDNA. PCR was performed using specific primers for GGT total mRNA, GGT mRNA type II and GAPDH. The figures represent a typical result from two-to-four independent experiments.

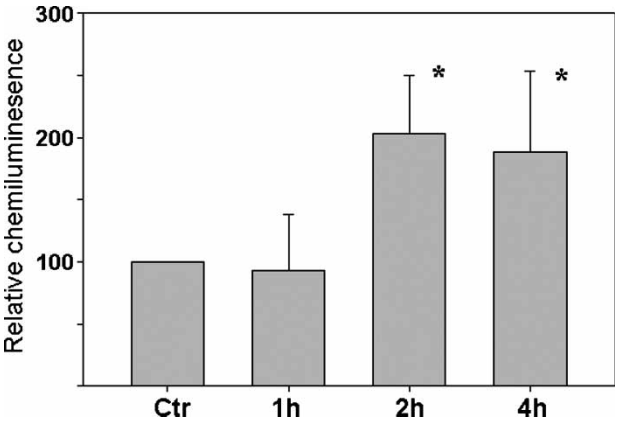

Figure 3. Increase in GTP-bound Ras after acute oxidative stress. Cells were exposed to menadione as described in Figure 1 and samples were collected after 30 min. The activation of Ras was measured using the GTP-bound Ras ELISA kit (Active Motif). The figure shows the mean chemiluminesence (+SD) relative to that of control cells from three experiments. The asterix * indicates a significant difference ($p < 0.05$) in mean value compared to the level of control cells.

The menadione-increased activity of GGT is mediated by the PI3-K, MEK1/2, p38 MAPK and JNK pathways

Several pathways, including the phosphatidylinositol-3 kinase-Akt, the ERK1/2-MEK1/2 and the p38 MAPK, operate downstream of Ras [40]. To determine whether these pathways are involved in the induction of GGT after exposure to acute oxidative stress, GGT activity was measured in untreated CC531 cells, menadione-treated cells and in cells that were exposed to specific inhibitors of PI3-K, MEK1/2 or p38 MAPK prior to and following the administration of menadione.

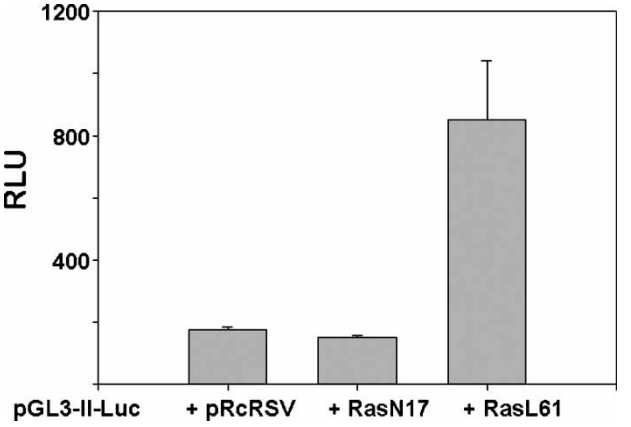

Figure 4. Constitutive activated Ras stimulates GGT promoter II activity. CC531 cells were co-transfected with 1 μg GGT-II-LUC reporter plasmid and 1 μg of empty expression vector (pRcRSV) or 1 μg expression plasmid for RasL61 (constitutive active Ras) or RasN17 (dominant negative Ras plasmid), respectively. The luciferase activity in cell extracts was determined 24 h after the transfection and expressed as relative luciferase units (RLU). The results of one experiment are shown and are the average of three independent parallels+SD. Similar results were obtained in another experiment.

Each of these protein kinase inhibitors significantly attenuated the menadione-induced increase in GGT activity. None of the inhibitors (administered separately) was able to completely block menadione-triggered GGT activation (Figure 5). This strongly indicates that GGT is regulated through several of the signal transduction pathways that operate downstream of Ras. Basal GGT activity was not significantly altered when cells were incubated with 10 μM of either inhibitor alone (data not shown). To further explore the role of these signal pathways in mediated menadione-induced GGT activation, the phosphorylation of status of Akt, ERK1/2 and p38 MAPK was examined by phosphorspecific antibodies. Phosphorylation of these protein kinases reflects their activation state and hence the activity of the signalling pathway in which they operate. Menadione triggered phosphorylation of all protein kinases examined. Pretreatment with specific kinase inhibitors abrogated menadione-provoked phosphorylation (Figure 6). Menadione treatment also resulted in increased JNK phosphorylation, but no reduction of phosphoJNK was observed in the presence of the specific JNK inhibitor SP600125 (data not shown).

Ras-induced activation of the GGT II promoter is blocked by over-expression of dominant negative mutants of p38 MAPK, Akt, and MEK

Menadione enhanced transcripts generated from the GGT II promoter and activated Ras induced this promoter (right panel, Figure 2 and Figure 3). This

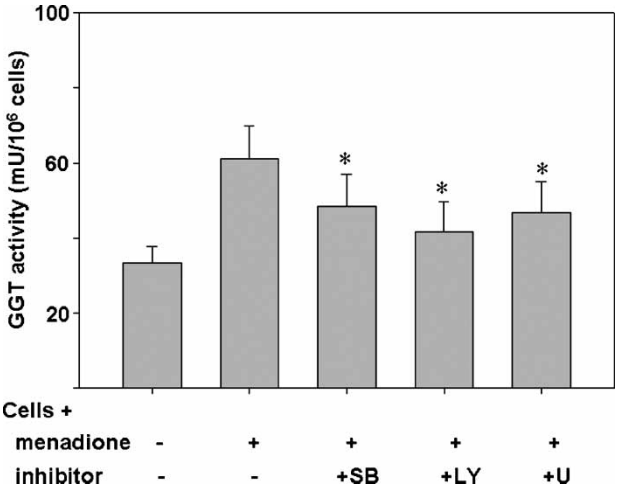

Figure 5. Effect of protein kinase inhibitors on oxidative stress-induced GGT activity. CC531 cells were exposed to menadione as described in Figure 1 and GGT activity was measured in cell homogenates after 24 h. In experiments where specific protein kinases were used, cells were pre-incubated with 10 μM of one the following protein kinase inhibitors: SB203580 (p38 MAPK), LY294002 (PI-3K) or U0126 (MEK1/2) in the medium 1 h prior to and after the oxidative stress period. Data are means (+ SD) of at least four independent experiments. The asterix * indicates a significant difference ($p < 0.05$) in mean value compared to the level of control cells.

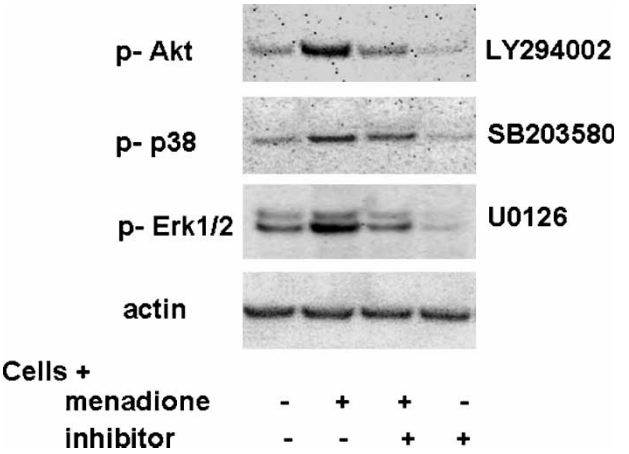

Figure 6. Menadione induces activation of the protein kinases Akt, ERK1/2 and p38 MAPK. CC531 cells were either untreated or pre-treated with the protein kinase inhibitors SB203580, LY294002 or U0126 before they were exposed to menadione as described in Figure 1. The cells were harvested after 30 min. Ten micromoles of the inhibitors was used. Proteins were subjected to Western blots using phosphospecific antibodies against the protein kinases to determine their activation state. To ensure equal loading and blotting of the proteins, membranes were re-hybridized with antibodies against actin. Data shown are typical findings from two-to-three experiments.

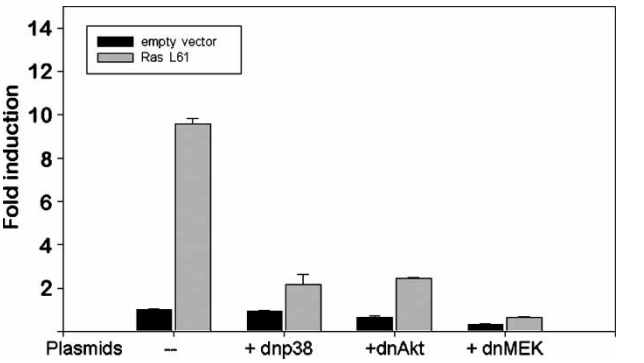

Figure 7. Activated Ras stimulates the GGT-IIpromoter in an Akt, MEK and p38 MAPK dependent manner. CC531 cells were co-transfected with GGT-II-LUC reporter plasmid and expression plasmids for RasL61 and either empty vector (pRcRSV) or vectors encoding dominant negative mutants of p38 MAPK (dnp38), MEK 1 (dnMEK) or Akt (dnAkt). The luciferase activity in cell extracts was determined 24 h after transfection. The luciferase activity in cells transfected with the empty vector pRcRSV was arbitrarily set as 1 and the luciferase activities in the other cells are represented as fold induction. The results shown are mean values of three independent parallels+SD.

induction seems to be mediated, at least partially, through the effector pathways PI3-K/Akt, MEK/ERK and p38 MAPK (Figures 5 and 6). To support a role for these pathways in menadione-induced activation of the GGT II promoter, transient transfection studies were performed with a GGT promoter II driven luciferase reporter plasmid and expression plasmids for dominant negative (dn) variants of Akt, MEK1 and p38α, respectively. Activated Ras strongly stimulated the GGT II promoter activity. Co-expression of dn p38, Akt or MEK had little or no effect on the basal activity of this GGT promoter, but strongly interfered with Ras-induced activation (Figure 7). These findings underscore a role of the PI3-K, MEK/ERK and p38 MAPK pathways in mediating menadione-Ras signalling to the GGT II promoter.

Discussion

Numerous stimuli, including oxidative stress, have been shown to upregulate GGT expression [10,29,30,32,37]. Induction of GGT may protect cells from oxidative stress, which in turn may provide advantages for tumour cells [2,41]. The mechanism underlying oxidative stress-triggered GGT activation remains elusive. In this study, we have shown that treatment of the rat colon cell line CC531 with the superoxide generator menadione led to increased GGT transcript and protein levels. In rat, GGT is encoded by a single copy gene with five alternative promoters that give rise to seven potential types of mRNA transcribed in a cell- and tissue-specific manner (for a recent review, see [16]). Menadione enhanced the total GGT mRNA levels in CC531 cells. Our previous studies have demonstrated that type II GGT transcripts are present in CC531 cells and that GGT type II mRNA levels were transiently augmented after butyrate treatment [31]. Elevated type II transcripts were also observed in menadione-exposed cells (this study). It was recently reported that GGT mRNA types I and V2, as well as GGT enzymatic activity, increased in rat lung epithelial type II cells treated with 4-hydroxy-2-nonenal [37,42]. An electrophile-response element in the proximal region of the GGT promoter V was responsible for 4-hydroxy-2-nonenal-induced promoter activity. This site was shown to bind the nuclear factor erythroid 2-related factor (Nfr) 1 and Nfr 2, c-Fos and Fra-1 after 4-hydroxy-2-nonenal stimulation. The induction of the GGT promoter V by 4-hydroxy-2-nonenal required activation of ERK1/2 and p38 MAPK, but not PI3-K. We did not examine the GGT type 5 transcripts, but GGT transcripts are reportedly expressed in a cell-specific manner [16]. The fact that PI3-K was activated by menadione in our cells, but not by 4-hydroxy-2-nonenal in rat alveolar epithelial cells may account for the differential activation of the distinct GGT promoters and hence upregulation of distinct GGT type transcripts. The GGT promoters contain a plethora of putative binding sites for transcription factors whose activities are modulated by different signalling pathways. The PI3-K/Akt pathway and the MEK/ERK, p38 and JNK MAPK cascades, all of which became activated upon menadione treatment of CC531 cells, converge to several of these transcription factors. Studies are in progress to determine which GGT promoter

elements are required to mediate menadione-induced activation of GGT expression in CC532 cells.

The signalling pathways that mediate oxidative stress-induced GGT expression have not been completely identified. Therefore, studies were initiated aiming at characterizing signalling pathways that regulate GGT expression in response to menadione. Our results demonstrate that menadione could activate Ras in the rat colon carcinoma cell line CC531. Because Ras can activate the MEK/ERK, p38 and JNK MAPK signalling cascades, as well as the PI3-K/Akt pathway, we focused on the putative involvement of these pathways in the modulation of oxidative stress-induced GGT expression. We found that all pathways were implicated (Figures 5–7 for MEK/ERK, p38 MAPK and PI3-K/Akt, and results not shown for JNK). Menadione seems to engage distinct signalling pathways in a cell-specific manner. Recently, it was reported that PI3-K activity and phosphoAkt levels in the kidney of young rats increased $\sim$1.5-fold 2 h after intraperitoneal injection of menadione, while phospho-ERK1/2 levels augmented 5-fold. Phospho-JNK1 levels rose modestly, while JNK2 phosphorylation was comparable in treated and untreated animals [43]. In accordance with these results, we registered an increase in phosphoAkt, phosphoERK1/2 (most profoundly ERK2) and p38 MAPK. The phosphorylation levels of p38 MAPK remained unchanged after menadione injection in the study by Jin et al. [43]. We also observed enhanced phosphoJNK1 and phosphoJNK-2 levels (data not shown). We analysed rat colon carcinoma cells after 30 min of menadione treatment, while in their study phospholevels were monitored in rat kidneys 1 and 2 h after administration of menadione. Treatment of rat hepatocytes with menadione also resulted in activation of ERK1/2 and JNK within 1 h and elevated phosphoERK and JNK activity was sustained for at least 4 h. As in the study by Jin et al. [43], but in contrast with our finding, no activation of p38 MAPK was detected [44]. Abdelmohsen et al. [45] also measured activation of ERK1/2 and PI3-K/akt in menadione-exposed rat liver epithelial cells. Menadione strongly activated ERK1/2, p38 MAPK and JNK in isolated rat pancreatic acinar cells, while ERK1/2 and p38 MAPK, but not JNK, were activated in rat macrophages [46,47]. Finally, treatment of the embryonal rat heart-derived cell line H9c2 with menadione triggered activation of ERK1/2, JNK and p38 MAPK [48]. None of these studies have, however, addressed a role for Ras in menadione-provoked activation of these protein kinases.

In conclusion, we have shown that Ras and the PI3-K/Akt, MEK/ERK and p38 MAPK pathways are implicated in menadione-triggered upregulation of GGT transcription and protein levels (which correlates with enzyme activity). These findings may have therapeutic potentials because an involvement of increased GGT expression in resistance of cancer cells to cytotoxic drugs has been envisaged [28]. Hence, specific inhibitors for e.g. Ras or the protein kinases PI3K, Akt, ERK1/2, JNK and p38 MAPK may offer a therapeutic strategy to repress GGT expression in human tumours. Several specific inhibitors against these protein kinases are currently tested in clinical trials involving cancer patients (reviewed in [49]).

Acknowledgements

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