Biochemical and Biophysical Research Communications 447 (2014) 77–82

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Sensitization of mesothelioma cells to platinum-based chemotherapy by GSTπ knockdown

Jianli Chen [a,b], Charalambos Solomides [c], Henry Simpkins [a,b,*]

[a] The Feinstein Institute for Medical Research, NS-LIJ Health System, 350 Community Drive, Manhasset, NY 11030, USA
[b] Department of Pathology and Laboratory Medicine at Staten Island University Hospital, 475 Seaview Avenue, Staten Island, NY 10305, USA
[c] Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Jefferson Medical College, 132 S. 10th Street, 260E Main, Philadelphia, PA 19107, USA

ARTICLE INFO

Article history:
Received 18 March 2014
Available online 29 March 2014

Keywords:
Mesothelioma
Cisplatin
Glutathione S Transferase π

ABSTRACT

It is predicted that the incidence of mesothelioma will increase and thus it is important to find new ways to treat this chemoresistant tumor. Glutathione-S-Transferase π (GSTπ) is found at significant levels in mesotheliomas and thus attenuating its intracellular levels may provide a means of sensitizing mesothelioma cells to chemotherapy.

GSTπ knockdowns were therefore prepared with shRNA (less off-target effects) employing two cell lines (211H, H2452) that were typed by immunohistochemistry to be of mesothelial origin.

The knockdowns exhibited a decrease in both total GST enzyme activity and GSTπ protein levels as well as an increase in both glutathione levels and sensitivity to cis and oxaliplatin. Cisplatin treatment of the knockdowns increased ROS levels significantly (as compared to the parental cells) and produced activation of the JNK/p38 pathways and activating transcription factor (ATF2). The degree of activation and increase in ROS appeared to correlate with the cell line's sensitivity to cisplatin. Treatment with N-Acetyl Cysteine decreased ROS production and JNK/p38 phosphorylation but had minimal affect on ATF2 suggesting a direct interaction of GTPπ with this transcription factor. Oxaliplatin treatment produced only minimal changes in ROS levels and activation of the JNK/p38 pathway.

Recently, new methods of siRNA delivery (nanoparticles) have been shown to be effective in decreasing the levels of target proteins in humans including candidate genes involved in drug resistance – thus this approach may have promise in sensitizing cisplatin-resistant tumors to chemotherapy.

1. Introduction

Mesothelioma is a malignant tumor arising from the pleura or peritoneum and has different phenotypes – epithelioid, sarcomatoid and mixed [1] with epithelioid being the most common – (60–70%). There is evidence of a causal relationship between the development of mesothelioma and asbestos exposure [2] and its incidence is predicted to rise in Europe, Japan and Australia due to the long latency between exposure and tumor expression [3]. It responds poorly to therapy [4] and cisplatin has been used in combination with anti-folates, nucleoside analogs and topoisomerase and mitotic inhibitors to improve response rate [5–7]. The standard chemotherapy protocol is a combination of pemetrexed and cisplatin [8], although some investigators have replaced cisplatin by oxaliplatin due to better tolerability and ease of administration [9].

The role of a Glutathione-S-Transferase π (GSTπ) in cisplatin resistance was first investigated in the early 1990's with equivocal results – British, Japanese and Polish groups showed a correlation between enzyme levels and resistance to cisplatin-based chemotherapy in ovarian cancer [10–12], but the results were disputed by groups in Japan, Holland and Germany [13–15]. Recent studies with head and neck carcinomas showed that elevated levels of GSTπ as measured by FISH amplification correlated to poor prognosis [16]. Conflicting results were reported with gastric cancer in Japan [17,18], although a study with lung cancer did report a relationship [19] and elevated levels in high grade osteosarcoma were related to a high relapse rate [20].

Recently, a GSTπ inhibitor 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio) hexanol (NBDHEX) was proposed as adjunct chemotherapy in malignant mesothelioma [21]. An earlier study had showed that the inhibitor was cytotoxic to hematologic, and cell lines derived from small cell lung carcinoma and hepatocarcinoma cell lines

* Corresponding author at: The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA. Fax: +1 718 226 4816.
E-mail addresses: jianli_chen@siuh.edu (J. Chen), charalambos.solomides@jefferson.edu (C. Solomides), hsimpkins@siuh.edu (H. Simpkins).

http://dx.doi.org/10.1016/j.bbrc.2014.03.100
0006-291X/

where it activated the JNK pathway via an ROS independent mechanism (although some ROS-mediated apoptosis occurred by the p38 pathway [22]). NBDHEX also ameliorated drug resistance in over twenty osteoscarcoma cell lines [23]. Glutathione-S-Transferase π also inhibited As_2O_3-induced apoptosis in lymphoma cells by modulating intracellular H_2O_2 levels [24].

An investigation was therefore undertaken to determine if GSTπ knockdown with shRNA (less off-target effects than with siRNA) could sensitize mesothelioma cells lines to cisplatin/oxaliplatin. Changes in ROS levels and activation of the JNK/p38 pathways were monitored following drug treatment. The role of activating transcription factor 2 (ATF2) was also investigated since it has been hypothesized that GSTπ can moderate JNK activity by direct interaction with this transcription factor [25].

2. Materials and methods

2.1. Cell lines

Cisplatin and oxaliplatin were obtained from the Sigma Chemical Company (St. Louis, MO) and N-Acetyl Cysteine from Fisher Scientific (Pittsburgh, PA).

The cell culture reagents and gentamicin were obtained from Cellgro (Herndon, VA). The cell lines – H226, 211H (CRL-2081) and H2452 (CRL-5946) were purchased from ATCC (Manassas, VA) and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and gentamicin at a final concentration of 10 μg/mL.

2.2. Immunohistochemistry

Cell pellets were treated with Bouin's solution (3 ml) and then embedded in paraffin. The sections were reacted with calretinin, synaptophysin, pancytokeratin, CK7, CK5/6, p63, TTF and CD56 antibodies obtained from Ventana Medical Systems (Phoenix, AZ), and BerEP4 from Biocare (Concord, CA). The staining procedures were performed as described by the manufacturer. Thrombomodulin staining was performed by Integrated Oncology (New York, NY). The slides were scored on a 0/4+ scale by two pathologists.

2.3. Cell growth assays

Cell growth assays were performed as described previously [26]. Briefly, 4000 cells were seeded into 96-well plates in triplicate with different concentrations of each drug and incubated for 72 h at 37 °C. 10 μl of MTT (5 mg/ml) was then added to each well at the end of the incubation period and incubated for a further 5 h. The plate was then scanned at 570 nm in a 96-well plate reader. Each experiment was performed at least three times in triplicate.

2.4. Colony forming assays (CFA)

Cells (1×10^5) were seeded in 6-well plates incubated for 24 h and then treated for 4 h with different drug concentrations. The cells were then washed twice with drug-free medium and trypsinized with 0.25% Trypsin-0.2% EDTA to obtain a single-cell suspension – 200 cells were seeded into 60 mm dishes in duplicate, and incubated for two-weeks in a drug-free complete medium to allow for colony growth. At the end of incubation period, the culture medium was aspirated and the cells fixed and stained with 0.5% methylene blue in 50% ethanol for 40 min at room temperature. Thereafter, the plates were gently washed with water and allowed to air-dry. Visible colonies (containing 50 or more cells each) were counted to determine the percent colony formation for each drug treatment. IC_{50} values were expressed as the mean ± S.D. (standard deviation) from triplicate experiments.

2.5. ROS measurements

The effect of drug treatment on intracellular ROS levels was measured with a flow cytometer using the fluorescent probes, dihydroethidium (DHE) (AnaSpec, San Jose, CA) and 5,6-dichloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (H_2DCFDA) (AnaSpec, San Jose, CA). Cells (1×10^5) were pre-incubated for 24 h in RMPI-1640 medium with 10% FBS and then treated with different concentrations of drug for an additional 24 h. DHE (2 μM) was added for 15 min. or H_2DCFDA (1 μM) for 30 min at 37 °C [27]. The cells were washed, suspended in HBSS buffer and flow cytometry performed with a BD FACSVerse (excitation wavelength 488 nm: emission wavelengths 525 nm for H_2-DCFDA; 575 nm for DHE).

2.6. Glutathione transferase enzyme activity and glutathione concentration

The GST assay kit was obtained from Sigma (Saint Louis, MO) and its activity measured with the cell lysate with 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate (Candler, NC) [28]. The GSH concentration was determined with a kit obtained from BioVision (Milpitas, CA). Lysate protein concentrations were assayed with the BCA protein kit (Pierce, Rockford, IL).

2.7. Stable GSTπ Knockdown

GSTπ knockdown was performed with a shRNA mixture (Santa Cruz Biotechnology, Dallas, TX) which contained three target-specific lentiviral vector plasmids, each encoding 19–25nt shRNAs designed to knockdown GTPπ gene expression (sequences described in the Supplementary data section). Each plasmid contains a puromycin-resistant gene for selection of cells stably expressing the shRNA. The control shRNA plasmid – A encodes a scrambled shRNA sequence that will not lead to specific degradation of any known cellular mRNA.

Cells (2×10^5) were seeded into 6 well plates and preincubated for 24 h in 0.8 ml of Opti-MEM. siPORT-NeoFX (6 μl) (Ambion, Austin, TX) was added to 100 μl of Opti-MEM with 1% fetal calf serum, mixed gently by pipetting and allowed to stand at RT for 10 min. GSTπ shRNA or control shRNA (6.6 μl) was pipetted into 100 μl of Opti-MEM/1% fetal calf serum and allowed to stand at RT for 10 min and then mixed with the previously prepared siPORT-NeoFX for 10 min at RT. This mixture was added to the cell suspension and incubated for a further 72 h. The transfected cells were trypsinized, counted and aliquots (200 cells) seeded into 100-mm tissue culture dishes with puromycin (5 μg/mL). Clones were isolated, cultured and analyzed by western blotting for GSTπ expression.

2.8. Western blots

Cells at a density of 1×10^6/ml, were washed with PBS at 4 °C (3×) and a whole cell lysate prepared from each cell line by scraping the cells into a buffer containing 20 mM Tris–HC1 pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% (v/v) Triton X-100, 0.5% (v/v) Nonidet P40, 2.5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 50 mM sodium fluoride and 1× protease inhibitor cocktail and incubated on ice for 30 min. The lysate was then centrifuged at 13,000g for 10 min and the supernatant transferred to a fresh tube and stored at −80 °C until use. Proteins were separated by SDS–PAGE and were transferred to a PVDF membrane. The different antibodies were applied (at concentrations

described by the manufacturer) to the PVDF membrane and the bands identified by enhanced chemiluminescence reagents (Pierce Biochemicals, Rockford, IL). The antibodies utilized were rabbit polyclonal GSTπ (Enzo Life Sciences-Plymouth Meeting, PA), rabbit polyclonal JNK, phospho-JNK, p38, phospho-p38 (Santa Cruz Biotechnology-Santa Cruz, CA), mouse monoclonal Actin (Ab-1) (Calbiochem-San Diego, CA), rabbit polyclonal anti-ATF2 (Thermo Scientific, Rockford, IL) mouse monoclonal anti-phospho-ATF2 (Millipore, Billerica, MA) and mouse monoclonal anti-GAPDH (Fisher Scientific, Pittsburgh, PA). The secondary antibodies were anti-rabbit and anti-mouse (Pierce Biochemical, Rockford, IL) and rabbit anti-mouse (Thermo Fisher Scientific, Rockford, IL). Densitometry analysis was performed with UN-SCAN-IT gel 6.1 software.

2.9. Statistical analysis

The linear regression analysis and paired *t*-test were performed using the SigmaStat Statistical Analysis System, Version 1.01. $P < 0.05$ was considered significant.

3. Results

First, the tumor cell origin of the cell lines were characterized by the current immunohistochemical techniques used in surgical pathology. This is important since many older cell lines were cultured from tumors that had been characterized by only light microscopy and recently DNA profiling has shown misidentification of some ovarian and endometrial cell lines [29]. All three cell lines were positive for calretinin and pancytokeratin but negative to BerEP4; H2452 was also positive for thrombomodulin (Table 1). H226 and H2452 were positive for CD7. They did not express synaptophysin (small cell lung carcinoma), TTF (epithelial lung cancers) or p63 (squamous cell carcinomas). The results support a mesothelial cell of origin for all three cell lines. Cell block morphology also showed 211H to be comprised of two cell types but H2452 of one – consistent with the ATCC typing of biphasic and epithelial.

Westerns blots showed GSTπ was present in all three cell lines (Fig. 1A). Attempts to prepare knockdowns were successful with the 211H and H2452 cell lines (doubling time – 40–50 h) but not with H226 (doubling time over 90 h). Knockdown produced significant decreases in GSTπ as assessed by western blots (Fig. 1B–D) and total GST enzyme activity was also diminished but to a lesser extent (Fig. 1E and F). Glutathione levels were increased (Fig. 1G and H). Western blots of GTPπ in control knockdowns showed no significant differences from the parental cells (Supplementary data).

The effect of cisplatin and oxaliplatin was measured by cell growth and colony forming assays and the knockdowns showed significant decreases in their IC_{50} to both drugs (Fig. 2A and B). ROS levels were then monitored following drug treatment – it was found that the only drug which substantially increased ROS was cisplatin (Fig. 2C) (only small changes with oxaliplatin were observed (Fig. 2D)). Drug treatment produced higher ROS levels with the 211H than the H2452 cells corresponding to their greater sensitivity to cisplatin (Fig. 2C). Significant changes were only observed with the DHE probe which is specific for O_2^- (the reaction product intercalates into DNA, producing a red shift in its fluorescence and does not require esterase activation [30]) but not DCF (which measures primarily H_2O_2 [31]). ROS elevations produced by cisplatin are markedly decreased with N-Acetyl Cysteine (a ROS scavenger) to levels similar to those observed with the parental cells (Fig. 2C). (ROS production was similar in the control knockdowns as compared to the parental cells – Supplementary data.)

Cisplatin treatment of the GSTπ knockdowns of both cell lines showed significant increases in the degree of phosphorylation of both JNK (primarily JNK1), p38 and ATF2 when compared to the parental cell lines (Fig. 3A and B). It is also evident that increased p38 phosphorylation is observed at lower cisplatin concentrations in the 211H/G8 cells reflecting their greater sensitivity to cisplatin. Oxaliplatin treatment (c.f. ROS levels) produced minimal/undetectable effects (Fig. 3C). Treatment with N-Acetyl Cysteine, inhibited JNK and p38 phosphorylation (Fig. 3A and B) and AFT2 phosphorylation was mildly decreased in the more sensitive 211H cells.

4. Discussion

A comprehensive review in 2003 by Townsend and Tew [32] described studies involving GSTπ and its role in drug resistance and the use of GSTπ inhibitors. Recently, new specific GSTπ inhibitors (primarily NBDHEX) have been shown to exhibit significant cytotoxic activity in different cell lines [22] including mesothelioma [21].

In 1996, antisense cDNA to GSTπ was found to sensitize a drug-resistant colon carcinoma cell line to adriamycin, cisplatin, melphalan and etoposide [33]. Recent technologic advances (packaging siRNA into lipid nanoparticles) have resulted in significant decreases in the target protein with only mild infusion – related side reactions (e.g. transthyretin siRNA [34]). Thus, antisense therapy may have a potential role in cancer chemotherapy (circumventing drug resistance) and was the rationale for this investigation. A very recent study showed that nanoparticle codelivery of siRNA and a cisplatin prodrug [35] produced enhanced tumor cell killing reinforces this hypothesis.

This study has shown that downregulation of GSTπ in two mesothelial cell lines produced sensitization to cisplatin which was accompanied by upregulation of ROS production and concurrent activation of the JNK/p38 pathways and ATF2. Previous work has shown that cisplatin can activate the JNK pathway in HT116 cells (colon carcinoma cells) via ROS generation. [36]. Arsenic trioxide-induced apoptosis in mesothelioma cells was reported to involve JNK activation [37] and in lymphoma cells, GST π inhibited As_2O_3-induced apoptosis by modulating ROS generation [24]. Dissociation of JNK from GSTπ [38] was also thought to potentiate As_2O_3 apoptosis in a promyelocytic leukemia cell line (NB4).

Activating transcription factor 2, a cyclic AMP response element binding protein and a member of the leucine zipper protein family regulates gene transcription by interacting with the ATF/cyclic AMP response elements and plays a role in many cellular responses, (including stress). Upregulation of ATF2 in melanomas [39] mammary carcinomas [40] and head and neck carcinomas [41] appeared to correlate with poor prognosis. Initially ATF2 was thought primarily to act via the p38 pathway [42] but effects on the JNK pathway were reported in a breast cancer cell line [43].

Table 1
Characterization of mesothelioma cell lines by immunohistochemistry. The techniques and origin of the antibodies are described in Section 2 (Pancyto–pancytokeratin).

Cell line	Calretinin	BerEP4	Pancyto	CK7	CK5/6	P63	TTF	CD56	Synaptophysin	Thrombomodulin
H226	3+	0	4+	4+	0/1+	0	0	0	0	0/1+
211H	2+	0	2+/3+	0	0	0	0	0	0	0
H2452	1+	0	3+/4+	3+	0/1+	0	0	0/1+	0	2+

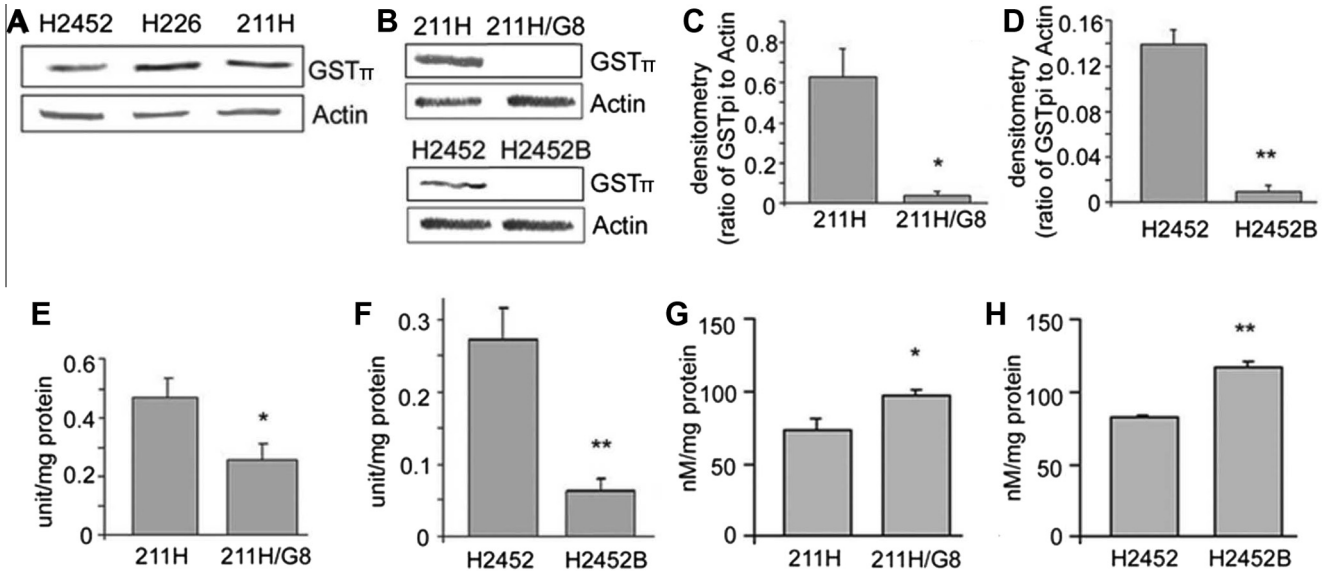

Fig. 1. (A) Western blots showing GSTπ expression in the H2452, H226, and 211H mesotheloma cell lines. (B) GSTπ expression in 211H, GSTπ-knockdown (211H/G8) and H2452 and its GSTπ-knockdown (H2452B); (C and D) densitometry of the western blots, *, **$P < 0.05$ when compared to the parental cells – the results represent three separate experiments-error is expressed as mean ± S.D.; (E and F) total GST enzyme activity in the 211H, 211H/G8, H2452 and H2452B cells, *, **$P < 0.05$ when compared to the parental cells, experiments performed twice in triplicate-error is expressed as mean ± S.D.; (G) and (H) GSH concentration in the 211H and 211H/G8, H2452 and H2452B cells, experiments performed twice in triplicate-error is expressed as mean ± S.D. *, **$P < 0.05$ when compared to the parental cells.

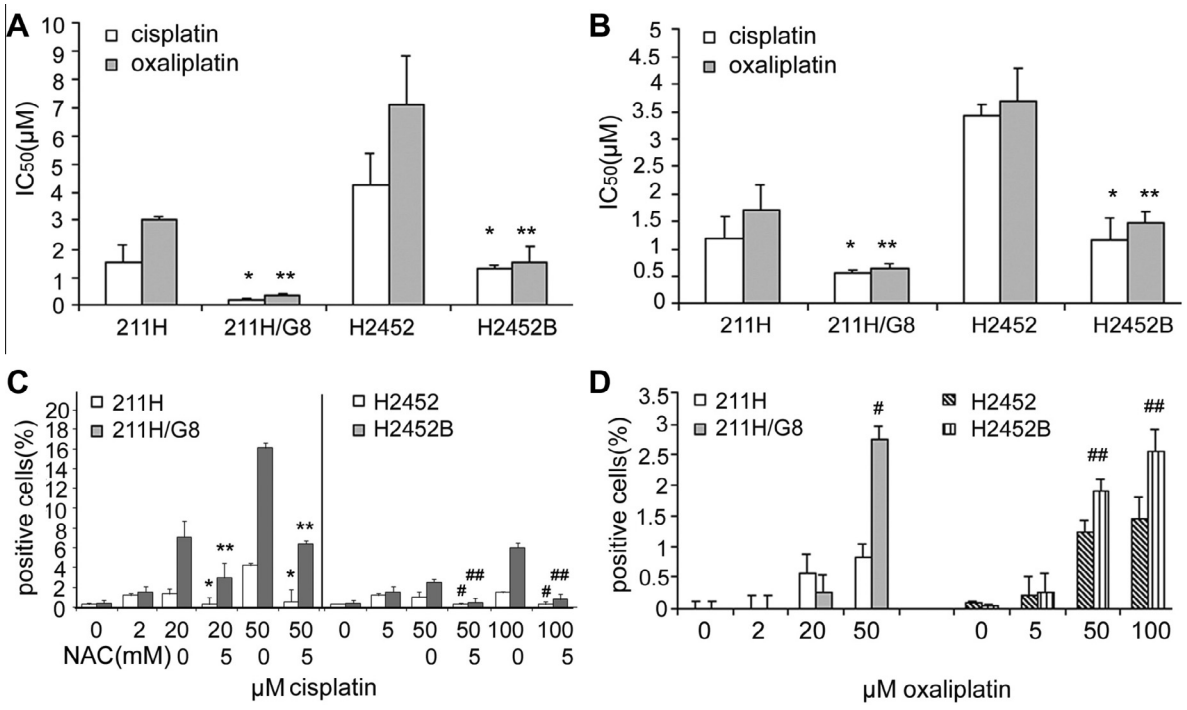

Fig. 2. (A) IC_{50} values by MTT assay of the 211H and H2452 parental cell lines and GSTπ-knockdowns (211H/G8, H2452B) when treated with cisplatin and oxaliplatin. Results from three separate experiments in triplicate, *, **$P < 0.05$, when compared to the parental cells. Error is expressed as mean ± S.D.. (B) IC_{50} values by CFA of the four cell lines when exposed to cisplatin and oxaliplatin. Results represent three separate experiments, performed in triplicate, error is expressed as mean ± S.D., *, **$P < 0.05$ when compared to the parental cells. (C) DHE fluorescence of the 211H, 211H/G8, H2452 and H2452B cells (expressed as the percentage of cells expressing increased fluorescence) following exposure to cisplatin(±) N-Acetyl-Cysteine: *, **$P < 0.05$ when compared to the percentage without NAC treatment in 211H and 211H/G8 cells; #, ##$P < 0.05$ when compared to the percentage without NAC treatment in H2452 and H2452/B cells; and following treatment with oxaliplatin (D) #, ##$P < 0.05$ when compared to the parental cells. Results represent four separate experiments in duplicate.

GSTπ can interact with the phosphorylated form of JNK1; an interaction with non-phosphorylated JNK can only occur if ATF2 is present but it can also interact directly with ATF2 [25]. However, these reports have been recently challenged since GSTπ may able to interact with unphosphorylated JNK and the GSTπ inhibitor, NBDHEX decreased this interaction [45]. DNA damaging agents can also result in ATF2 migrating to the cytoplasm and being phosphorylated by protein kinase C [44].

Thus the work presented here has shown that GSTπ levels in mesothelioma cells can alter ROS levels and activation of the p38

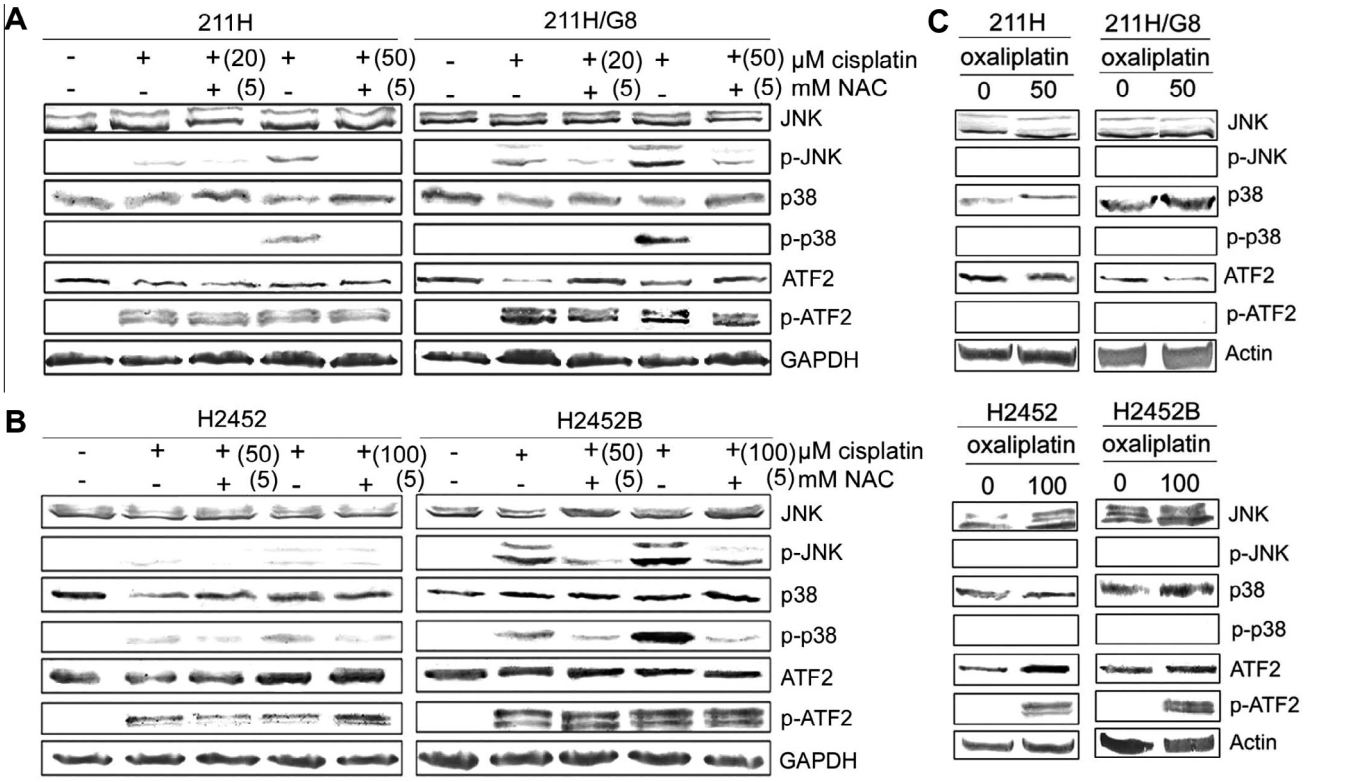

Fig. 3. Western blotting analysis of p-JNK, p-p38, and p-ATF2 following treatment for 24 h at different cisplatin concentrations ±N-Acetyl Cysteine with the 211H, 211H/G8 cells (A) and H2452, H2452B cells (B). Treatment with 50 and 100 μM oxaliplatin is also presented (C). Experiments were performed at least twice.

and JNK pathways following treatment with cisplatin. The JNK pathway may be attenuated by the direct interaction of GSTπ with p-JNK and phosphorylation of transcription factor ATF2, although the p38 pathway also appears to play a role. It is significant that N-Acetyl Cysteine, an ROS scavenger inhibited ROS generation and p38 and JNK activation.

Finally, the increased sensitivity of the GSTπ knockdowns to oxaliplatin was found not to correlate with JNK/p38 activation; a not unexpected finding since its mechanism of action is different and poorly understood. Oxaliplatin is more effective in treating colon cancer then cisplatin but it forms less DNA adducts than cisplatin in colon carcinoma cell lines (although the adducts are more bulky). The DNA repair pathways including nucleotide excision (NER) and mismatch repair (MMR) cannot explain the difference in the cytotoxicity of oxaliplatin as compared to cisplatin in different cell lines [46]. However, post-replication bypass mechanisms involving several DNA polymerases – (especially polymerase β) do show differences in some cell lines [47]. Additionally, oxaliplatin may interact directly with intracellular and nuclear proteins – the DACH hydrophobic moiety may interact with the hydrophobic pockets in the proteins in contradistinction with to the more hydrophilic drug, cisplatin [46].

Conflict of interest statement

None declared.

Acknowledgments

The work was supported by the National Institutes of Health United States (Grant R01-CA098804, Funding Agency: National Cancer Institute) and Staten Island University Hospital Research Fund.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.03.100.

References

[1] B.W. Robinson, R.A. Lake, Advances in malignant mesothelioma, N. Engl. J. Med. 353 (2005) 1591–1603.
[2] J.P. van Meerbeeck, A. Scherpereel, V.F. Surmont, et al., Malignant pleural mesothelioma: the standard of care and challenges for future management, Crit. Rev. Oncol. Hematol. 78 (2011) 92–111.
[3] A. Peto, C. Decarli, F. La Vecchia, et al., The European mesothelioma epidemic, Br. J. Cancer 79 (1999) 666–672.
[4] R.A. Stahel, W. Weder, Improving the outcome in malignant pleural mesothelioma: nonaggressive or aggressive approach?, Curr Opin. Oncol. 21 (2009) 124–130.
[5] Y. Aelony, Raltitrexed and pemetrexed studies in mesothelioma have not shown improved quality of life nor prolonged survival compared with effective pleurodesis with thoracoscopic talc poudrage, J. Clin. Oncol. 24 (2006) 4667–4668.
[6] D.A. Fennel, J.P. Steele, J. Shamash, et al., Efficacy and safety of first-or second-line irinotecan, cisplatin, and mitomycin in mesothelioma, Cancer 109 (2007) 93–99.
[7] R. Maruyama, F. Shoji, T. Okamoto, et al., Triplet chemotherapy with cisplatin, gemcitabine and vinorelbine for malignant pleural mesothelioma, Jpn. J. Clin. Oncol. 35 (2005) 433–438.
[8] H.L. Kindler, Systemic treatments for mesothelioma: standard and novel, Curr. Treat. Options Oncol. 9 (2008) 171–179.
[9] A. Xanthopoulos, T.T. Bauer, T.G. Blum, et al., Gemcitabine combined with oxaliplatin in pretreated patients with malignant pleural mesothelioma: an observational study, J. Occup. Med. Toxicol. 3 (2008) 1–7.
[10] J.A. Green, L.J. Robertson, A.H. Clark, Glutathione-S-transferase expression in benign and malignant ovarian tumors, Br. J. Cancer 88 (1993) 235–239.
[11] X. Cheng, J. Kigawa, Y. Minagawa, et al., Glutathione-S-transferase π expression and glutathione concentration in ovarian carcinoma before and after chemotherapy, Cancer 79 (1997) 521–527.
[12] P. Surowiak, V. Materna, I. Kaplenko, et al., Augmented expression of metallothionein and glutathione-S-transferese π as unfavorable prognostic factors in cisplatin-treated ovarian cancer patients, Virchows Arch. 447 (2005) 626–633.

[13] A.G.J. van der Zee, B. van Ommen, C. Meijer, et al., Glutathione-S-transferase activity and isoenzyme composition in benign ovarian tumors, untreated malignant ovarian tumors and malignant tumors after platinum/cyclophosphamide chemotherapy, Br. J. Cancer 66 (1992) 930–936.
[14] S. Ghazal-Aswad, L. Hogarth, A.G. Hill, et al., The relationship between tumor glutathione concentration, glutathione-S-transferase isoenzyme expression and response to single agent carboplatin in epithelial ovarian cancer patients, Br. J. Cancer 74 (1996) 468–473.
[15] K. Ikeda, K. Sakai, R. Yamamoto, et al., Multivariate analysis for prognostic significance of histologic subtype. GSTπ, MDR-1 and p53 in stages II-IV ovarian cancer, Int. J. Gynecol. Cancer 13 (2003) 776–784.
[16] K.J. Cullen, K.A. Newkirk, L.M. Schumaker, et al., Glutathione-S-transferase π amplification is associated with cisplatin resistance in head and neck squamous cell carcinoma cell lines and primary tumors, Cancer Res. 63 (2003) 8097–8102.
[17] T. Okuyama, Y. Maehara, K. Endo, et al., Expression of glutathione-S-transferase pi and sensitivity in human gastric cancer cells to cisplatin, Cancer 74 (1994) 1230–1236.
[18] Y. Kodera, K.-I. Isobe, M. Yamauchi, et al., Expression of glutathione-S-transferase α and π in gastric cancer: a correlation with cisplatin resistance, Cancer Chem. Pharm. 34 (1994) 203–208.
[19] F. Bai, Y. Nakanishi, M. Kawasaki, et al., Immunohistochemical expression of glutathione-S-transferase π can predict chemotherapy response in patients with non-small cell lung carcinoma, Cancer 78 (1996) 416–421.
[20] M. Pasello, F. Michelacci, I. Scionti, et al., Overcoming glutathione-S-transferase pi-related cisplatin resistance in osteosarcoma, Cancer Res. 68 (2008) 6661–6668.
[21] A. De Luca, F. Pellizzari Tregno, A. Sau, et al., Glutathione S-transferease P1-1 as a target for mesothelioma treatment, Cancer Sci. 104 (2013) 223–230.
[22] P. Turella, C. Cerella, G. Filomeni, et al., Proapoptotic activity of new glutathione S-transferase inhibitors, Cancer Res. 65 (2005) 3751–3761.
[23] M. Pasello, F. Michelacci, I. Scionti, et al., Overcoming glutathione S-transferase P1-related cisplatin resistance in osteosarcoma, Cancer Res. 68 (2008) 6661–6668.
[24] L. Zhou, Y. Jing, M. Styblo, et al., Glutathione S-transferase π inhibits As_2O_3-induced apoptosis in lymphoma cells: involvement of hydrogen peroxide catabolism, Blood 105 (2005) 1198–1203.
[25] A.F. Thevenin, C.L. Zony, B.J. Bahnson, et al., GST pi modulates JNK activity through a direct interaction with JNK substrate, ATF2, Protein Sci. 20 (2011) 834–848.
[26] H. Parekh, H. Simpkins, Cross-resistance and collateral sensitivity to natural product drugs in cisplatin-sensitive and -resistant rat lymphoma and human ovarian carcinoma cells, Cancer Chem. Pharm. 37 (1996) 457–462.
[27] E. Takada, K. Hata, J. Mizuguchi, C-Jun-NH_2-terminal kinase potentiates apoptotic cell death in response to carboplatin in B lymphoma cells, Cancer Chem. Pharm. 62 (2008) 569–576.
[28] W.H. Habig, M.J. Pabst, W.B. Jakoby, Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation, J. Biol. Chem. 249 (1974) 7130–7139.
[29] C. Korch, M.A. Spillman, T.A. Jackson, et al., DNA profiling analysis of endometrial and ovarian cell lines reveals misidentification, redundancy and contamination, Gynecol. Oncol. 127 (2012) 241–248.
[30] A.K.S. Camara, M.L. Riess, L.G. Kevin, et al., Hypothermia augments reactive oxygen species in the guinea pig isolated heart, Am. J. Physiol. (Heart Circ. Physiol.) 286 (2004) H1289–H1299.
[31] C. Loetchutinat, S. Kothan, S. Dechsupa, et al., Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2′,7′-dichlorofluorescein diacetate assay, Radiat. Phys. Chem. 72 (2005) 323–331.
[32] D.M. Townsend, K.D. Tew, The role of glutathione-S-transferase in anti-cancer drug resistance, Oncogene 22 (2003) 7369–7375.
[33] N. Ban, Y. Takahashi, T. Takayama, et al., Transfection of glutathione-S-transferase (GST)-π antisense complementary DNA increases the sensitivity of a colon cancer cell line to adriamycin, cisplatin, melphalan and etoposide, Cancer Res. 56 (1996) 3577–3582.
[34] T. Coelho, D. Adams, A. Silva, et al., Safety and efficacy of RNAi therapy for transthyretin amyloidosis, N. Engl. J. Med. 369 (2013) 819–829.
[35] X. Xu, K. Xie, X.-Q. Zhang, et al., Enhancing tumor cell response to chemotherapy through nanoparticle-mediated codelivery of siRNA and cisplatin prodrug, Proc. Natl. Acad. Sci. 110 (2013) 18638–18643.
[36] P. Bragado, A. Armesilla, A. Silva, et al., Apoptosis by cisplatin requires p53 mediated p38α MAPK activation through ROS generation, Apoptosis 12 (2007) 1733–1742.
[37] R. Eguchi, Y. Fujimori, H. Takeda, et al., Arsenic trioxide induces apoptosis through JNK and ERK in human mesothelioma cells, J. Cell. Physiol. 226 (2011) 762–768.
[38] S. Bernadini, M. Nuccetelli, N.I. Noguera, et al., Role of GSTP1-1 in mediating the effect of As_2O_3 in the acute promyelocytic leukemia cell line NB4, Ann. Hematol. 85 (2006) 681–687.
[39] A.J. Berger, H.M. Kluger, N. Li, et al., Subcellular localization of activating transcription factor 2 in melanoma specimens predicts patient survival, Cancer Res. 63 (2003) 8103–8107.
[40] S. Knippen, T. Loning, V. Muller, et al., Expression and prognostic value of activating transcription factor 2 (ATF2) and its phosphorylated form in mammary carcinomas, Anticancer Res. 29 (2009) 183–190.
[41] D. Duffey, S. Dolgilevich, S. Razzouk, et al., Activating transcription factor-2 (ATF2) in survival mechanisms in head and neck carcinoma cells, Head Neck 33 (2011) 1586–1599.
[42] A. Bhoumik, V. Ivanov, Z. Ronai, Activating transcription factor 2-derived peptides alter resistance of humor tumor cell lines to ultraviolet irradiation and chemical treatment, Clin. Cancer Res. 7 (2001) 331–342.
[43] J. Hayakawa, C. Depatie, M. Ohmichi, D. Mercola, The activation of c-Jun NH_2-terminal kinase (JNK) by DNA-damaging agents serves to promote drug resistance via activating transcription factor 2 (ATF2)-dependent enhanced DNA repair, J. Biol. Chem. 278 (2003) 20562–20592.
[44] E. Lau, Z.A. Ronai, ATF2-at the crossroads of nuclear and cytosolic functions, J. Cell Sci. 125 (2012) 1–10.
[45] A. De Luca, L. Federici, M. De Canio, et al., New insights into the mechanism of JNK1 inhibition by glutathione transferase P1-1, Biochemistry 51 (2012) 7304–7312.
[46] E. Raymond, S. Faivre, S. Chaney, et al., Cellular and molecular pharmacology of oxaliplatin, Mol. Cancer Ther. 1 (2002) 227–235.
[47] J. Yang, J. Parsons, N.H. Nicolay, et al., Cells deficient in the base excision repair protein, DNA polymerase beta, are hypersensitive to oxaliplatin chemotherapy, Oncogene 29 (2010) 463–468.