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Targeting pancreatic cancer with a G-quadruplex ligand

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ABSTRACT

The integrity of telomeres in most cancer cells is maintained by the action of the telomerase enzyme complex, which catalyzes the synthesis of telomeric DNA repeats in order to replace those lost during replication. Telomerase is especially up-regulated in metastatic cancer and is thus emerging as a major therapeutic target. One approach to telomerase inhibition involves the sequestration of the single-stranded 3′ ends of telomeric DNA into higher-order quadruplex structures. We have recently shown that tetra-substituted naphthalene diimide compounds are potent quadruplex-stabilizing molecules with telomerase inhibitory activity in cells. We show here that one such compound, BMSG-SH-3, which has been optimized by computer modeling, has significant in vivo antitumor activity against a model for pancreatic cancer, a cancer that is especially resistant to current therapies. A large reduction in telomerase activity in treated tumors was observed and the naphthalene diimide compound was found to be selectively localized in the treated tumors. We find that the expression of the therapeutically important chaperone protein HSP90, a regulator of telomerase is also reduced in vivo by BMSG-SH-3 treatment. The compound is a potent stabilizer of two G-quadruplex sequences found in the promoter region of the HSP90 gene, as well as a G-quadruplex from human telomeric DNA. It is proposed that the simultaneous targeting of these quadruplexes may be an effective anti-tumor strategy.

1. Introduction

Pancreatic cancer remains one of the most intractable of the more common human cancers world-wide,[1] with ca 3% five-year survival in the UK.[2] It is also of increasing prevalence in many countries, for reasons that are as yet poorly-understood. It is the fourth most common cause of cancer mortality. Current chemotherapy for pancreatic cancers utilises 5-fluouracil and/or its derivatives gemcitabine and capecitabine[3]; more recently these agents have been used in combination with newer drugs such as Tarceva. However the onset of resistance is common and survival is rarely prolonged for more than short time-scales. A small percentage of pancreatic cancers appear to have a heritable component, with some carriers of BRCA1 and BRCA2 mutations having been identified with the disease[4,5], and the gene PALB2 has more recently been identified as a susceptibility factor.[6] More common are mutations in the k-ras gene[7–10], although approaches to targeting k-ras and components of its signaling transduction pathway have not been clinically successful to date. Recent advances in treatment have highlighted use of the tyrosine kinase inhibitor sunitinib[11] for a rare form of the disease, pancreatic islet cell cancer, where clinical trials have produced encouraging results to date.

A defining characteristic of ca 80% of all cancer cells is the up-regulation of hTERT, the catalytic component of the telomerase enzyme complex.[12] This maintains telomere length as a result of its reverse transcriptase activity and telomere capping function and contributes to cancer cell immortalization.[13] A study[10] of the genomic status of 13 patients with pancreatic adenocarcinoma, has found that telomerase is expressed at low levels during early development of pancreatic cancer whereas expression is markedly increased once the disease becomes invasive. Telomerase is thus a potential target for pancreatic cancer, in accord with the promising results to date from several vaccine approaches to treatment using antibodies targeting hTERT.[14] Telomerase inhibition, leading to selective cancer cell senescence and apoptosis, can be achieved in several ways, notably with complementary oligonucleotide hybridization to the telomerase RNA template (hTR)—such a molecule, GRN163L (Imetelstat), is currently in early-stage clinical trials in a range of human cancers.[15] A distinctive approach to telomerase inhibition involves targeting the substrate for telomerase, the 3′ single-stranded end of telomeric DNA.[16–18] This region of the telomere is normally associated with multiple copies of the single-stranded specific protein hPOT1, and its displacement with appropriate small molecules results in the folding of the DNA into

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stable quadruplex structures, which are unable to hybridize with hTR and thus inhibit telomerase function. Quadruplex formation also elicits a senescence response and chromosomal end-to-end fusions[19] as well as a selective DNA damage response[20,21], which may contribute to the potent in vitro inhibition of cell proliferation observed with several of these G-quadruplex ligands, as well as to the in vivo anti-tumour activity found in xenograft models with the acridine derivatives BRACO-19[22] and RHSP4.[23]

A large number of quadruplex-binding ligands have been reported to date,[24] yet remarkably few have been progressed to the point of being lead candidates in a cancer drug discovery programe. We have previously reported on a novel series of highly-substituted naphthalene diimide compounds, several of whom show exceptional quadruplex affinity and selectivity.[25–27] Crystallographic studies on complexes between a naphthalene diimide compound and human telomeric quadruplexes[28] have provided a starting-point for rational optimization of these properties, and previous cell-based studies have indicated high potency in short-term assays for inhibition of cell proliferation in a range of cancers.[27] The high percentage of primary pancreatic cancers positive for hTERT (typically >80%)[29], combined with the clear unmet clinical need for new therapies, has prompted us to focus attention on this particular human cancer type.

One particular naphthalene diimide derivative, BMSG-SH-3 (*N*,*N*′-Bis(3-(4-methylpiperazin-1-yl)propylamino)-2,6-bis(3-(4-methylpiperazin-1-yl)propylamino)-1,2,5,8-naphthalene-tetracarbo xylic acid diimide: Fig. 1), has been designed by us[27] using molecular modeling on the basis of crystallographic data, with the aim of enhancing quadruplex affinity and selectivity. This compound shows sub-micromolar cell growth inhibitory activity in a range of pancreatic (and other) cancer cell lines, and also inhibits telomerase in these cells.[27] We have previously shown that tetra-substituted naphthalene diimide compounds show rapid uptake into the nuclei of MCF7 cancer cells.[25] More recently we have shown that BMSG-SH-3 can be visualized in cell nuclei of Mia-Pa-Ca2 cells in culture after 30 min of exposure (Hampel et al., to be published).

We report here on studies that demonstrate significant anti-tumour activity for this compound in a pancreatic cancer xenograft model, accompanied by changes in telomerase activity and in telomere maintenance. Possible changes in the expression of two other proteins that are implicated in pancreatic tumors have also been examined. Bcl-2 is over-expressed in at least 70% of pancreatic cancers[30] and HSP90 is a ubiquitously expressed chaperone protein responsible for the correct folding of a large number of cellular proteins including those involved in oncogenesis.[31] HSP90 is itself a current target for therapeutic intervention in a number of cancers.[32] It has been proposed that some promoter sequences in cancer-related genes contain guanine tracts capable of forming quadruplex structures, and that these may be stabilized by ligand binding, thereby inhibiting transcription of the gene.[33] We show here that BMSG-SH-3 binds two putative quadruplex sequences in the HSP90 promoter and provide evidence suggesting that these may also be targets for this compound.

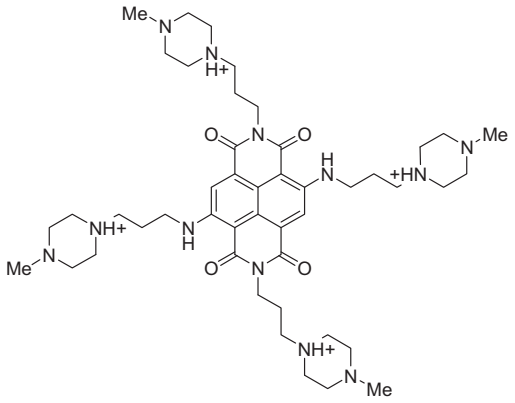

Figure 1. Structure of BMSG-SH-3.

2. Materials and methods

The naphthalene diimide compound derivative used here contains a *N*-methyl-piperazine group at the termini of each of the four side-chains (Fig. 1). It was synthesized and purified by hplc as described previously,[27] and was analytically pure as judged by NMR, mass spectral and chromatographic data (99.9% purity). It was used as the free base. Animals were housed in groups of five at 19–23 °C, with a 12-hour light–dark cycle, and fed with a conventional diet. Experimental work was carried out in accordance with UK Home Office regulations, local Ethics committee rules and the document 'Guidelines for the welfare and use of animals in cancer research'.[34]

2.1. Dose ranging studies

An initial study to determine the maximum tolerated dose was performed in female CD1 mice, with an initial mean weight of 25 g (Harlan UK Ltd,). The naphthalene diimide derivative BMSG-SH-3 was dissolved in PBS and administered intraperitoneally to the mice ($n = 2$). The body weight was recorded daily and the animals were observed for clinical symptoms.[35] The starting dose (10 μg/kg) was increased step-wise. It was found that a safe therapeutic dose was 15 mg/kg given as a single injection. The same dose was also well tolerated after intravenous administration. In order to design a therapeutic regime, animals were subsequently treated intravenously with BMSG-SH-3, in a repetitive dosing study. Three different schedules were established: 1/2 of the MTD twice per week, 1/5 of the MTD every 48 h, and 1/10 of the MTD every 24 h. Only the first schedule was found not to be well tolerated, with a loss of animal body weight being observed.

2.2. In vivo efficacy in mouse xenografts

MIA-Pa-Ca-2 and HPAC cells were obtained from the European Collection of Cell Cultures (ECACC) and cultured as recommended (DMEM, 2 mM glutamine and 10% Fetal Bovine Serum). Five to six weeks old immuno-deficient Swiss nude mice (Charles River UK Ltd) were maintained in individually ventilated caging (IVC) systems. Tumors were inoculated by subcutaneous injection of tumor cells (MIA-Pa-Ca-2 1×10^7, HPAC 5×10^6) in a mixture of 50:50 (v/v) medium and Matrigel® (BD Biosciences) in each flank. Tumor development was monitored by serial calliper measurement with tumor volumes calculated as

$$V = (d^3\pi)/6 \quad (1)$$

30 days after inoculation (=day 1, median tumor volume of ~100 mm^3) animals were randomised into a control and a treatment group ($n = 5$). Treated animals received 3 mg /kg of BMSG-SH-3 three times a week (days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26 for Mia-Pa-Ca2 and days 1, 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, 29, 32, 34, respectively). The median relative tumor volume, that is, change in size relative to starting size, was plotted against time. Upon termination of the experiment (day 26), BMSG-SH-3-treated tumor tissue and control tumours were excised, and snap-frozen in liquid nitrogen for further analysis.

2.3. Protein extraction

Tumor samples were excised into small pieces and frozen in liquid nitrogen. Approximately 5 mg of tumor was lysed in RIPA lysis buffer (1×) containing protease cocktail inhibitors, PMSF and sodium orthovanadate (Santa Cruz Biotechnology) according to manufacturer's instruction. Cell pellets from in vitro experiments were also lysed as above with appropriate amount of lysis buffer. Total protein concentration was determined using the Pierce BCA protein assay kit according to manufacturer's instructions.

2.4. Determination of in vivo telomerase activity

Telomerase activity was determined using the TRAP-LIG assay[36], a modified telomere repeat amplification protocol that ensures that there is no carry-over of ligand into the second PCR step of the assay. Briefly, 1000 ng of protein from untreated and treated samples were incubated with TS forward primer (0.1 μg of 5′-AATCCGTCGAGCAGAGTT-3′) at 30 °C for 10 min to allow the initial elongation to take place. Elongated products were purified using QIA quick nucleotide purification kit (Qiagen) according to the manufacturer's instructions. The eluted samples were freeze-dried and re-dissolved in PCR grade water. Redissolved PCR products were subjected to amplification in master mix containing ACX reverse primer (1 μM, 5′-GCGCGG $[CTTACC]_3$CTAACC-3′), TS forward primer (0.1 μg, 5′-AAT CCGTCGAGCAGAGTT-3′), TRAP buffer, BSA (5 μg), 0.5 mM dNTPs, and 2 U of TAQ polymerase (RedHot, ABgene, Surrey, U.K.) for 35 cycles of 94 °C for 30 s, at 61 °C for 1 min, and at 72 °C for 1 min. Samples were separated on a 12% PAGE and visualized with SYBR green (Aldrich) staining. Gels were quantified using a gel scanner and gene tool software (Sygene, Cambridge, U.K.). Intensity data were obtained by scanning and integrating the total intensity of each PCR product ladder in the denaturing gels. Background readings were corrected against a negative control and telomerase activity was expressed as a percentage of activity relating to activity in untreated sample.

2.5. Immunoblotting experiments

Total protein from samples were loaded onto pre-cast SDS–PAGE gels (Bio Rad) and transferred onto a Nitrocellulose membrane (Invitrogen) and the membranes were probed with primary antibodies against bcl-2, PARP (BD Pharmingen), HSP90, hTERT, k-ras and β-actin (Santa Cruz Biotechnology). For the detection of γH2AX nuclear protein was extracted TEB lysis buffer (PBS containing 0.5% Triton 100× (v/v), 2 mM phenylmethylsulfonylfluoride (PMSF), 0.02% (w/v) sodium azide) for 10 min on ice. The resulting lysate was centrifuged at 400 g for 10 min. The nuclear pellet was resuspended in TEB buffer and centrifuged again. Nuclear protein was extracted with 0.2 N HCl overnight at 4 °C. The supernatant was recovered following centrifugation at 400 g for 10 min and protein was separated on PVDF membrane before incubating with γH2AX primary antibody (Millipore). Following incubation with the appropriate secondary antibodies the membranes were visualized with the horseradish peroxidase luminescent visualization system (National Diagnostics).

2.6. In vivo multi-spectral fluorescence imaging study

Tumor exposure and biodistribution of the fluorescent BMSG-SH-3 were analyzed using spectral fluorescence (Maestro In Vivo Imaging System, CRi Inc.). Animals bearing HPAC tumours which had been treated for 5 cycles (3 mg/kg i.p. 3 times per week) were sacrificed 48 hours after the final dose and tumour and key organs imaged ex vivo Optical image sets were acquired with a red filter for acquisition of one complete image cube. Spectral libraries for BMSG-SH-3 were imported and the respective spectra unmixed, using commercial software (Maestro software).

2.7. FRET melting study

The complete HSP90 sequence is:
5′-AGGGCGGGCCAAAGGGAAGGGGTGGGC-3′ (33mer)
The two tagged DNA sequences used were overlapping 21-mer sequences taken from this 33-mer:
HSP90a:
5′-Fam-GGGCCAAAGGGAAGGGGTGGG-Tamra-3′
and HSP90b:
5′-Fam-GGGCGGGCCAAAGGGAAGGGG-Tamra-3′
The ability of BMSG-SH-3 to stabilize these two G-quadruplex DNA sequences was investigated using a fluorescence resonance energy transfer (FRET) assay modified to be used as a high-throughput screen in a 96-well format.[37] The labeled oligonucleotides had attached the donor fluorophore FAM: 6-carboxyfluorescein and the acceptor fluorophore TAMRA: 6-carboxytetramethylrhodamine). The FRET probe sequences were diluted from stock to the correct concentration (400 nM) in a 60 mM potassium cacodylate buffer (pH 7.4) and then annealed by heating to 85 °C for 10 min, followed by cooling to room temperature in the heating block. The compound was stored as a 10 mM stock solution in DMSO; final solutions (at 2× concentration) were prepared using 10 mM HCl in the initial 1:10 dilution, after which 60 mM potassium cacodylate buffer (pH 7.4) was used in all subsequent steps. The maximum HCl concentration in the reaction volume (at a ligand concentration of 20 μM) is thus 200 μM, well within the range of the buffer used. Relevant controls were also performed to check for interference with the assay. 96-Well plates (MJ Research, Waltham, MA) were prepared by aliquoting 50 μl of the annealed DNA into each well, followed by 50 μl of the compound solutions. Measurements were made on a DNA Engine Opticon (MJ Research) with excitation at 450–495 nm and detection at 515–545 nm. Fluorescence readings were taken at intervals of 0.5 °C in the range 30–100 °C, with a constant temperature being maintained for 30 s prior to each reading to ensure a stable value.

Final analysis of the data was carried out using a script written in the program Origin 7.0 (OriginLab Corp., Northampton, MA). The advanced curve-fitting function in Origin 7.0 was used for calculation of ΔT_m values. Esds in ΔT_m are ± 0.1° C.

3. Results

3.1. BMSG-SH-3. has anti-cancer activity

Initial in vivo studies to determine the maximum tolerated dose (MTD) show that the animals readily tolerate BMSG-SH-3 at doses of at least 15 mg/kg while showing significant anti-tumour activity at 3 mg/kg, using a three times weekly dose schedule. The xenograft studies (Fig. 2a) demonstrate that tumour growth is decreased by ca 50% over the period of the experiment, and that this is not accompanied by any significant weight loss in the treated animals (Fig. 2b). Furthermore, activity was also seen in the HPAC pancreatic tumour model where a 30% reduction of tumour growth was achieved (data not shown).

3.2. BMSG-SH-3. localizes in tumors

The ligand is strongly fluorescent and this property has been exploited to examine its localization in treated animals. Fig. 3 shows that uptake is mostly in the tumors and pancreas, with lesser amounts in the lung. Only trace fluorescence was observed in other organs such as heart, kidneys, liver and spleen.

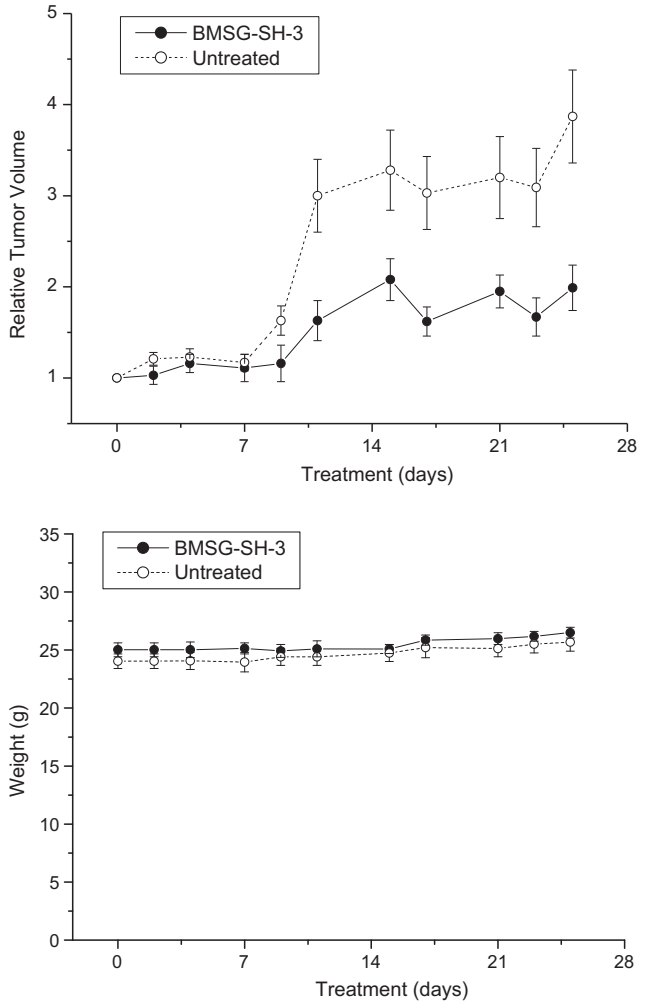

Figure 2. Experimental therapy of established murine MIA-Pa-Ca2 flank xenograft tumours treated with BMSG-SH-3. Animals received 3 mg/kg intraperitonally 3/week (closed symbols) and were compared to age matched untreated control animals (open symbols). The top graph shows development of tumour volume over time. The bottom graph shows the change in body weight over time.

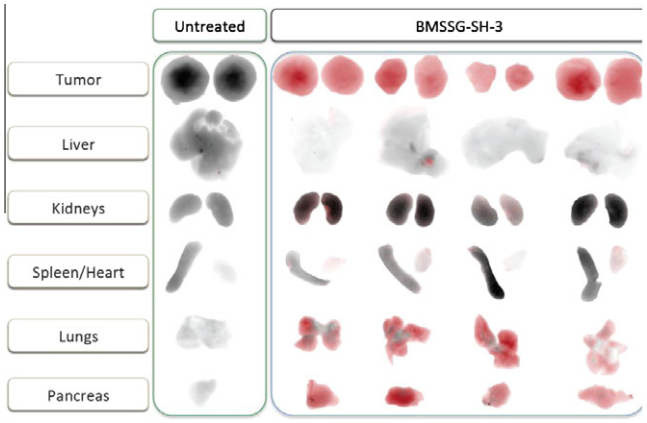

Figure 3. Biodistribution of BMSG-SH-3 in major organs. Fluorescent BMSG-SH-3 was visualized ex vivo 48 h after the last of four cycles of 3 mg/kg given 3 times/week intraperitoneally.

3.3. BMSG-SH-3. stabilizes HSP90 promoter quadruplexes

The compound is a potent stabilizer of human telomeric quadruplexes, with a ΔT_m of 28.3 °C at 0.5 μM concentration.[27] The stabilization observed here for the two HSP90 quadruplexes is exceptionally high:

ΔT_m at 0.5 μM BMSG-SH-3: HSP90a 34.9 °C, HSP90b 31.0 °C

ΔT_m at 1.0 μM BMSG-SH-3: HSP90a 36.3 °C, HSP90b 32.0 °C

(More extensive biophysical data showing that these two sequences form stable quadruplexes under physiological conditions will be presented elsewhere).

3.4. BMSG-SH-3. is an in vivo telomerase inhibitor

The activity of telomerase in tumour samples treated with BMSG-SH-3 for 25 days was decreased by ca 50% compared to untreated tumour samples (Fig. 4). The levels of HSP90 and hTERT protein, both of which are associated with the regulation and activity of the telomerase enzyme complex, have also been examined (Fig. 5). The expression of both these proteins was reduced by ca 30% following treatment. No significant changes in k-ras protein levels were observed in any of the tumour samples. A small (10%) down-regulation of bcl-2 protein was observed (Fig. 6a, c), which is at the limit of statistical significance. In our hands we have observed that MIA-Pa-Ca-2 cells express high levels of bcl-2 protein compared to other human pancreatic cancer cell lines such as BxPc3 and HPAC (Fig. 7a). Fig. 8 shows that no changes were detected in levels of the DNA damage-associated nuclear proteins PARP and γH2AX during the xenograft experiments.

4. Discussion

Elevated levels of bcl-2 protein expression and mutated/up-regulated k-ras have been correlated with poor response to chemotherapy and development of drug resistance in the clinic for human pancreatic cancers. The lack of any significant change in expression levels for either of these proteins strongly suggests that they are not targets for BMSG-SH-3.

We have previously shown that the small molecule BMSG-SH-3 is an effective stabilizer of human telomeric quadruplex structure, a potent inhibitor of telomerase function in vitro and is able to displace the single-strand binding protein hPOT1 from telomeric ends.[27] We have taken these various lines of evidence as supportive (though not definitive proof) of the concept that BMSG-SH-3 can also bind to the single-strand telomeric DNA overhang in cells and in vivo, and thus can inhibit the telomerase enzyme complex. The data presented here though suggests that this is not the sole contributor to the observed anti-tumor action of this compound.

A large number of small molecules that stabilize G-quadruplex nucleic acid motifs have been reported, and evidence of in vivo anticancer activity has been found for a few, notably the acridine derivatives BRACO-19 and RHSP4.[18,20,22,23] A variety of xenograft models for human cancer have been found to be sensitive to these agents, and telomere targeting has been observed to be associated with the anticancer activity.[18] Quarfloxin (CX-3543) is the sole G-quadruplex ligand to have been reported[38] to demonstrate activity to date in a pancreatic cancer model (the MIA-Pa-Ca-2 xenograft, as studied here). However Quarfloxin does not appear to act at the telomere level and instead inhibits rRNA biogenesis via a quadruplex-mediated mechanism. The established G-quadruplex ligands BRACO-19 and RHSP4 have shown single-agent antitumor activity in xenograft models of, for example, breast, lung and vulval cancer but to our knowledge BMSG-SH-3 is the first G-quadruplex ligand to show in vivo anticancer activity associated with telomerase inhibition in a pancreatic cancer model.[18] The suppression of HSP90 protein expression may be a consequence of telomerase inhibition since the two proteins are associated together.[39] However the evidence presented here, suggested that quadruplex sites in the HSP90 promoter are significantly stabilized by BMSG-SH-3 supports

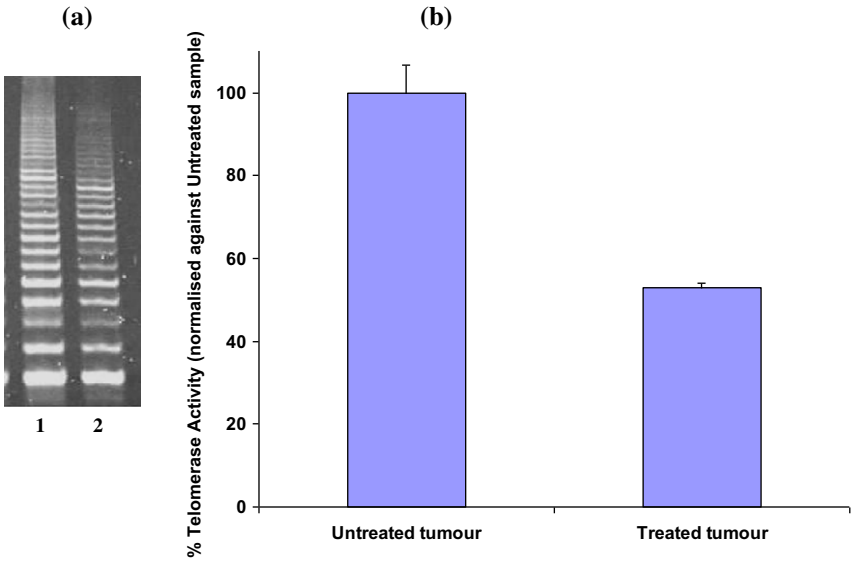

Figure 4. (a). Gel showing in vivo telomerase activity in MIA-Pa-Ca-2 xenograft tumour samples, estimated by the TRAP-LIG assay. Lane 1; untreated tumour, lane 2; tumour treated with BMSG-SH-3; (b) Graph showing in vivo telomerase activity normalized against untreated tumour sample.

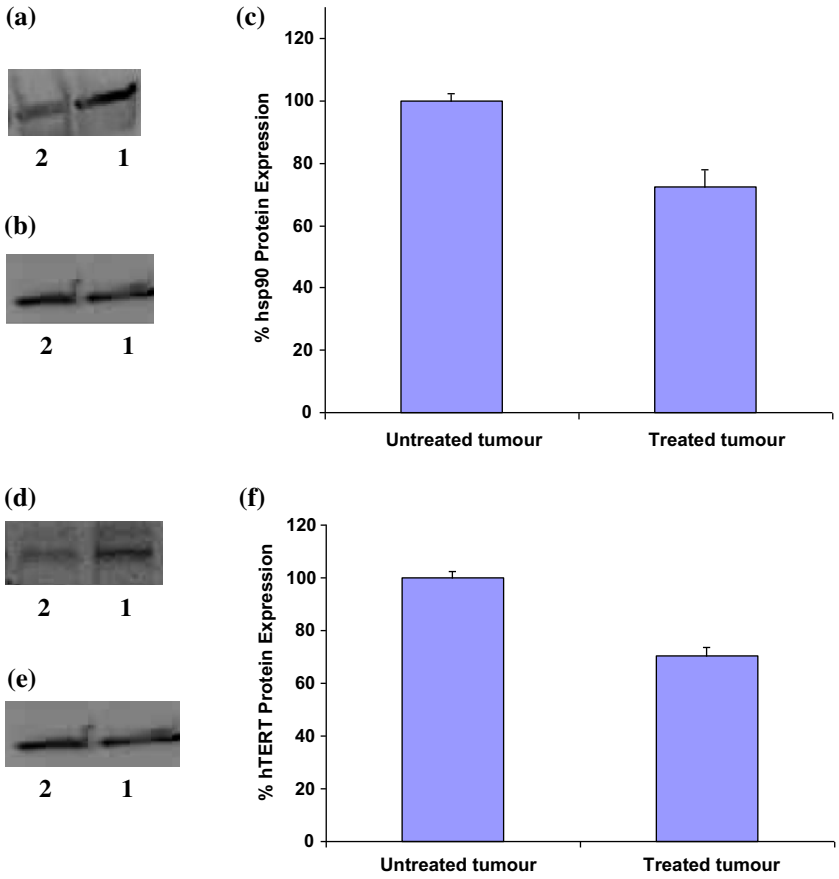

Figure 5. Immunoblots showing expression levels in MIA-Pa-Ca-2 tumours exposed to BMSG-SH-3. (a) expression of HSP90 protein, Lane 1; untreated tumour, lane 2; tumour treated with BMSG-SH-1; (b) expression of β-actin loading control, Lane 1; untreated tumour, lane 2; tumour treated with BMSG-SH-3; (c) quantified expression levels of HSP90 protein normalized against expression levels of the control β-actin; (d) expression of hTERT, Lane 1; untreated tumour, lane 2; tumour treated with BMSG-SH-3 (no difference in these loading controls was observed); (e) expression of β-actin. Lane 1; untreated tumour, lane 2; tumour treated with BMSG-SH-3; (f) quantified expression levels of hTERT protein normalized against β-actin expression levels.

the hypothesis that direct down-regulation of HSP90 expression may also be involved, although more evidence for this will be required before firm conclusions can be drawn. There is evidence that telomerase activity is HSP90-dependent[40], and a further hypothesis to be explored is that at least some of the down-regulation of telomerase expression observed here is related to HSP90

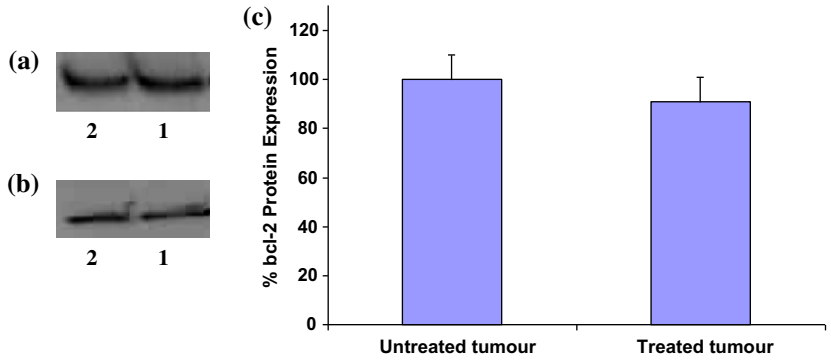

Figure 6. (a) Expression of bcl-2 protein in MIA-Pa-Ca-2 tumour xenografts exposed to BMSG-SH-3. Lane 1; untreated tumour, lane 2; tumour treated with BMSG-SH-3; (b) expression of β-actin; (c) graph showing bcl-2 protein expression normalized against control β-actin expression levels.

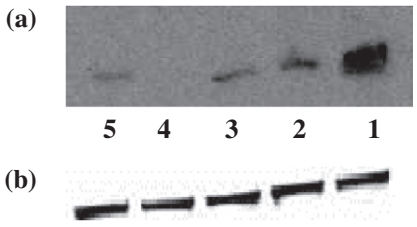

Figure 7. Expression levels of bcl-2 protein (a) and β-actin control; (b) from cells treated with BMSG-SH-3. In Lane 1; GIST48, human gastrointestinal tumour cells, lane 2; MIA-Pa-Ca-2 human pancreatic tumour cells, lane 3; BxPc3, human pancreatic tumour cells, lane 4; Panc1, human pancreatic tumour cells and lane 5; HPAC, human pancreatic tumour cells. An equal volume of total protein was loaded in each lane.

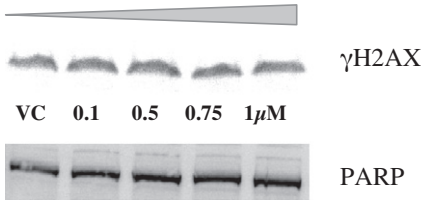

Figure 8. Western blots of γH2AX and PARP proteins in MiaPaCa-2 cells treated with BMSG-SH-3 for 8 h. PARP was obtained from whole cell extract and γH2AX was extracted from the cell nuclei with TEB buffer.

down-regulation—this is given credence by the unexpected non-observation of γH2AX and PARP DNA damage responses. Changes in these markers have been observed for other telomeric quadruplex-binding ligands.[21,23] (There is also a validated quadruplex-forming sequence in the hTERT promoter[41], and it is plausible that the observed hTERT down-regulation is due to BMSG-SH-3-induced quadruplex stabilization, although this has not yet been explored).

BMSG-SH-3 is not an obvious drug candidate molecule—it has a higher molecular weight than is normally acceptable and it carries four positive changes at physiological pH. However it is rapidly taken up into cancer cell nuclei (Hampel et al., to be published) and the significant responses that it produces in the MIA-Pa-Ca-2 xenograft model indicates that its intracellular tumour distribution is at least adequate in this xenograft model. This conclusion is reinforced by the striking observation of pancreatic tumor localization, although no information on penetration within the tumour mass is as yet available. Some localization in lung tissue is apparent (Fig. 4a), suggesting that BMSG-SH-3 may also be of use in targeting lung carcinomas. BMSG-SH-3 is not localized in heart tissue and does not have significant hERG activity[42] (an indicator of possible cardiotoxicity) and observations to date of treated animals do not indicate any other major off-target toxicities. The surprising lack of DNA damage responses also suggest that this toxicity pathway is not involved in the cellular response to BMSG-SH-3, by contrast with other G-quadruplex ligands[20,21] such as RHSP4. Telomerase inhibition can target cancer stem cells[43], suggesting that this may be a viable approach for long-term tumour ablation and therapeutic benefit. We conclude that the simultaneous targeting of several quadruplexes results not only in the telomerase inhibition observed here, but also in therapeutically-beneficial HSP90 down-regulation, and is a positive outcome of the inherent broad quadruplex affinity of BMSG-SH-3. Future studies will aim to optimize this naphthalene diimide series with respect to its pharmacological properties, and will examine further implications of multi-quadruplex targeting. It will also be interesting to examine the in vivo antitumor activity of other recently-described G-quadruplex ligands that have been designed with the aid of computer modeling, such as a methylene blue derivative.[44]

Acknowledgments

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