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ORIGINAL ARTICLE

Efficacy of SSG and SSG/IFNα2 against human prostate cancer xenograft tumors in mice: a role for direct growth inhibition in SSG anti-tumor action

Jing Li · Daniel J. Lindner · Carol Farver · Ernest C. Borden · Taolin Yi

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Abstract
Background Pre-clinical activity of SSG against melanoma and renal cancer has been identified recently although the drug's mechanism of action and activity against tumors of additional histological-types remain undefined.
Methods The effects of SSG and SSG combination with other agents on DU145 human prostate carcinoma xenograft tumors in mice and on DU145 cell subpopulations of differential SSG sensitivities were evaluated.
Results DU145 tumor growth was inhibited by SSG (69%), IFNα2 (33%) or the combination (80%) that induced complete regression of WM9 human melanoma tumors. DU145 cells in culture were also partially growth inhibited by SSG at killing doses (200–800 μg/ml) for WM9 cells, indicating a correlation of SSG inhibition of cancer cell growth in vitro and in vivo. DU145 cells formed multiple micro tumors in mice treated with SSG or SSG/IFNα2 in contrast to the single large tumors in the control or IFNα2-treated mice, suggesting the existence of an SSG-resistant subpopulation in DU145 cells. Indeed, DU145 but not WM9 cells formed colonies (~4% frequency) when cultured in the presence of SSG. Single cell clone (DU145–7) isolated from DU145 cells showed SSG-resistant growth in culture, unassociated with cross-resistance to IFNα2 and converted to SSG-responsive cells by BSO that inhibited intracellular glutathione levels.
Conclusions These results implicate a role for direct growth inhibition in SSG anti-tumor action, provide novel insights into the mechanism of tumor resistance to the drug and suggest a therapeutic potential for SSG and its combinations with IFNα2 or BSO for prostate cancer that warrants further investigation.

Keywords Prostate cancer · Sodium stibogluconate · IFN · BSO and drug-resistance

Abbreviations

SSG	Sodium stibogluconate
IFNα2	Interferon-α2b
BSO	L Buthionine-sulfoximine
Mϕ	Macrophage

Introduction

Prostate cancer is the most common malignancy for men in USA and accounts for approximately 30,000 annual deaths in this country. Although the majority of the newly diagnosed advanced prostate cancer patients respond initially to androgen therapy, virtually all eventually develop androgen-independent disease [15]. Currently, few treatment options are available to prostate cancer refractory to hormone therapy [34]. Additional therapeutic strategies are needed to reduce mortality from prostate cancer.

J. Li · D. J. Lindner · E. C. Borden · T. Yi (✉)
Department of Cancer Biology, Lerner Research Institute, The Cleveland Clinic, 9500 Euclid Avenue, NB4-67, Cleveland, OH 44195, USA
e-mail: yit@ccf.org

D. J. Lindner · E. C. Borden · T. Yi
Taussig Cancer Center, The Cleveland Clinic, Cleveland, OH 44195, USA

C. Farver
Department of Pathology, The Cleveland Clinic, Cleveland, OH 44195, USA

Anti-tumor activity of SSG (sodium stibogluconate) has been identified in recent pre-clinical studies [10, 42], providing rationale for investigation in clinical trial. This drug has been used for decade as an anti-leishmania therapeutic based on an undefined mechanism of action [16]. Our prior molecular studies demonstrated its activity to inhibit SHP-1 protein tyrosine phosphatase (PTPase) [26, 27, 42]. Furthermore, it has had anti-tumor activity against renal cancer [10] and melanoma [42] in mice that apparently functioning through distinct cellular mechanisms. SSG inhibited growth of murine renal cancer (Renca) in mice via a T-cell-dependent immune mechanism despite its lack of a growth inhibitory activity against Renca cells in vitro [10]. This SSG anti-tumor action, based on immunity and independent of direct inhibition of tumor growth, might have predominantly resulted from SSG inhibition of SHP-1 [27] that negatively regulates the activation of the key anti-tumor immune cells such as T cells [7, 18, 29, 43] and NK cells [5, 6]. This mechanism of action for SSG as an SHP-1-targeted molecule is supported by the activity of SSG at a therapeutically achievable dose to inhibit SHP-1 [26, 27, 42] and to activate T cells in vitro and in vivo [10]. It is further supported by the observation that SSG induced systemic macrophage (Mϕ) expansion in mice [10], a prominent feature of mice with genetic SHP-1-deficiency [32, 33]. This mechanism of action for SSG also provides an explanation for the synergistic interaction of SSG with IL-2 in anti-Renca tumor action [10] given the negative role of SHP-1 in IL-2 signaling [23]. Moreover, it designates activated immune cells as potentially significant endpoints for clinical investigation of SSG as a novel anti-cancer therapeutic.

In contrast to the T cell-dependent anti-Renca tumor action [10], SSG effectively inhibited the growth of WM9 human melanoma xenograft tumors in T cell-deficient nude mice and acted in synergy with IFNα2 to eradicate WM9 tumors [42]. This anti-melanoma action was therefore independent of the conventional T immune cells. It might be mediated via direct growth inhibition of WM9 tumors by SSG given that the proliferation of WM9 cells in culture was blocked by SSG in vitro [42]. Such an action mechanism of direct growth inhibition was unrelated to SHP-1 because the absence of SHP-1 expression in WM9 cells [42] excluded it as a target molecule in the melanoma cells. Additional studies are needed to assess the significance and mechanism of SSG-induced growth inhibition of cancer cells in its anti-tumor action. It is also important to investigate whether SSG has activity against tumors of other histology-types besides renal cancer and melanoma.

We have evaluated in this work the activities of SSG and SSG/IFNα2 in mice against xenograft tumors consisting of DU145 human prostate cancer cells. DU145 cells were partial growth inhibited in culture by SSG at doses inducing complete kill of WM9 cells. Our results demonstrated that DU145 tumors were partially inhibited by SSG/IFNα2 treatment that induced complete regression of WM9 tumors. This partial response of DU145 tumors to the treatment was due to the formation of multiple micro-tumors, which apparently resulted from an SSG-resistant DU145 subpopulation that was sensitized to the drug in culture by γ-glutamylcysteine-synthetase inhibitor BSO [14]. These data further demonstrate the significance of direct inhibition of malignant cell growth in SSG anti-tumor action and provide novel insights into the mechanism of tumor resistance to the drug. Moreover, they also suggest the potential of SSG, SSG/IFNα2 and SSG/BSO as novel therapeutic strategies for prostate cancer that need to be evaluated in future clinical investigation.

Materials and methods

Cells, cell culture and reagents

Human prostate cancer cell line DU145 [22] and human melanoma cell line WM9 [12] were obtained from colleagues at the Cleveland Clinic. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Recombinant human IFNα2 (interferon-α2b, specific activity 2×10^8 units/mg protein, Intron A, Schering-Plough) and SSG (100 mg Sb/ml, used to designate SSG concentration hereafter) have been described previously [42]. L-Buthionine-sulfoximine (BSO) was purchased from a commercial source (Sigma-aldrich).

Cell growth inhibition assays and glutathione assays

For determination of the effects of SSG and/or IFNα2 on cell growth, cells were cultured in the absence (control) or presence of various amounts of SSG and/or IFNα2 for 6 days prior to quantification of viable cells by MTT assays as described [17]. The effects of BSO on cell growth were assessed by culturing cells for 4 days in the absence or presence of BSO and/or SSG. Viable cells were then quantified by MTT assays. Percentage of growth inhibition was calculated by comparing the viable cells in cultures containing SSG and/or IFNα2 to those of the control (100%). For quantification of glutathione, cells were cultured in the absence or presence of BSO (400 μM) for 18 h, washed twice with PBS and

then harvested for glutathione measurement using a commercial glutathione assay kit (World Precision Instruments, Sarasota, FL) following the manufacturer's procedures.

Isolation and characterization of single cell clones

For quantification of SSG resistant cells in DU145 cell line, DU145 cells were seeded at differential cell numbers into culture dishes and cultured for 6 days in the presence of SSG at 100 μg/ml. The dishes were then washed in PBS twice and stained with crystal violet [8] prior to manual counting the numbers of colonies in the dishes and data analysis by linear regression to calculate the relative number of SSG-resistant cells in the DU145 cell line.

For isolation of single cell clones from DU145 cell line, DU145 cells were suspended in culture medium without SSG, seeded at one cell/ml in 24 well plates and cultured for 2 weeks to allow outgrowth of colonies. Individual wells containing only a single colony were selected by microscopy. The cells from these wells were subsequently transferred and expanded as single cell clones. The single cell clones were cultured in the absence or presence of SSG for 6 days prior to quantification of viable cells by MTT to determine their relative growth.

Animal studies

Nude mice (4 weeks old, female, Taconic) were inoculated (s.c.) in the flanks with DU145 or WM9 cells (3×10^6 cells/site) on day 1. Starting on day 4, the mice were subjected to PBS (Control) or treatment with IFNα2 (500,000 U, s.c., daily), SSG (12 mg, s.c., daily) or both (SSG/IFNα2). The doses of IFNα2 and SSG used for treatment were comparable to those used in a previous study [42] that demonstrated their tolerance and efficacy in mice. Tumor volume was measured every 2 days and calculated using the formula for a prolate spheroid ($V = 4/3\ \pi$ a2b) [20]. Mouse viability (daily) and body weights (weekly) were recorded. Tumors and tumor inoculation sites of the mice were harvested at the end of the experiment. Hematoxylin + Eosin (HE) stained tissue sections of tumors and tumor inoculation sites tissues of the mice were prepared and subject to microscopic evaluation. Student's t test was used to assess the significance of the tumor volume differences. All studies involving mice were approved by the Institutional Animal Care and Use Committee.

Results

SSG and SSG/IFNα2 inhibits the growth of DU145 human prostate cancer xenograft tumors in nude mice

To assess the potential of SSG in prostate cancer treatment and investigate the mechanism of SSG antitumor action, we determined the effects of SSG and its combination with IFNα2 against 4 day established DU145 tumors in nude mice. DU145 xenograft tumors grew aggressively in the absence of treatment (Fig. 1a) and were modestly growth inhibited (~33%) by IFNα2 therapy (Fig. 1). SSG as a single agent was more effective than IFNα2 in suppressing the growth of DU145 tumors and induced approximately 69% of growth inhibition (Fig. 1). SSG combination with IFNα2 induced 80% of growth inhibition of DU145 tumors, a significant ($P < 0.01$) increase in comparison to those induced by SSG or IFNα2 individually (Fig. 1). Consistent with a previous report [42], the treatments of SSG, IFNα2 and SSG/IFNα2 combination were tolerated in the mice, which all survived until the end of the study with comparable body weights (data not shown).

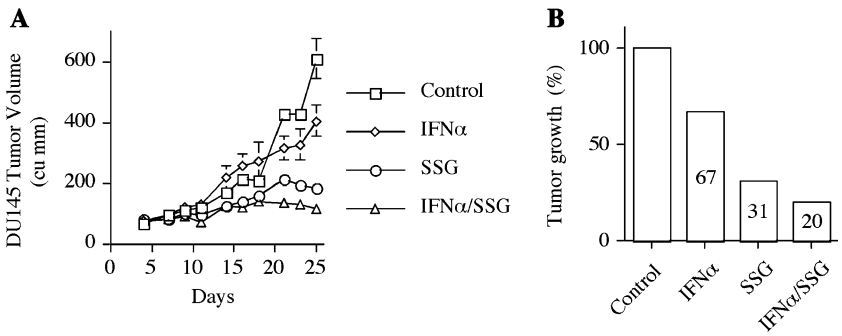

Fig. 1 Inhibition of DU145 tumor growth by IFNα2, SSG and IFNα2/SSG combination. Athymic nude mice (female, 6 week old) bearing 4 day established tumors (s.c.) of DU145 human prostate cancer cells were treated with PBS (control), IFNα2 (5×10^5 U daily, s.c), SSG (12 mg daily, s.c.) or the combination. **a** Tumor volumes (mean ± SD, $n = 8$) in the differentially treated mice. **b** Relative tumor growth in the mice calculated based on data in **a**

These results identified a growth inhibitory activity of SSG against DU145 tumors in mice. This SSG activity was superior to that of IFNα2 under the experimental conditions and interacted with IFNα2 against DU145 tumors.

SSG or SSG/IFNα2 induced formation of DU145 micro-tumors in mice

Despite inhibition of DU145 tumors (Fig. 1), SSG and SSG/IFNα2 failed to cure of established DU145 tumors in mice under the experimental conditions (Fig. 1). To understand the partial response of DU145 tumors to SSG and SSG/IFNα2, we evaluated the histology of the tumor inoculation sites in the differentially treated mice.

A single large tumor was evident at the inoculation site of the control mice and had substantial central necrosis often associated with rapid tumor growth (Fig. 2a). In IFNα2-treated mice, a more compact single tumor was formed in consistent with reduced tumor growth induced by IFNα2 therapy (Fig. 2b). In contrast, several distinct small tumors were present at the inoculation site of SSG-treated mice (Fig. 2c). Such micro tumors, but with reduced number, were also present at the inoculation site of mice treated with SSG/IFNα2 combination (Fig. 2d).

We also determined the efficacy of SSG/IFNα2 against WM9 tumors under comparable conditions and whether WM9 melanoma cells form micro tumors in the SSG/IFNα2-treated mice. Consistent with the previous report [42], SSG/IFNα2 induced regression of WM9 tumors that grew aggressively in the control mice (Fig. 3a). Histological evaluation of the WM9 cell inoculation site in the mice from the treatment group failed to detect tumors (Fig. 3b).

These results suggested that DU145 cells might harbor a subpopulation insensitive to SSG and formed the micro tumors during the treatments, resulting in partial tumor response to the treatments.

DU145 cell line harbors an SSG-resistant subpopulation

To derive more direct evidence of an SSG-resistant subpopulation in DU145 cells, we compared the in vitro growth of DU145 and WM9 cells in the presence of SSG. In addition, we also assessed colony formation of the cancer cell lines in the presence of SSG.

Unlike WM9 cells that were completely killed in culture by SSG at concentrations above 200 μg/ml, DU145 cells were partially growth inhibited in the presence of the drug at 800 μg/ml (Fig. 4a). DU145 cells seeded in culture medium containing SSG (100 μg/ml) readily formed distinct colonies after 6 days (Fig. 4b) while WM9 cells failed to do so (data not shown). Based on the numbers of colonies out-grown in such cultures, it was calculated that approximately 4% of DU145 cells were capable of SSG-resistant growth in vitro (Fig. 4b).

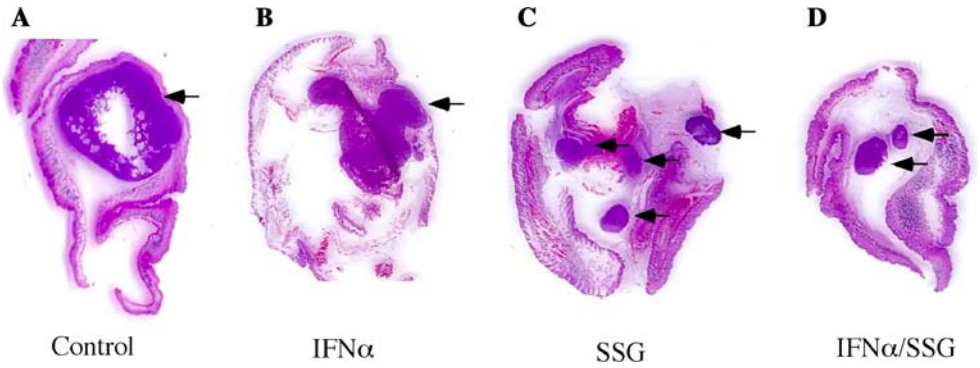

Fig. 2 DU145 cells form micro-tumors in mice treated with SSG or IFNα2/SSG combination. Histological section (H&E) of DU145 inoculation sites in mice subjected to differential treatment (day 25). Positions of DU145 tumors are indicated by the *arrows*

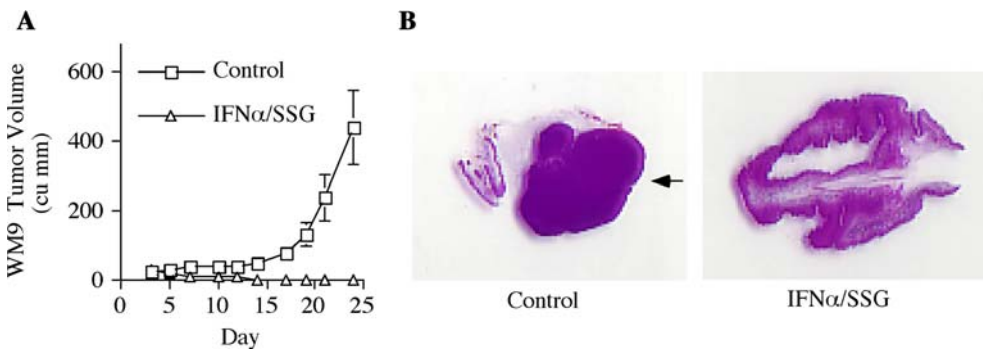

Fig. 3 SSG/IFNα2 induces complete regression of WM9 human melanoma tumors in mice. **a** Tumor volumes of nude mice bearing 4 day established WM9 tumors subjected to no treatment or SSG/IFNα2 treatment as described in Fig. 1. **b** Histology (H&E) of WM9 inoculation sites in mice (day 25). WM9 tumor is indicated by the *arrow*

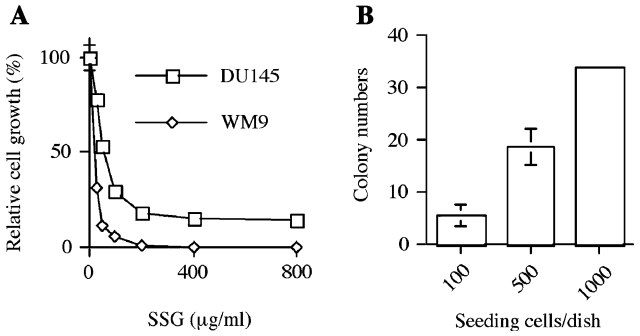

Fig. 4 DU145 cell line harbors a subpopulation resistant to SSG in culture. **a** Relative cell growth of DU145 and WM9 cells in the absence or presence of various amounts of SSG as determined by MTT assays in day 6 culture. **b** Numbers of colonies formed in culture dishes seeded with DU145 cells after cultured in the presence of SSG (100 μg/ml) for 6 days. Data represent the mean ± SD of triplicate samples

This observation demonstrated the pre-existence of a small subpopulation within DU145 cells that was resistant to SSG at a level capable of killing WM9 cells in culture.

Isolation and characterization of SSG-sensitive and -resistant DU145 clones

We next isolated single cell clones from DU145 cells without SSG selection and identified clones with differential sensitivities to SSG in culture. Parental DU145 cells were seeded at one cell/well in culture in the absence of SSG to allow the out growth of single cell clones, 12 of which were randomly selected and individually expanded for further characterization. The clones were initially screened by in vitro growth assays in the presence of high dose SSG (800 μg/ml). Clone DU145-7 was identified as markedly resistant to SSG in culture, retaining approximately ~52% growth in the presence of SSG at 800 μg/ml. The other clones were killed completely (e.g., DU145-8) or showed little growth inhibition when exposed to a comparable SSG level.

DU145-7 and DU145-8 clones were further compared for growth inhibition by SSG or IFNα2. DU145-7 cells in culture were minimally affected by SSG at concentrations at less than 100 μg/ml and had inhibited only ~40% by SSG at 200 μg/ml (Fig. 5b). In contrast, DU145-8 cells were growth inhibited by SSG in a dose-dependent manner with complete kill induced by SSG at 200 μg/ml (Fig. 5b). Despite their differential responses to SSG, the two clones grew comparably in the absence of the drug (Fig. 5a). Interestingly, they were comparably growth inhibited by IFNα2 (Fig. 5c).

These results demonstrated that single cell clones from DU145 cells had markedly different SSG sensitivities, which did not result from differential growth rates of individual clones and was not associated with differential responses to IFNα2.

Sensitization of SSG-resistant DU145-7 cells to SSG by BSO in association with inhibition of intracellular glutathione synthesis

As an initial effort to understand and overcome SSG-resistance in DU145 cells, we sought to identify agents that could sensitize the DU145-7 cells to SSG in culture. Among the compounds investigated was BSO, an inhibitor of γ-glutamylcysteinesynthetase [14] critical in glutathione production [28].

In the presence of BSO, growth of DU145-7 cells was inhibited dose-dependently by SSG with complete cell kill in day 4 culture occurring at 200 μg/ml (Fig. 6a), an SSG dose that induced only ~20% growth inhibition as a single agent under comparable conditions (Fig. 6a). BSO alone had little effects on DU145-7 cells (Fig. 6a) and failed to augment IFNα2-induced growth inhibition of the DU145-7 cells (data not shown). BSO augmented SSG-induced growth inhibition of DU145-8 cells (Fig. 6b) and inhibited the growth of DU145-8 cells (~50%) as a single agent (Fig. 6b). Cells of the two clones expressed glutathione at comparable levels that were markedly reduced after treatment of the cells with BSO for 18 h (Fig. 6c),

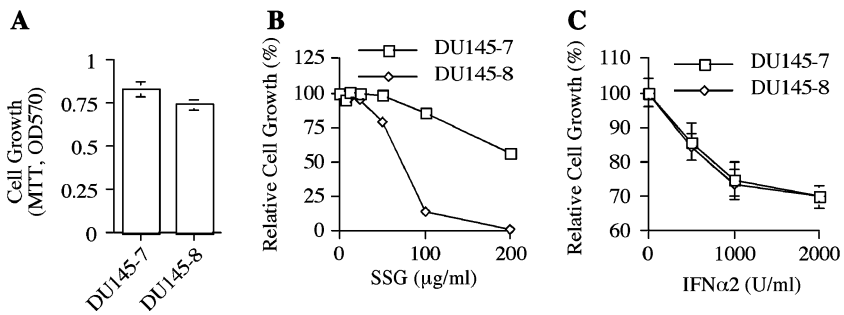

Fig. 5 Growth rates of single cell clones of DU145–8 and DU145-7 in the absence or presence of SSG or IFNα2 in culture. Growth of the DU145 clones in the absence (**a**) or presence of SSG (**b**) or IFNα2 (**c**) in day 6 cultures as quantified by MTT assays. Data represent mean ± SD of replicate samples

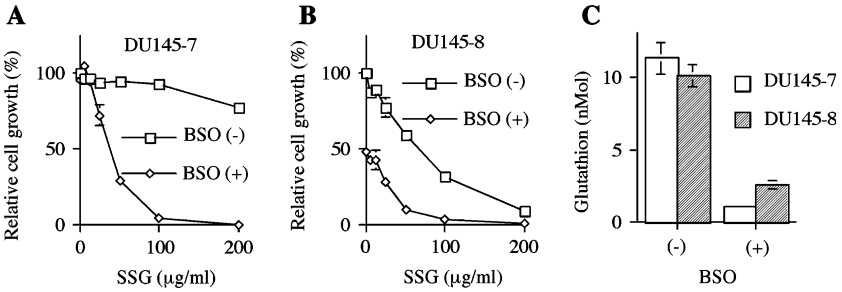

Fig. 6 BSO sensitizes DU145-7 and -8 cells to SSG in culture in association with inhibition of intracellular glutathione synthesis. Relative growth of DU145-7 (**a**) and DU145-8 (**b**) cells in the absence (−) or presence (+) of BSO (50 μM) and SSG in day 4 culture as quantified by MTT assays. Data represent mean ± SD of replicate samples. DU145-7 and -8 cells cultured in the absence or presence of BSO were also harvested for quantification of intracellular glutathione levels (**c**). Data (μM/mg cellular proteins) represent mean ± SD of replicate samples

consistent with the reported activity of BSO as an inhibitor of glutathione synthesis [14].

These results identified BSO as a sensitizing agent that converted the SSG-resistant DU145-7 cells into SSG-responding cells in culture, implicated glutathione in SSG action and suggested therapeutic potential of BSO/SSG combination.

Discussion

SSG had significant anti-tumor activity against melanoma [42] and renal cancer [10] in pre-clinical studies and might have potential as a novel therapeutic for human malignancies. However, its mechanism of action is not fully defined. It is also unclear whether the drug has pre-clinical activity against tumors of additional histological-types.

SSG markedly inhibited the growth of DU145 human prostate cancer xenograft tumors in mice (Fig. 1a). This activity of SSG as a single agent was detected at a tolerated dose and resulted in 69% tumor growth inhibition, superior to that of IFNα2 monotherapy (33% growth inhibition) (Fig. 1b). The combination of SSG and IFNα2 induced a further increase in tumor growth inhibition (80%). IFNα2 is a cytokine with therapeutic effects in hematopoietic malignancies and solid tumors, approved for the treatment of several malignant diseases [41]. It has been investigated in early-phase clinical trials in combination with other therapeutics in prostate cancer patients [19, 21, 31, 40]. Our results suggest a therapeutic potential SSG and SSG combination with IFNα2 for prostate cancer that warrants further investigation in clinical studies. Given the marked growth inhibition of DU145 tumors by SSG or SSG/IFNα2 combination, the treatments might also prolong survival of tumor-bearing mice in comparison to control. Whereas the potential effect represents a key quantification of preclinical efficacy, it was not assessable in this study in which no mouse death occurred due to the relatively short study period of 25 days (Fig. 1).

Our results also provided novel insights into the mechanism of SSG anti-tumor action and designated an important role for direct growth inhibition of tumor cells by the drug. Previous studies have indicated an anti-tumor immune mechanism for SSG that functioned effectively in a mouse renal tumor model in the absence of direct growth inhibition of the renal tumor cells by SSG [10]. On the other hand, SSG was active in inhibiting growth of melanoma tumors in T-cell-deficient nude mice and directly inhibited the melanoma cell growth in culture [42]. It was unclear whether SSG anti-melanoma action was mediated via other immune cells in the nude mice or resulted from direct growth inhibition of melanoma cells by the drug. Indeed, SSG was found to induce tumor-infiltrating Mϕ in mice [10] and activate NK cells (unpublished results). Both types of immune cells are active in T cell-deficient athymic nude mice [4].

Several lines of evidence from this work indicate that SSG action against DU145 tumors and WM9 melanoma was mediated in a substantial effect by direct growth inhibition. A correlation existed between SSG anti-tumor efficacy in mice and SSG inhibition of tumor cell growth in culture. SSG in vitro partially inhibited DU145 cell growth at doses that completely killed WM9 cells (Fig. 4a). It induced only incomplete regression of DU145 tumors even when combined with IFNα2 (Fig. 1) whereas such therapy achieved cure of established WM9 tumors (Fig. 3). Moreover, an SSG-resistant subpopulation was identified in DU145 cells but not in the WM9 cell line. This subpopulation was minimally growth inhibited in culture by SSG at doses effective against the SSG-sensitive population in DU145 cells (Figs. 4, 5, 6)

or WM9 cells (Fig. 4a). Interestingly, DU145 cells in mice treated with SSG or SSG/IFNα2 formed micro tumors instead of the single large tumor in control animals (Fig. 2). These micro tumors possibly resulted from the SSG-resistant subpopulation in DU145 cells and responsible for the incomplete regression of DU145 tumors in mice. These results taken together underscore a role for direct growth inhibition of tumor cells in SSG anti-tumor action.

A mechanism of action via growth inhibition has implications regarding potential clinical application of the drug and development of refined anti-cancer agents from SSG analogs. Tumors susceptible to SSG growth inhibition may prove more responsive to SSG therapy. Moreover, tumor growth responses might be exploited for prognosis and for identification of potential indications among malignancies. Further such a mechanism of action for SSG also underscores the significance of growth inhibitory activity in identification of SSG analogs with anti-tumor potential and highlights the needs for better understanding of growth inhibitory mechanism of the drug. However, the growth inhibition mechanism identified here does not diminish a role for immune cell activation as a distinct and effective means in anti-tumor action of SSG. Both mechanisms may be needed for optimal SSG anti-tumor effects.

Another significant part of our work was identification and isolation of SSG-resistant and -sensitive DU145 cell clones that have distinct growth responses to the drug. In addition to supporting a role for direct growth inhibition in SSG anti-tumor action, they may also provide mechanistic explanation for the formation of DU145 micro tumors and for the differential responses of DU145 and WM9 tumors to SSG. Moreover, these clones could prove valuable resources for elucidation of SSG-resistance mechanism and essential tools for developing strategies to overcome resistance. Indeed, initial characterization of chemical compounds using the SSG-resistant DU145 clone has led to the identification of BSO that was capable of sensitizing SSG-resistant DU145-7 cells to SSG in culture (Fig. 6). Unfortunately, the slow growth of DU145-7 tumors in mice (our unpublished data) prevented further assessment of the effects of SSG/BSO in vivo. This characteristic of DU145-7 tumors also raises the possibility that slow tumor growth might be a potential mechanism for SSG-resistance and warrants more detailed investigation in the future. It is also worth noticing that high doses of SSG might be ineffective to overcome SSG-resistance given the resistance of DU145 subpopulation to SSG up to 800 μg/ml in culture (Fig. 4).

Our results demonstrate an absence of association of SSG-resistance with a cross-resistance to IFNα2, indicating a beneficial effect of cytokine against SSG-resistant tumors and suggesting mechanistic insights. These notions are illustrated by the observation that the SSG-resistant DU145 cells were growth inhibited in culture by IFNα2 at a level similar to that of SSG-sensitive cells (Fig. 5c). They are further supported by in vivo evidence of reduced number of DU145 micro tumors in mice treated with SSG/IFNα2 in comparison to that in SSG-treated mice (Fig. 2). Since the signaling pathway for IFNα2 growth inhibition was apparently intact in the SSG-resistant cells, it suggests further that SSG inhibition of tumor cell growth might be mediated via a mechanism distinct from the IFNα2 signal pathways. Alternatively, the lack of cross-resistance might also be explained by SSG-resistance resulted from a defect in drug trafficking across tumor cell member or converting into its active form.

Our intriguing finding that BSO sensitized SSG-resistant and -responsive DU145 cells to SSG might also have significant implications. BSO is an inhibitor of γ-glutamylcysteine-synthetase [14], a rate-limiting enzyme in glutathione production [28], and has been used extensively as an experimental tool to manipulate cellular glutathione levels [1]. It was also investigated clinically [2, 3, 25] in phase I trials as a potential anti-cancer agent in combination with chemotherapeutics based on the association of high levels of glutathione expression in cancer cells with drug-resistance [30]. Our finding that DU145-7 and -8 cells had similar levels of intracellular glutathione (Fig. 6c) does not support such a simple association of glutathione levels with SSG-resistance in DU145-7 cells. Nevertheless, the capacity of BSO to sensitize SSG-resistant and responsive DU145 cells to SSG suggests the potential of SSG/BSO combination as a novel therapeutic strategy that might be of particular value against secondary tumors resistant to SSG monotherapy or tumor types of intrinsic SSG-resistance.

Interestingly, our results also provided evidence supporting a cross-resistance of the DU145 clones to SSG and BSO. BSO as a single agent failed to inhibit the growth of SSG-resistant DU145-7 (Fig. 6a) while it had significant activity against the SSG-responsive DU145-8 cells (Fig. 6b). This observation suggests that both agents might target a common signaling molecule or pathway. Whereas BSO inhibits glutathione production [14], SSG has inhibitory activity against selective protein tyrosine phosphatases [27], including oncogenic SHP-2 involved in human malignancies [35–39] and mitogenic signaling [11, 13, 24]. Thus SSG might inactivate oncogenic protein tyrosine phosphatases to induce cancer cell growth arrest and death. It was proposed that phosphatase inhibition by SSG might be

mediated via covalent (e.g., oxidative) modification of a catalytic cysteine conserved in phosphatases by the pentavalent antimony in SSG [42]. On the other hand, glutathione is an antioxidant [9] that might re-activate oxidative phosphatases or inactivate SSG. Therefore glutathione deprivation by BSO might potentiate SSG activity to inhibit oncogenic phosphatases in cancer cells. Based on a similar mechanism, BSO alone might also inactivate oncogenic phosphatases via depleting cellular glutathione to increase intracellular oxidation. Accordingly, it could be speculated that cancer cells depending on oncogenic phosphatases for growth and survival might be more sensitive to SSG or BSO while the independent ones could be more resistant. In this regard, our work has provided a basis for more detailed characterization of the drug sensitive and resistant clones for potentially differential expression/activities of oncogenic phosphatases to define the mechanism of SSG inhibition of tumor cell growth and tumor resistance to the drug.

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