

Depletion of drug-surviving glioma cells by a second phase treatment with low concentration of salinomycin

Zahid M. Delwar,¹ Dimitrios Avramidis,¹
Åke Siden,^{1,2} Mabel Cruz,¹
Juan Sebastian Yakisich¹

¹Department of Clinical Neuroscience,
Karolinska Institute, Stockholm;

²Department of Neurology, Karolinska
University Hospital, Stockholm, Sweden

Abstract

Standard treatment for glioma includes surgery, radiotherapy and chemotherapy but the outcome of patients is very poor. Antineoplastic drugs are usually administered alone or in combination for variable times (continuously or in cycles) in a single phase schedule. In this study we explored *in vitro* the antiproliferative effect of a 2 phases treatment. In the first phase, glioma cells were treated for 3-4 weeks with hydroxyurea (HU) or aphidicolin and then for 4-8 weeks with salinomycin, a drug that preferentially inhibits the proliferation of cancer stem cells. We found that salinomycin, is able to slowly deplete the fraction of glioma cells that survive the exposure to HU or aphidicolin. Surviving cells were killed at salinomycin concentrations lower than those required to kill untreated cells. The fraction of surviving cell showed traits of senescence including increased activity of the senescence associated β -galactosidase (SA- β -gal) marker. Our data suggest that drug-induced senescent cells may constitute a novel target for cancer treatment and can be exploited in a two phases therapeutic regimen.

Introduction

The standard treatment for gliomas includes surgery, radiation and chemotherapy.¹ Antineoplastic drugs are administered alone or in combination. Typical administration of temozolomide, the most used drug for gliomas, as single agent is 5 days every 28-day cycle.² This regimen offers little benefit.^{3,4} Alternative regimens are undergoing clinical trials⁵ but the hope to find a successful treatment is low since a result from a 21 days every 28 days regime does not improve survival.⁶ When used in combination, 2 or more antineoplastic drugs are often administered simultaneously.^{2,7} Despite numerous studies evaluating different

drugs combinations for short cycles or prolonged continuous administration the prognosis of patients carrying malignant gliomas is poor and most patients die within 14 months.^{1,3,4} Therefore, it is important to develop new therapeutic regimens and/or find new targets for treatment. In addition, the presence of chemotherapy resistant cells associated with a stem cell phenotype suggests that successful treatment of gliomas will require the elimination of 100% of cancer cells.^{8,9} We recently showed that a fraction of glioma cells are able to survive prolonged exposure to high concentration of HU or aphidicolin. The surviving cells were able to resume growth when the drugs were removed from the culture media.¹⁰ We speculated that the fraction of surviving cells, although resistant to HU or aphidicolin, may have become sensitized and could be eliminated using a second phase treatment with a different drug. On the other hand, the potential specific effects of salinomycin on cancer stem cells (*see below*) make this substance a good candidate to eliminate the fraction of surviving cells that are usually associated with the fraction of stem cells present in cell lines and tumours.¹¹ Salinomycin is a polyether antibiotic commonly used as an anticoccidial drug, it is a highly selective potassium ionophore and a p-glycoprotein inhibitor¹² that acts as a specific inhibitor of cancer stem cells¹³ and overcomes ABC transporter-mediated multidrug and apoptosis resistance in stem-like cells.¹⁴ However, the effective salinomycin concentration against cancer stem cells may be highly toxic.^{15,16} and this could prevent its use as conventional anticancer drug. The aim of this study was develop a novel *in vitro* two phase treatment to eliminate 100% of cancer cells by depleting HU- or aphidicolin-resistant glioma cells with lower (and likely less toxic) concentrations of salinomycin.

Materials and Methods

Reagents and enzymes

Dimethylsulfoxide (DMSO), hydroxyurea (HU), aphidicolin, salinomycin and temozolomide were purchased from Sigma (Sweden). All other reagents were of analytical grade or the highest grade available.

Cell lines

Stock cultures of human DBTRG-05MG glioma cell line were obtained from the European Collection of Cell Culture (ECACC). Cells were routinely cultured as previously described in RPMI-1640 supplemented with 10 % serum, 1% HT, 2 mM glutamine and 1 mM Sodium pyruvate.¹⁰

Correspondence: Juan Sebastian Yakisich, Department of Clinical Neuroscience R54, Karolinska Institute, Karolinska University Hospital, Sweden. S-141 86, Stockholm, Sweden. Tel. +46.8.585.89.533 - Fax: +46.8.585.87010. E-mail: sebastian.yakisich@ki.se

Key words: cancer, gliomas, senescence, cell proliferation, salinomycin.

Acknowledgements: this study was supported by grants from the Swedish Research Council and the Karolinska Institute.

Received for publication: 8 March 2011.

Revision received: 9 June 2011.

Accepted for publication: 14 June 2011.

This work is licensed under a Creative Commons Attribution NonCommercial 3.0 License (CC BY-NC 3.0).

©Copyright Z. Delwar et al., 2011
Licensee PAGEPress, Italy
Drugs and Therapy Studies 2011; 1:e7
doi:10.4081/dts.2011.e7

Preparation of drugs

Aphidicolin, salinomycin and temozolomide were prepared as stock solutions (2.5 mM, 10 mM and 100 mM respectively) in DMSO and stored at -20 °C. HU was diluted in distillate sterile water and stored at -20 °C as 1 M stock solution. The final dilutions were done in culture media, keeping the DMSO concentration below 1% (v/v).

Experimental procedures

Short term proliferation assay

DBTRG.0.5 MG cells were plated in 96 well microplates (~5,000 cell/well) and allowed to adhere overnight. Drugs at the appropriate concentration were added and incubated for 72 hours. Cell viability was measured by the cell counting kit (CCK Kit, Sigma, Sweden) following manufacturer's instructions. Drug effects were tested in three independent experiments performed by quadruplicates.

Long term proliferation assay

For prolonged effect of drugs on cell cultures, cells were plated in 96 well microplates (~5,000 cells/well) and allowed to grow for 3-4 days. Drugs were then added and maintained for 1-2 weeks (media and drugs were changed twice a week). After that, cells were incubated in drug-free media (changed twice a week) for 2-4 weeks. Re-growth was evaluated using a routine inverted microscope as previously described.^{10,17,18} This long term assay was repeated independently and the number of wells used for each treatment and controls are indicated in the respective figures.

Detection of senescence associated β -galactosidase (SA- β -gal) activity

Staining was performed as described by Dimri *et al.*¹⁹ Drug-treated cells were washed twice with PBS and then fixed for 4 minutes at room temperature in freshly prepared solution of 3% formaldehyde in PBS. The cells were washed again with PBS and incubated at +37°C (without CO₂) with X-gal staining solution, consisted of 1 mg/mL of 5-bromo-4-chloro-3-indolyl b-D-galactoside, 40 mM citric acid sodium phosphate pH 6.0, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl and 2 mM MgCl₂. Staining (characteristic blue color) was observed within 2h but maximal detection was noted at 12-16h. As negative control, untreated cells seeded at ~55,000 cells/mL, were allowed to proliferate for 3 days and processed simultaneously with experimental samples.

Statistical analysis

One-way ANOVA with Newman-Keuls multiple comparisons post test was performed using GraphPad Prism version 5.04 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

Results

DBTRG.05MG cells survived prolonged exposure to HU and aphidicolin and resumed growth in a stochastic manner

We previously reported that DBTRG.05MG human glioma cells can survive prolonged exposure (up to one month) to high concentration HU (10 mM) or aphidicolin (2.5 μ M) and can resume growth forming a monolayer when re-incubated in drug-free media as early as within 2 weeks.¹⁰ We report here that the fraction of surviving cells when re-incubated in drug free media re-enter the cell cycle in a stochastic way: in some wells re-growth (proliferation) was observed as early as after 2 weeks of incubation in drug-free media while in other wells the surviving cells remained viable for longer and variable periods and proliferation was observed after any time point between 3-10 weeks. We also observed that in some wells, cells remained viable, without evidence of proliferation for up to 3-4 month.

In a typical experiment for the present study, performed in 96 wells, re-growth was observed after two weeks in few wells (2/96 and 4/96 for 10 mM HU and 2.5 μ M aphidicolin, respectively). Additional re-growth was observed in other wells at week 3 and 4 (Figure 1). The treatment for 4 weeks with either HU or aphidicolin left very few surviving cells in all wells suggesting that the very low percentage

of re-growing cells might be due to the harsh conditions. For this reason, we next treated cell with aphidicolin for only 3 weeks and evaluated regrow after 1-4 weeks. In this experiment, a higher number of surviving cells in each individual wells was found and, as expected, a high number of regrowing cells was observed (Supplementary Table 1).

Salinomycin inhibited proliferation of DBTRG.05MG human glioma cells

We evaluated the antiproliferative effect of salinomycin on glioma cells. In short term assays (72 h) the IC₅₀ was 1 μ M (Figure 2A). The effect was similar in complete and serum free media (that usually favour the growth of cancer stem or stem-like cells). Because the IC₅₀ predicts the LC₁₀₀ by interpolation, we determined the RC₀ parameter experimentally. The RC₀ determines the minimum time and concentration required to kill 100% of cells using a long term assay.^{10,18} We found that exposure to 5 μ M salinomycin for at least one week was required to kill 100 % of cells and to prevent re-growth (RC₀= 5 μ M, one week, Figure 2B).

Salinomycin prevented re-growth and slowly depleted surviving cells

We tested the effect of salinomycin on the fraction of cells that survived prolonged exposure to HU. In these experiments, cells were

treated as described before for 4 weeks and then incubated with salinomycin (0.1, 0.25 or 0.5 μ M) or DMSO alone (control) for another 8 weeks. It was found that 0.5 μ M salinomycin was able to prevent re-growth of surviving cells. Moreover, this salinomycin concentration slowly killed these cells. In drug-free media wells (control cells); at week 6 after re-incubation 1/24 wells showed re-growth and in the rest of the wells (23/24) some cells remained viable. In contrast, in 0.5 μ M salinomycin treated wells, the number of cells gradually decreased with time until no visible viable cells was observed in any well (0/24, Figure 3A and 3B, panel I). Evaluation of the same experiment at week 8 showed re-growth in one additional control well and non-viable cells were observed in only one well. At this time point, no additional re-growth was observed in 0.5 μ M salinomycin treated wells (Figure 3B, panel II). A lower salinomycin concentration (0.25 μ M) was not able to kill surviving cells from all wells but prevented re-growth. At the lowest salinomycin concentration tested (0.1 μ M), viable cells were observed in all wells and re-growth was observed in one well. Similar results were observed in three independent experiments. The stochastic nature and low number of re-growth of surviving cells treated with vehicle (DMSO) alone (Figure 1) makes statistical analysis difficult. For this reason, we used the number of wells containing viable cells as a better parameter to statistically analyse the effects of different salinomycin concentrations

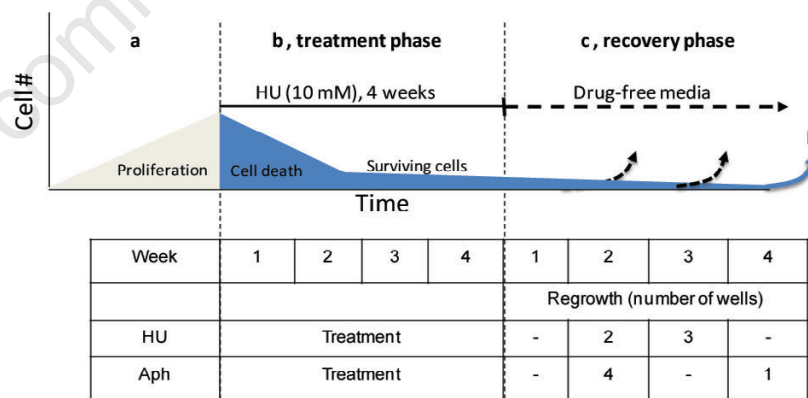


Figure 1. Stochastic re-growth of DBTRG.05MG human glioma cells after prolonged treatment with hydroxyurea (HU) or aphidicolin (Aph). DBTRG.05MG cells were grown to semi-confluency in 96 well plates and treated with HU (10 mM) or Aph (2.5 μ M) for 4 weeks. Media and drugs were changed twice a week. Top panel Schematic representation of events in a single well: After a period of proliferation in drug-free media (a), exposure to drugs (e.g. HU 10 mM) for 4 weeks killed most of the cells but a fraction of surviving cells were observed (b), (treatment phase). When re-incubated in drug free-media for 4 weeks (c), (recovery phase), re-growth were observed at variable times points (indicated as "R" in exiting arrow). Broken arrows indicate that re-growth might occur at any time point. In other wells, some cells were clearly viable (prolonged arrest) but they did not proliferate while few cells died in the next 8 weeks observation period. (Accompanying table) In a typical experiment performed in 96 wells, when cells were treated for 4 weeks with either HU or Aph we could observe re-growth in few wells as early as two weeks after incubation in drug-free media. In other wells, cells resumed proliferation at week 3 and 4.

on surviving cells. The rationale for this choice is based on the fact that in wells containing non-viable cells re-growth will not be observed while in well containing viable cells re-growth can be observed at variable times (Figure 1). This is important, because a single event of re-growth in any well is clinically equivalent to relapse of a tumour. Figure 3C shows that treatment of surviving cells with 0.25-0.5 μM salinomycin for 6 weeks, significantly reduce (0.5 μM completely eliminates) the number of wells containing viable cells compared to vehicle (DMSO) alone. For comparison, treatment of HU- or aphidicolin surviving cells with low concentration (2 μM) of temozolomide does not reduce the number of wells containing viable cells (*data not shown*).

Since, as mentioned above, the treatment with either HU or aphidicolin for 3 weeks left higher number of surviving cells compared to treatment for 4 weeks (Figure 2), we evaluated the effect of salinomycin in glioma cells previously treated with aphidicolin for 3 weeks. In this experiment, despite the fact that 100% of surviving cells stained positive for the SA- β -gal marker (Figure 4), we observed re-growth in higher number of control wells (8/24, aphidicolin-treated for 3 weeks). In salinomycin (0.25-0.5 μM) treated wells few viable cells were observed compared to control wells and there was no evidence of re-growth (*data not shown*).

Long term exposure to high HU concentration induced expression SA- β -gal in 100 % of surviving cell fraction

In DBTRG.05MG glioma cells, typical senescent cells were detected in HU or aphidicolin treated cultures after one week. Microscopic examination revealed that at week 1 a fraction of cells without typical morphology of senescence expressed the SA- β -gal marker (*data not shown*). At week 2, 100% of the cells expressed the SA- β -gal marker but with variable intensity: e.g. the intensity of the marker was very weak in some cells (Figure 4, arrows). Prolonged treatment with HU for 3-4 weeks reduced the number of viable cells: e.g., at week 4 few cells survived and the intensity of the SA- β -gal signal was strong in all surviving cells (Figure 4).

Discussion

In the present study we report that DBTRG.05 human glioma cells that survived prolonged exposure (3-4 weeks) to high concentration of HU (10 mM) or aphidicolin (2.5 μM) when re-incubated in drug-free media were able to re-enter the cell cycle and resume

proliferation in a stochastic manner (Figure 1). The DBTRG.05 cell line have similar sensitivity to patient derived cell lines when treated with a menadione alone, vitamin C alone or a combination of menadione: vitamin C.¹⁸ HU and aphidicolin are commonly used to synchronize cells for cell cycle studies. HU is also used as anticancer drug. We show here that salinomycin slowly depleted surviving cells (Figure 3). Remarkably, about 10 times lower salinomycin concentration than that necessary to kill HU- or aphidicolin-untreated cells (Figure 2 and literature data)¹²⁻¹⁴ was able to prevent re-growth and slowly killed surviving cells (Figure 3). We chose salinomycin for a second phase because it was shown to target stem cells and it is widely accepted that surviving cells that are more resistant to chemotherapy may have stem cell properties.²⁰ The potential use of salinomycin for monotherapy as conventional anticancer agent may be limited by its toxicity to normal cells since relatively high concentration are needed to kill stem-like cells, e.g. i) in short term assays 1-5 μM salinomycin is required to induce apoptosis and inhibit prolif-

eration of cancer cells,¹² ii) in long term assays, a small fraction of human leukemia stem cell-like KG-1a cells are able survive exposure up to 10 μM salinomycin for 12 weeks.¹⁴ These data are in agreement with our results that showed that while the IC₅₀ for DBTRG.05MG human glioma cells was around 1 μM , exposure to concentration >5 μM for at least one week was required to prevent re-growth (Figure 2). However, the higher sensitivity of HU- or aphidicolin-surviving cells to salinomycin compared to untreated cells open the possibility to use this drug for cancer treatment in a two-stage (or 2 Phases Treatment, 2PT) treatment regime. Similar as shown in Figure 3, the first phase, using conventional anticancer drugs (or cocktails) would aimed at killing most cancer cells and maybe, if only sub lethal concentration would be reaching inside the tumour, to induce senescence in the fraction of surviving cells. The second phase using salinomycin would prevent re-enter of surviving cells into the cell cycle and/or kill them all. In mice, up to 72.5 \pm 3.6 ng/mL (96.5 μM) salinomycin can be reached in plasma after an iv

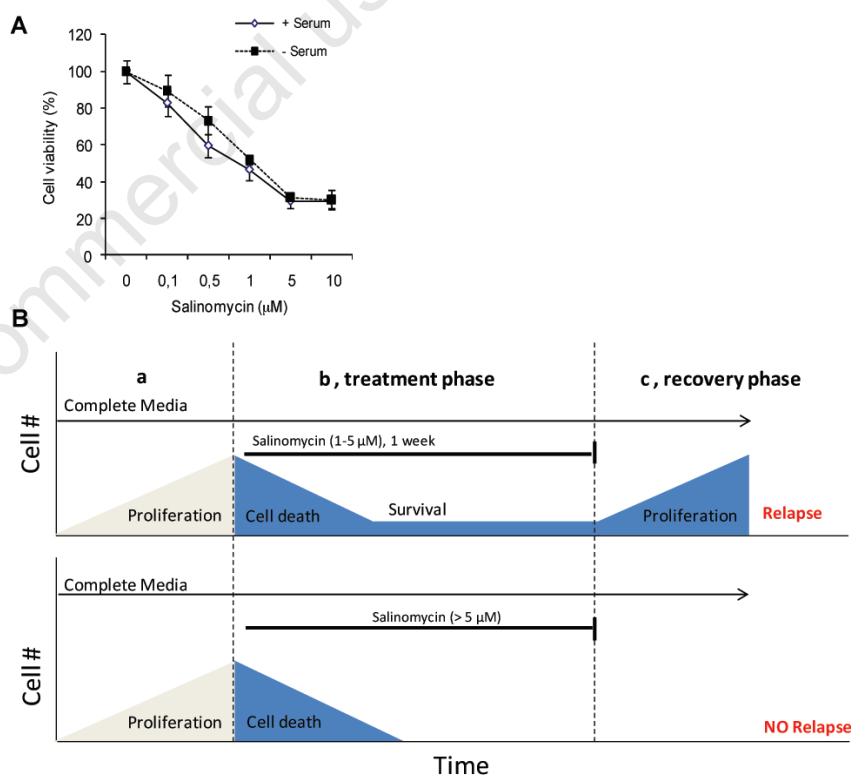


Figure 2. Effect of short (72h) and prolonged (1 week) treatment of salinomycin on DBTRG.05MG glioma cells. (A) DBTRG.05MG glioma cells were incubated with the indicated concentration of drugs for 72 h. Cell proliferation was measured by the CCK kit. Results are representative of two independent experiments performed by quadruplicates. (B) (top panel) Exponentially growing cells were incubated in complete media for 2-3 days (a), exposed to 1-5 μM salinomycin for 1 week (b). During this period the media and the drugs were changed twice a week. A clear decrease in the cell density was observed by microscopic examination indicating extensive cell death followed by a stable low cell density (b). When the drug was removed, the surviving cells resumed proliferation and formed a monolayer indicated as re-growth (c). (B) (bottom panel) same as top but cells were incubated with salinomycin at > 5 μM for 1 week.

administration of 1 mg/kg²¹ without signs of acute toxicity. Considering a brain/plasma ratio of 0.13±0.01, concentration of up to 12.5 µM can be reached in mice brain.²¹ Humans might be more sensitive to salinomycin since this dose (by ingestion) was reported to produce severe toxicity.¹⁶ The very low concentration (0.5 µM) required to deplete surviving cells (Figure 3) suggests that salinomycin might be useful since, at least in mice, the concentration required to deplete surviving cells compared to the concentration that can be reached in brain and plasma are between 25-50 and 193-386 times lower (12.5/0.5-0.25 and 96.5/0.25-0.5) respectively. Our finding that salinomycin, at concentration lower than the RC₀ depleted surviving cells, encourage further studies to evaluate its potential use as a second phase agent for cancer treatment and guarantee further studies to expand our *in vitro* results and evaluate its potential toxicity to normal cells. The latter should be done in animal models rather than in normal cell lines (glial cells) to determine the toxicity to other types of neural cells. At the cellular level, tumour cells must remain viable for long time but without undergoing cell division which is a characteristic of senescent cells, in contrast to the temporarily arrested cells that re-enter the cell cycle within few days. Indeed, the fraction of HU surviving cells, showed three traits of senescence: i) arrested cell division; ii) expression of the SA-β-gal marker and iii) adoption of flat morphology by some cells (Figure 4). Some of these surviving cells regrew when incubated in drug-free media. Thus, it is likely that surviving cells escape drug-induced cell death activating senescence (drug-induced senescence) and these cells escape the senescent state in a stochastic way when they are re-incubated in drug free media. Escape of DIS has recently been reported in lung cancer stem cells.²² However, due to the fact that, in order to induce senescence, the authors treated the cells for only 120 h (5 days) but (as they showed) at least 7 days were required to induce senescence in 100% of the cells, it is possible that a small percentage of non-senescent cells were temporarily arrested. After 5 days of treatment, the authors detected that more than 95 % (but not 100%) of cells were positive for the SA-β-gal marker. Evasion from senescence is relatively common in cancer cells and cancer stem cells.²³ Two possibilities can explain their results: i) the small fraction of non-senescent cells (temporarily arrested) might simply re-enter the cells cycle when incubated in drug-free media or ii) the small fraction of SA-β-gal negative might have evaded senescence. In both scenarios it is difficult to conclude that cells escaped from the senescence state because those cells were never senescent.

Another study that reported escape from DIS

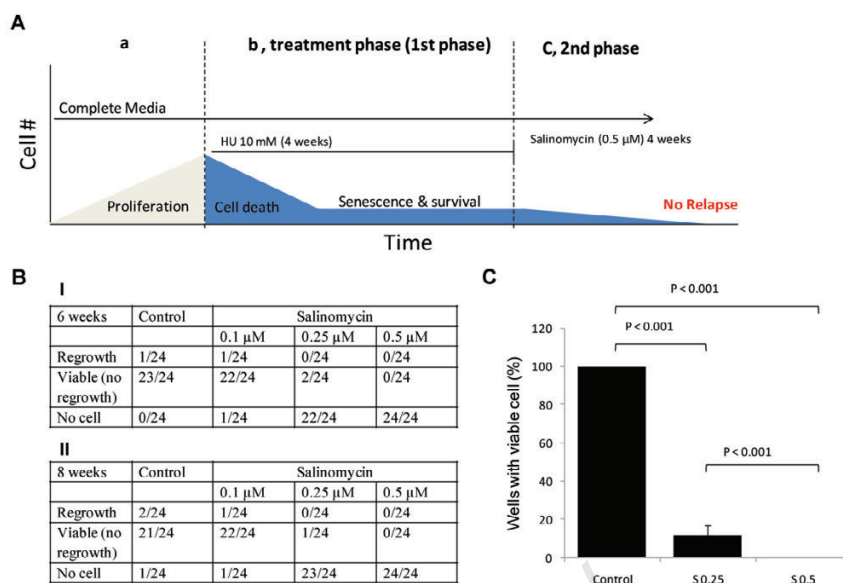


Figure 3. Salinomycin prevents re-growth of surviving cells. DBTRG.05MG cells were grown to semi-confluency in 96 well plates and treated with HU (10 mM) for 4 weeks. Media and drugs were changed twice a week. (A) Schematic representation of events in a single well: After a period of proliferation in drug-free media (a), exposure to 10 mM HU killed most of the cells but a fraction of cells survived (b) treatment phase (1st phase). Post-treatment with 0.5 µM salinomycin slowly depleted surviving cells within the next 4 weeks (c), 2nd phase). (B) I: Summary of the effect of 6 weeks exposure to salinomycin (0.1, 0.25 or 0.5 µM) on re-growth of HU-treated cells in a typical 96 well plate. Wells were evaluated for the presence of re-growth, viable cell or no cell. II. Same experiment but evaluated after 8 weeks of treatment. (C) Statistical analysis of the effect of 0.25 and 0.5 µM salinomycin (S 0.25 and S 0.5, respectively) on the number of wells containing viable cells. See text for details.

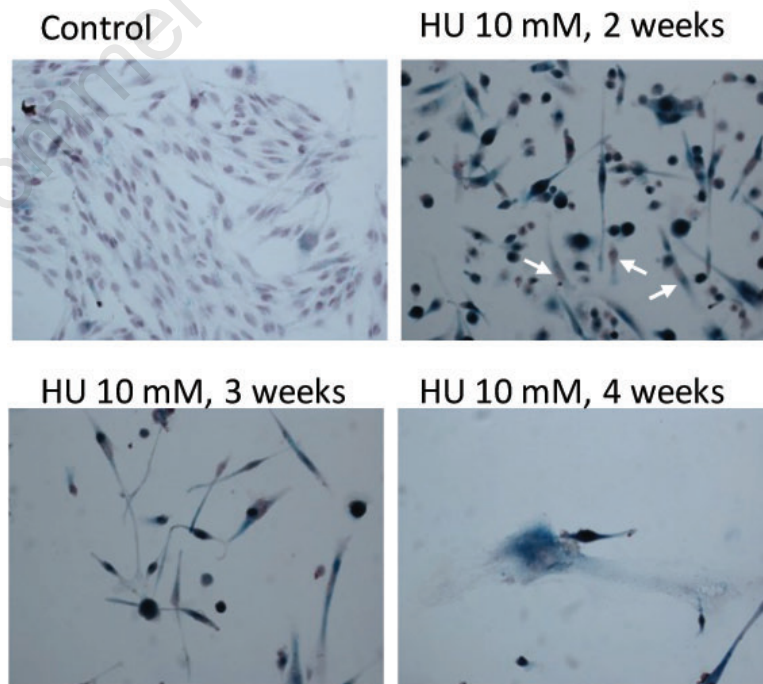


Figure 4. Prolonged HU exposure induces senescence in the fraction of surviving cells. DBTRG.05MG human glioma cells were treated for 2-4 weeks with HU (10 mM) and stained for the senescence associated SA-β-galactosidase marker (SA-β-gal). The fraction of surviving cells becomes senescent since they stop dividing, and express SA-β-gal. Some cells also adopt a flat shaped structure typical of senescent cells. For control, cells were grown for three days in drug-free media (plus vehicle) and processed simultaneously with experimental samples.

also used short term exposure to drugs (3-4 days) in order to induce senescence²⁴ making it difficult to discriminate between a true escape from senescence state and evasion or temporary arrest. To circumvent this limitation, in the present study we used prolonged incubation time with anticancer drugs (HU and aphidicolin for at least 3-4 weeks) as well as prolonged re-incubation time in drug free media to observe for re-growth. This strategy makes more likely that 100 % of the surviving cells are senescent since, in addition to stop dividing all surviving cells expressed the SA- β -gal marker (Figure 4). Thus, we provide evidence that drug-treated surviving cells can either remain in the senescent state for long time or re-enter the cell cycle (escape senescence) in a stochastic manner (Figure 1). We conclude that activation of the senescence program is important for surviving continuous exposure to high concentrations of HU or aphidicolin and that senescent cells may be more sensitive to salinomycin compared to non-senescent cells. It is important to mention that in our experiments, glioma cells were grown in routine media suggesting that escape from senescence might not be restricted to cancer stem cells²² but may also occur in any cancer cell.

However, we recently developed a model (Stemness Phenotype Model, SPM) that proposes that all glioma cells have stem cell potential depending on the microenvironment.²⁵ If the SPM is true, any glioma cell might be able to escape DIS and, sooner or later form a new tumour. In a clinical context, the stochastic re-entering into the cell cycle and re-growth of the tumour might explain why tumour relapse occurs at variable times after the primary cancer treatment. Therefore, depleting surviving (senescent?) cells or preventing them to re-enter the cell cycle will be necessary in order to prevent tumour relapse.

Although our study was limited to glioma cells, our findings i) open the possibility to develop a two phases therapeutic regime for other cancers that may deplete 100% of cancer cells preventing relapse and, ii) encourage further studies to explore the role of senescence in chemotherapy resistance in order to develop specific senescent cell targeting drugs.

References

- Stupp R, Tonn JC, Brada M, Pentheroudakis G and ESMO Guidelines Working Group. High-grade malignant glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010;21:v190-3.
- Walbert T, Gilbert MR, Groves MD, et al. Combination of 6-thioguanine, capecitabine, and celecoxib with temozolomide or lomustine for recurrent high-grade glioma. *J Neurooncol* 2011;102:273-80.
- Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol* 2009;10:459-66.
- Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987-96.
- Wick W, Platten M, Weller M. New (alternative) temozolomide regimens for the treatment of glioma. *Neuro Oncol* 2009;11:69-79.
- Robinson CG, Palomo JM, Rahmathulla G, et al. Effect of alternative temozolomide schedules on glioblastoma O(6)-methylguanine-DNA methyltransferase activity and survival. *Br J Cancer* 2010;103:498-504.
- Zhang YH, Yue ZJ, Zhang H, et al. Temozolomide/PLGA microparticles plus vatalanib inhibits tumour growth and angiogenesis in an orthotopic glioma model. *Eur J Pharm Biopharm* 2010;76:371-5.
- Hatiboglu MA, Wei J, Wu AS, Heimberger AB. Immune therapeutic targeting of glioma cancer stem cells. *Target Oncol* 2010;3:217-27.
- Yakisich JS. Pre-clinical anticancer drugs screening: perspectives from emerging models of glioma biology. *Drugs Ther Stud* 2011;1:e1.
- Avramidis D, Cruz M, Sidén Å, et al. Regrowth Concentration Zero (RC0) as complementary endpoint parameter to evaluate compound candidates during pre-clinical drug development for cancer treatment. *J Cancer Sci & Ther* 2009;1.
- Ropolo M, Daga A, Griffero F, et al. Comparative analysis of DNA repair in stem and nonstem glioma cell cultures. *Mol Cancer Res* 2009;7:383-92.
- Riccioni R, Dupuis ML, Bernabei M, et al. The cancer stem cell selective inhibitor salinomycin is a p-glycoprotein inhibitor. *Blood Cells Mol Dis* 2010;45:86-92.
- Gupta PB, Onder TT, Jiang G, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell* 2009;138:645-59.
- Fuchs D, Daniel V, Sadeghi M, et al. Salinomycin overcomes ABC transporter-mediated multidrug and apoptosis resistance in human leukemia stem cell-like KG-1a cells. *Biochem Biophys Res Commun* 2010;394:1098-104.
- Boehmerle W, Endres M. Salinomycin induces calpain and cytochrome c-mediated neuronal cell death. *Cell Death Dis* 2011;2:e168.
- Story P, Doube A. A case of human poisoning by salinomycin, an agricultural antibiotic. *N Z Med J* 2004;117:U799.
- Delwar ZM, Avramidis D, Follin E, et al. Cytotoxic effect of menadione and sodium orthovanadate in combination on human glioma cells. *Invest New Drugs* 2011 May 10. Epub ahead of print.
- Vita MF, Nagachar N, Avramidis D, et al. Pankiller effect of prolonged exposure to menadione on glioma cells: potentiation by vitamin C. *Invest New Drugs* 2010 Jul 13. Epub ahead of print.
- Dimri GP, Lee X, Basile G, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci USA* 1995;92:9363-7.
- Gupta PB, Chaffer CL, Weinberg RA. Cancer stem cells: mirage or reality? *Nat Med* 2009;15:1010-2.
- Lagas JS, Sparidans RW, van Waterschoot RA, et al. P-glycoprotein limits oral availability, brain penetration, and toxicity of an anionic drug, the antibiotic salinomycin. *Antimicrob Agents Chemother* 2008;52:1034-9.
- Sabisz M, Skladanowski A. Cancer stem cells and escape from drug-induced premature senescence in human lung tumour cells: implications for drug resistance and in vitro drug screening models. *Cell Cycle* 2009;8:3208-17.
- Karimi-Busheri F, Rasouli-Nia A, Mackey JR, Weinfeld M. Senescence evasion by MCF-7 human breast tumour-initiating cells. *Breast Cancer Res* 2010;12:R31.
- Roberson RS, Kussick SJ, Vallieres E, et al. Escape from therapy-induced accelerated cellular senescence in p53-null lung cancer cells and in human lung cancers. *Cancer Res* 2005;65:2795-803.
- Cruz M, Sidén Å, Tasat DR, Yakisich JS. Are all Glioma Cells Cancer Stem Cells? *J Canc Sci Ther* 2010;2:100-6.