Sulfuraphane potentiates gemcitabine mediated cytotoxicity towards pancreatic cancer stem-like cells

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Introduction: Despite intense efforts to develop high effective treatments against pancreatic cancer, agents that cure this disease are still not available. Cancer stem cells may be responsible for high resistance and early metastasis of pancreatic cancer. Considerable attention has focused on broccoli compound sulfuraphane, which is suggested for combination therapy targeting pancreatic cancer stem cells. Since increasing evidence suggests that chemotherapeutic treatment alone results in an enrichment of cancer stem cells, we investigated whether the natural compound sulfuraphane can increase drug-mediated cytotoxicity in the pancreatic cancer cell line MIA-PaCa2, which is highly enriched in cancer stem cell characteristics (CSCs).

Methods: The effect of single and combined treatment was examined by MTT-test, Annexin-V staining and colony formation assay. To investigate cytotoxicity towards CSCs we performed spheroid formation assays and evaluated ALDH activity and protein expression in CSCs.

Combined treatment with sulfuraphane and gemcitabine reduced tumor cell viability

A: CSCs MIA-PaCa2 pancreatic cancer cells were left untreated (CO), or were treated with sulfuraphane (SF) or gemcitabine (GEM) alone or in a combination (SF+GEM) for 72 h and representative pictures were shown. B: CSCs MIA-PaCa2 cells were seeded in 6-well tissue culture plates and treated as indicated. Seventy-two hours later apoptosis induction was evaluated by annexin-V staining and flow cytometry and is presented as percentage of annexin-V positive cells. Decreased cell viability and high levels of apoptosis induction after combined treatment admixtures increased cytotoxic effects.

Sulfuraphane and gemcitabine together strongly inhibited long term survival and tumor growth in vivo

A: Long term effects of combined treatment were assessed by colony forming assays. Following 72 h treatment with SF and GEM alone or in combination (SF+GEM), CSCs MIA-PaCa2 cells were reseeded and cultured in absence of treatment. The quantification of colonies revealed that SF alone and in combination with GEM strongly inhibited colony formation. B: CSCs MIA-PaCa2 cells were treated for 72 hours with SF, GEM and SF+GEM. Following treatment, cells were injected subcutaneously into nude mice. Tumor growth was measured and tumor take was evaluated. Treatment with GEM delayed the tumor growth whereas SF and combination therapy abrogated tumor growth.

Long term treatment reduced ALDH1 activity

A: Following long term (21 d) or short term treatment (72 h), ALDH1 activity was analyzed by flow cytometry and the percentage of ALDH1-positive cells is presented. B: After long term treatment (21 d), expression of ALDH1 protein was analyzed by Western blot. Expression of β-actin served as loading control. C: Following long term treatment (21 d) cells were subjected to immunofluorescence for ALDH1. The reduction of the stem cell marker ALDH1 suggest that combined treatment reduced the number of CSCs within the population.

Concentration: In this study we demonstrate that SF potentiates cytotoxicity of GEM towards pancreatic CSCs in vitro and in vivo. Moreover, combined treatment with SF and GEM targeted CSC characteristics such as clonogenic potential, self renewal and ALDH1 activity. CSC sensitizing mechanism of SF might be a down regulation of proteins which are involved in resistance and self renewal. SF has no side effects on normal cells and combination treatment with GEM might be a promising therapeutic strategy in patients.