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SULFORAPHANE ENHANCES EFFECTS OF SORAFENIB, QUERCETIN AND CHEMOTHERAPY TOWARDS PANCREATIC CANCER STEM-LIKE CELLS

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Background: Despite intense efforts to develop treatments against pancreatic cancer, agents that cure this highly resistant and metastasizing disease are not available. Considerable attention has focused on broccoli compound sulforaphane, which is suggested as combination therapy for targeting of pancreatic cancer stem cells. However, there are concerns that anti-oxidative agents such as sulforaphane may interfere with cytotoxic therapy – as suggested e.g. for vitamins. **Material and methods:** The effects of sulforaphane upon combination with various standard chemotherapeutics, the dietary agent quercetin and the multi kinase inhibitor sorafenib were evaluated using *in vitro* and *in vivo* models of pancreatic tumor cells with stem-like phenotype. CSC-marker expression, ALDH1 activity, self-renewal potential, Notch signaling, migratory activity, apoptosis induction, viability, proliferation, NF-κB-signaling, and angiogenesis were analyzed. **Results:** While each therapeutic agent alone diminished the stem-like characteristics, elimination of highly aggressive stem-like cells was not complete. However, combination with sulforaphane led to an additive effect of each single agent. This was due to inhibition of self-renewal activity and sensitization to apoptosis by inhibition of Notch, NF-κB, caspases, clonogenicity, spheroid-forming, migratory activity and downregulation of anti-apoptotic and EMT-related proteins. *In vivo,* combination treatment was most effective and totally abolished growth of cancer stem-like xenografts. No pronounced side effects were observed in mice. Our data suggest that sulforaphane increases the effectiveness of various cytotoxic drugs, sorafenib and quercetin against cancer stem cells without inducing additional toxicity in mice. **Conclusions:** Our data suggest the combination sulforaphane with conventional or novel cancer therapeutics is safe and a promising new concept for targeting of pancreatic cancer stem-like phenotype.

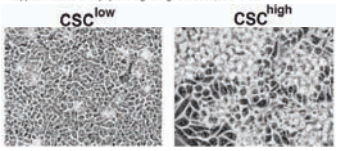
Characterization of CSC markers in established pancreatic cancer cell lines

Table CSC-characteristics of CSC^{low} MIA-PaCa2 and CSC^{high} BxPc-3 cells

	MIA-PaCa2	BxPc-3	Reference
ATCC No.	CRL-1420	CRL-1687	ATCC
Source	Primary tumor	Biopsy primary tumor	ATCC
Degree of tumor differentiation	Poor	Well	ATCC
Therapy resistance	High	Low	+
Colony-forming capacity	High	None	+
Spheroid-forming capacity	82%	None	Present study
Secondary spheroid formation	High	None	+
ALDH activity	Yes	No	+
Growth on nude mice	Yes, very fast	Yes, very slow	+
Serial transplantation *in vivo*	Yes, very fast	Yes, but not consistent	+
CD44$^+$/CD24$^-$	95.5 ± 3.7	17.5 ± 5.7	+
CD133	Yes, upon hypoxia	No	Data not shown
Differentiation potential	Yes	No	Present study
E-cadherin expression	Lost	High	Present study

Reference:

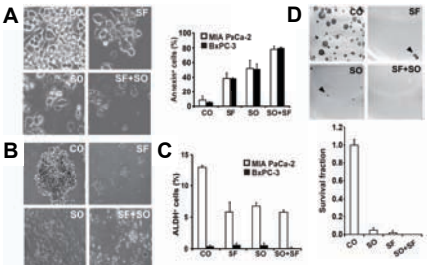
1 Kallifatidis G, Rausch V, Baumann B, Apel A, Beckermann BM, Groth A, Mattern J, Li Z, Kolb A, Moldenhauer G, Altevogt P, Wirth T, et al. Sulforaphane targets pancreatic tumour-initiating cells by NF-kappaB-induced antiapoptotic signalling. *Gut* 2009;58:949-63.



More spheroidal growing cells in CSC^{high} MIA-PaCa2

1

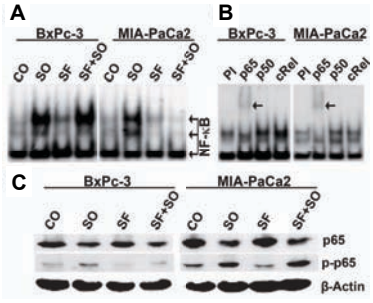
Sulforaphane (SF) enhances Sorafenib (SO)-induced inhibition of CSC self-renewal



We next investigated whether pre-treatment of cells with SF for 24 h followed by incubation with SO for additional 48 h resulted in a more pronounced induction of apoptosis as single SF or SO treatment by Annexin V-staining (A). SF pre-treatment has an additive effect to SO-induced apoptosis in pancreatic CSCs *in vitro and* leads to an almost complete eradication of features such as spheroid formation, clonogenicity and ALDH activity (B, C, D).

2

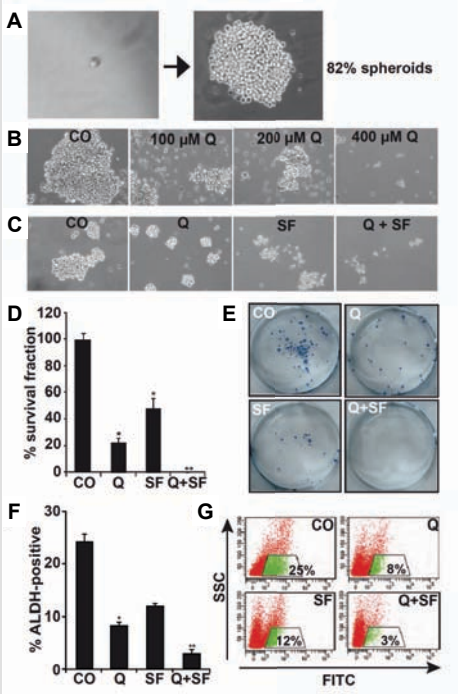
SF abolishes SO-induced NF-κB activity and EMT induction in CSCs



Enhanced NF-κB activity is involved in apoptosis resistance of CSCs as assayed by gel shift assays and Western blot analysis. Unexpectedly, we found strong upregulation of NF-κB binding by SO treatment in both, CSC^{low} and CSC^{high} cells, whereas SF mediated a marked reduction of SO-induced NF-κB binding in CSC^{low} cells and totally abolished it in CSC^{high} cells.

3

Quercetin (Q) effects on pancreatic CSCs are enhanced by SF



(A) 82% of CSC^{high} cells form spheroids (**B**) Q alone abolishes spheroids in a dose-dependent manner (**C**) SF (10 µM) co-treatment enhances spheroid-elimination by Q (200 µM). SF and Q together are strongest in elimininationn of (**D, E**) colony formation and (**F, G**) **ALDH1** activity.

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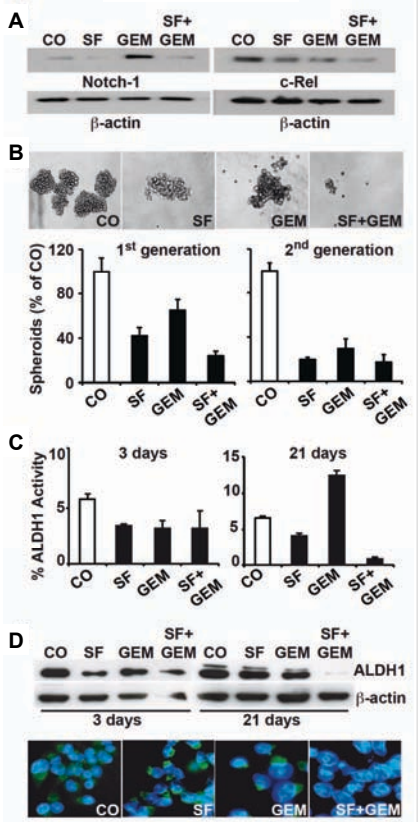
Gemcitabine effects on pancreatic CSCs are enhanced by SF



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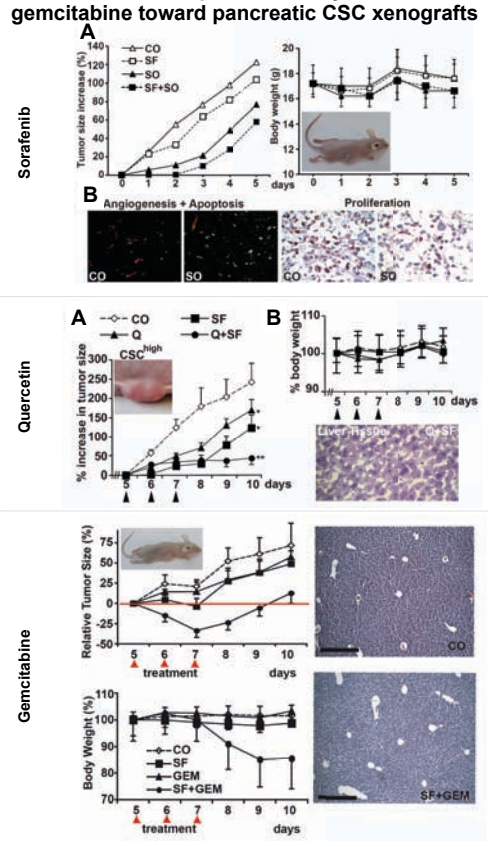
SF enhances therapeutic efficacy of SO, Q and gemcitabine toward pancreatic CSC xenografts



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