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Sulforaphane eradicates pancreatic cancer stem cells by NF-κB

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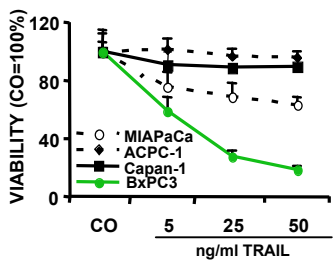
Introduction

Emerging evidence suggests that cancer stem cells (CSCs) play a central role in the pathogenesis of cancer. We identified CSCs in pancreatic cancer cell lines and patient tumors by expression of CSC markers and correlation to growth on nude mice, differentiation capacity, clonogenicity, sphere formation and therapy resistance. The chemopreventive agent sulforaphane prevented NF–κB binding in CSCs, downregulated apoptosis inhibitors and induced apoptosis along with prevention of clonogenicity.

Results

1

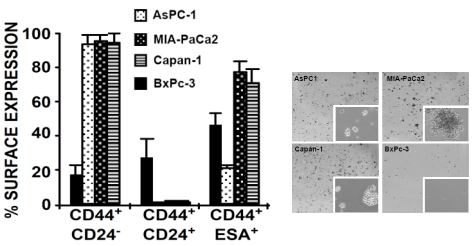
TRAIL-sensitivity in pancreatic tumor cell lines



Cells were left untreated (CO) or were treated with recombinant TRAIL (5, 25, 50 ng/mL) and 24 h later mitochondrial activity/viability was measured by MTT assay. Only BxPC3 cells were sensitive to TRAIL-induced apoptosis.

2

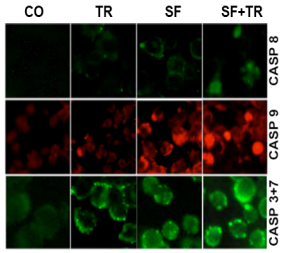
Expression of CSC surface markers in pancreatic cell lines



CSC-like characteristics in four human pancreatic cancer cell lines were classified by expression of specific surface markers, colony and spheroid forming capacity. We defined the CD44⁺/CD24⁻ population as CSC-like phenotype in pancreatic cancer cell lines.

3

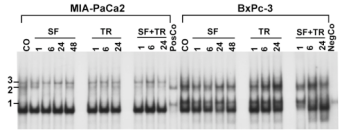
Sensitivity of CSCs to sulforaphane



MIA-PaCa2 cells were left untreated (CO), or were treated with Sulforaphane (SF). Twenty-four hours later, TRAIL was added to untreated (TR) or pre-treated cells (SF+TR) and activity of caspase 8, 9 and 3+7 was analyzed by fluorochrome-linked inhibitors of caspases (FLICA).

4

SF inhibits binding of transactivation competent NF-κB dimers in CSC^{high} cells



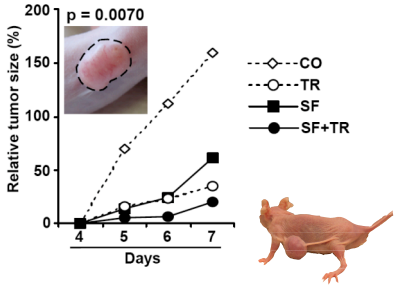
NFκB Complex

CSC^{high}	Complex 3	cREL cREL
CSC^{low}	Complex 1	p50 p50

Pancreatic cancer cell lines were left untreated (CO) or were treated with sulforaphane (SF), TRAIL (TR) or SF+TR for time points indicated. NF-κB binding was analyzed by EMSA. Three different bands became visible corresponding to three different NF-κB subunits binding to the oligonucleotide (1, 2, 3).

5

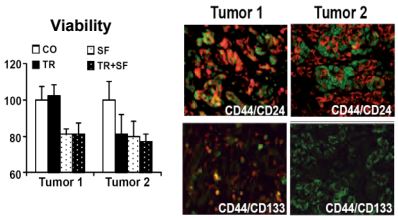
In vivo effect of Sulforaphane



Mice s.c. xenografted with MIA-PaCa2 cells received SF, TRAIL or both agents together at days 4 to 6 after tumor cell implantation. The tumor volumes were measured daily.

6

CD44⁺/CD24⁺ and CD44⁺/CD133⁺ CSCs correlate to *ex vivo* sensitivity of patient tumors



Isolated primary tumor cells were treated with SF ± TRAIL. Cells from tumor 2 were sensitive towards SF and TRAIL, while cells from tumor 1 were only sensitive towards SF. The high grade of resistance of tumor 1 corresponded to the presence of rare populations of CD44⁺/CD133⁺ and CD44⁺/CD24⁺ cells in the tissue sections.

Conclusions

CSCs are present in pancreatic tumors and are resistant towards chemotherapy. We observed specific binding of transactivation potent c-Rel containing NF-κB complexes in CSCs but not in non-CSCs. Sulforaphane prevented NF-κB binding along with strong induction of apoptosis. In a xenograft model, sulforaphane strongly blocked tumor growth and combination with TRAIL had an additive effect without obvious cytotoxicity to normal cells. Our data suggest combination of sulforaphane with TRAIL as promising strategy for targeting of pancreatic CSCs.

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