

Marine sponge *Crambe crambe* for efficient elimination of pancreatic cancer stem cells

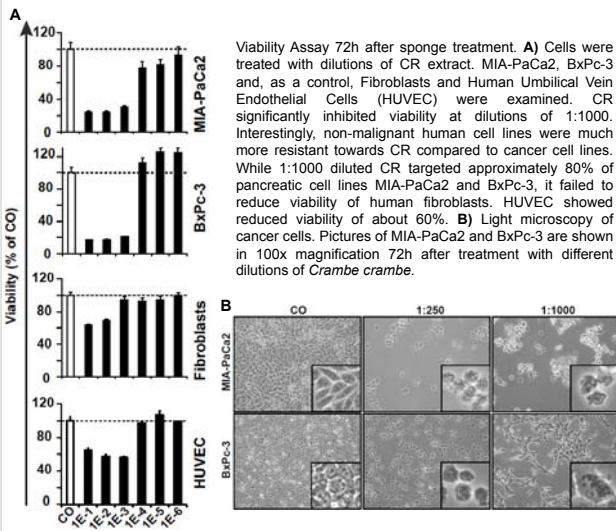
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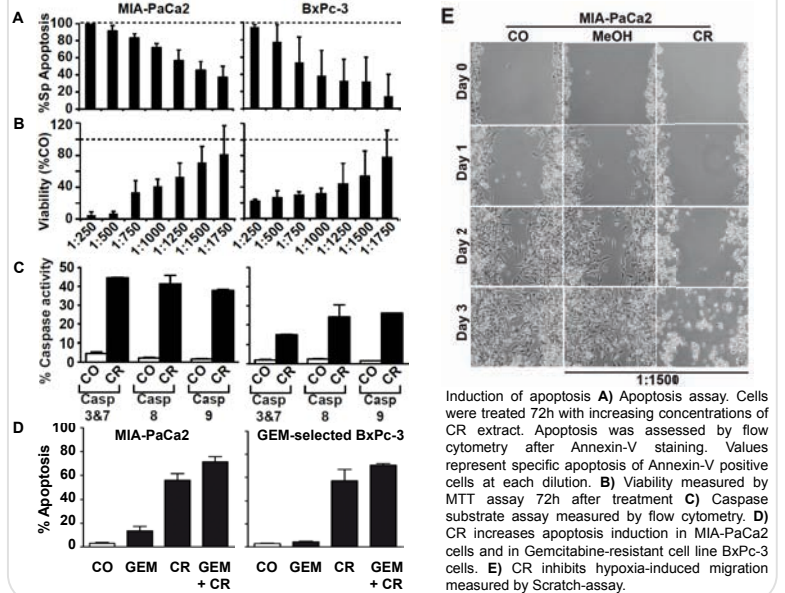
Marine organisms are known to produce a variety of secondary metabolites, from which the most are non characterized so far. Some of the characterized sponge metabolites exhibit bioactivity toward cancer cells and are therefore suggested for development of new anticancer therapeutics. We screened methanolic extracts from ten different marine sponges and one freshwater sponge for anticarcinogenic activity. Marine sponge *Crambe crambe* (CR) showed highest activity towards pancreatic cell lines MIA-PaCa2 and BxPc-3, with high (CSC^{high}) or low (CSC^{low}) content in cancer stem cell (CSC) markers, respectively. In contrast, nonmalignant cells like fibroblasts or HUVEC were not affected. CR induced efficiently apoptosis as measured by Annexin-V staining and detection of active Caspase 3, 7, 8 & 9. We could also show that CR treatment overcomes intrinsic and acquired resistance towards chemotherapy in CSC-enriched highly resistant pancreatic tumor cell lines. CR extract efficiently and specifically inhibits proliferation and induces cell cycle arrest. A long term effect on CSCs was observed in colony formation and spheroid assays. The side population defined for its CSC characteristics and multi-drug-resistance was reduced by CR treatment. In xenograft studies CR avoided growth *in vivo* indicating that CSC are eliminated.



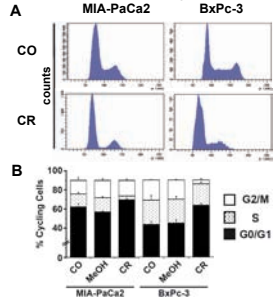
CR reduces viability of pancreatic CSCs



CR overcomes apoptosis resistance of pancreatic CSCs

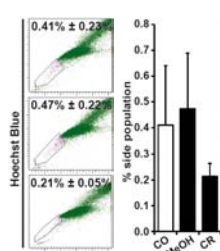


CR leads to cell cycle arrest



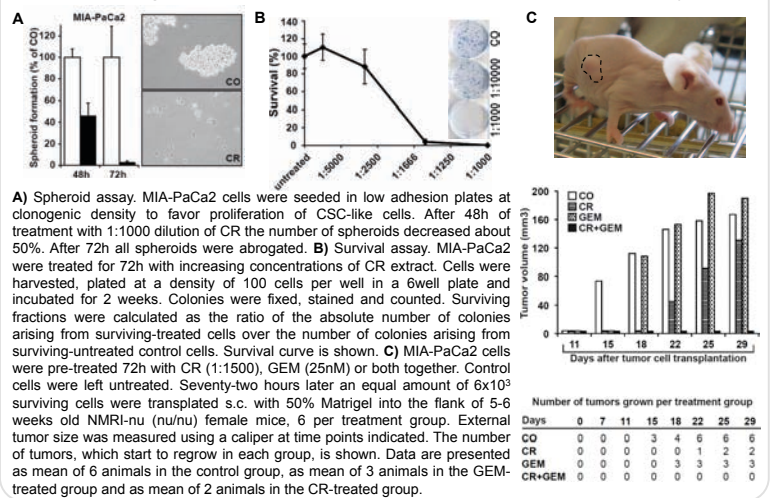
Cells were treated with a 1:1000 dilution of CR, methanol or were left untreated for 24h. Cell cycle analysis was performed after PI staining by flow cytometry. **A)** Representative graphs are shown. **B)** CR caused a significant G0/G1 arrest in MIA-PaCa2 and in BxPc-3 cell line. CR leads to a nearly complete breakdown of G2/M phase in BxPc3 cell line. Methanol treated cells did not show any effect on cell cycle compared to untreated cells.

Decrease of hoechst SP by CR



The side population (SP) phenotype is defined by ABC-transporter activity, which are known to efflux chemotherapeutics and are associated with multi drug resistance (MDR). The SP also shows CSC characteristics. MIA-PaCa2 was stained with hoechst 33342 dye in presence or absence of Verapamil. The SP was gated and is shown as percentage of viable cell population. After 24h treatment with CR, SP was reduced significantly. Treatment with vehicle control MeOH has no significant change to SP.

CR targets CSC characteristics and re-sensitizes to chemotherapy



Conclusion

In the present work we screened crude methanolic extracts from 11 different sponges and identified the species *Crambe crambe* as most effective. CR extract efficiently and specifically inhibits proliferation, cell cycle arrest and clonogenicity and induces apoptosis in pancreatic cancer cell lines with low or high content of CSC markers. In contrast normal cells were less affected. Furthermore, for the first time we demonstrate, that extracts from marine sponges potentially target stem cell characteristics in cancer stem cell-like cells, including self-renewal potential and hoechst-efflux population. CR also re-sensitizes Gemcitabine-selected highly resistant cancer cells to chemotherapy. Our study suggests *Crambe crambe* as a promising source for isolation of new therapeutics for elimination of pancreatic tumors enriched in CSCs.