Sulforaphane Renders Androgen-independent Stem-like Prostate Cancer Cells Sensitive To TRAIL

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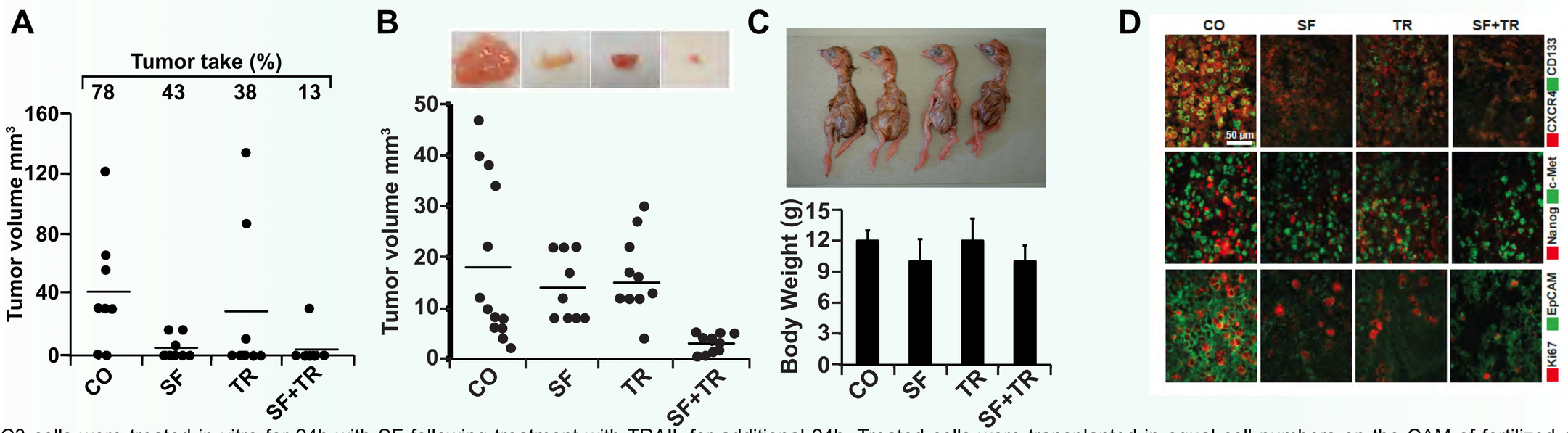
Introduction

Advanced androgen-independent prostate cancer (AIPC) is an aggressive malignancy with poor prognosis and no treatment options. The reason could be that AIPC cells have been identified as therapy resistant cancer stem cells (CSCs). The tumor necrosis factor (TNF)-related apoptosis ligand (TRAIL) is a new promising cancer-specific therapeutic option. There are indications that resistance to TRAIL may be due to CSCs. Recently, the dietary broccoli-derived agent sulforaphane (SF) showed properties against CSC features. Therefore we evaluate the efficiency of TRAIL alone or in combination with SF on CSCs in AIPC cells. The effect of single and combined treatment on CSCs was examined by colony formation assay, sphere assay, adipogenic differentiation assay, protein analysis via western blot and flow cytometry and transplantation models on fertilized chicken eggs.

In vivo

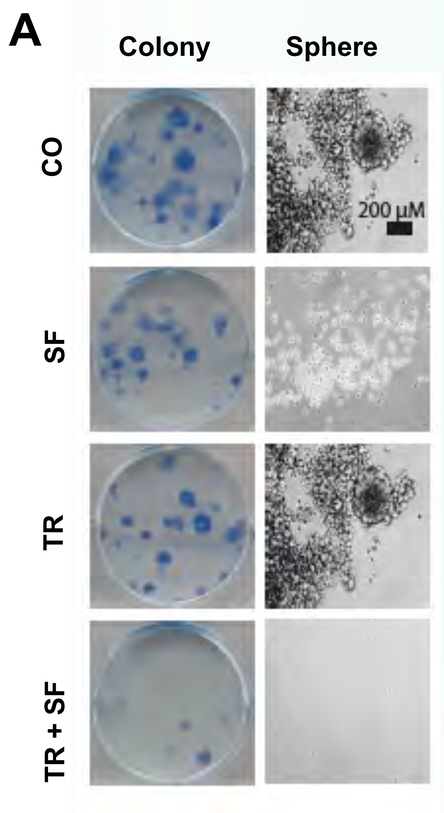
Co-administration with SF and TRAIL inhibits tumorigenicity and tumor progression in chicken embryos

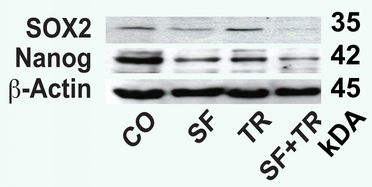


A: PC3 cells were treated in vitro for 24h with SF following treatment with TRAIL for additional 24h. Treated cells were transplanted in equal cell numbers on the CAM of fertilized chicken eggs. Ten days later tumor take and tumor volume were evaluated. The volumes of single tumors are presented as dots and the average volume of each treatment group as line.
B: Untreated PC3 cells were transplanted on the CAM of fertilized chicken eggs. Following engraftment the xenografts were left untreated or treated with SF, TRAIL or in combination. The diagramm shows single tumor sizes as dots and the average size of treatement groups as lines**. C:** Pictures of removed chicken embryos and average body size of each treatment group are presented. No effect on body weight could be observed. **D:** Pictures show stainings of removed chicken xenografts with relevant CSC markers. Results indicate that CSC marker expression strongly repressed by SF and TRAIL together.

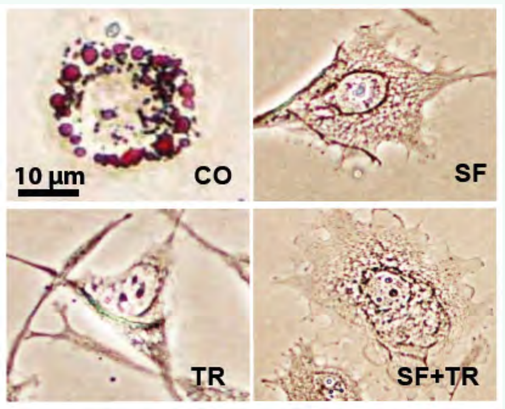
In vitro

Co-administration with SF and TRAIL strongly inhibited self renewal and differentiation capacity and CSC marker expression

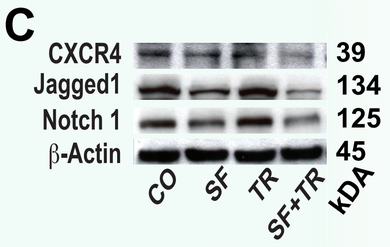


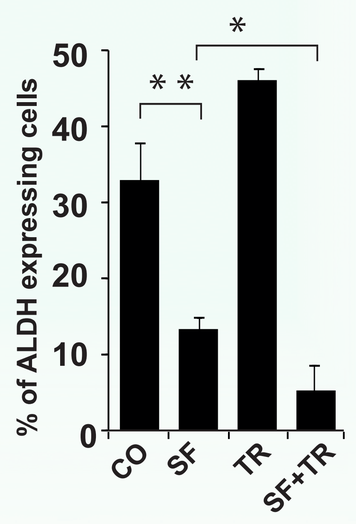


A: To analyze self renewal capacity untreated, SF, TRAIL or SF+ TRAIL treated cells were seeded at clonal density in normal medium for colony formation (left panel) or neural stem cell medium for sphere formation (middle panel). Expression of proteins important for self renewal was evaluated via western blot (right panel). Results indicate that SF and TRAIL together inhibited self renewal potential in CSCs.



B: Adipogenic differentiation assay was performed to investigate whether combined treatment targets pluripotent cells. Following treatment medium was exchanged to AdipoDiff medium to induce differentiation. After 14 days cells were stained with Oil Red O to detect fat droplets. Representative pictures demonstrate that all treatment groups could reduce adipogenic differentiation potential but with the stongest effect following SF and TRAIL co-administration.





C: Expression of CSC marker expression was evaluated. Following treatment and protein extraction, CXCR4, Jagged1 and Notch 1 expression was measured via western blot (upper panel). Following treatment the amount of cells with high ALDH 1 activity was analyzed by flow cytometry (lower panel).
Results suggest that combination of SF and TRAIL effectively blocks the expression of proteins important for tumor resistance and progression.

Conclusion

In this study we demonstrate that the pre-administration of SF strenghtes the effect of TRAIL against prostatic CSCs *in vitro* and *in vivo*. Combined treatment with SF and TRAIL inhibited CSC characteristics such as tumorigenicity, self-renewal, differentiation and expression of proteins important for resistance and metastasis. Therefore a diet enriched in SF or co-administration of SF might be promising therapeutic strategies to overcome TRAIL resistance in patients with AIPC

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