Immunoregulatory effects of sulforaphane in pancreatic cancer

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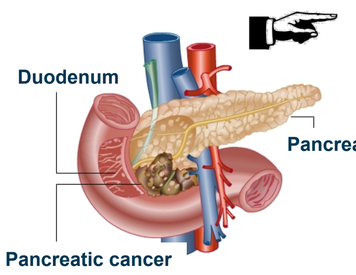
Introduction: Due to the advanced stage at the time of diagnosis and the quickly established resistance, the use of conventional therapies in pancreatic ductal adenocarcinoma (PDA) is limited. The isothiocyanate sulforaphane is considered a promising treatment option, as suggested by laboratory and animal studies as well as epidemiological research. However, its immunomodulatory properties in the human system are poorly investigated. PDA is characterized by chronic inflammation, tumor immune response and strong immunosuppression. Thus, we aimed to examine the effect of sulforaphane on parts of this assembly.

Pancreatic adenocarcinoma (PDA)



- Aggressive cancer
- Late diagnosis
- Early dissemination, extensive local invasion
- Cancer stem cells
- Resistant against chemo-radiotherapy
- Triggers strong immunosuppression on different levels
- Chronic inflammation

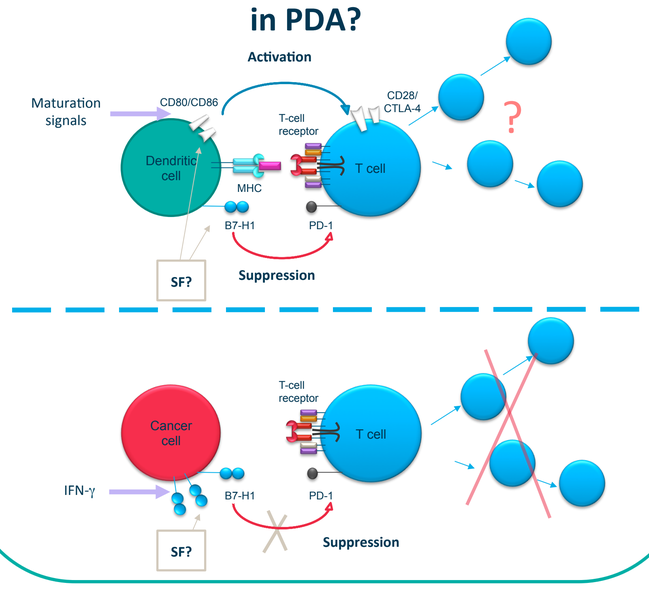
New therapeutic approaches?

Sulforaphane (SF)

- Isothiocyanate from cruciferous vegetables
- Lowers human cancer risk. Epidemiological evidences In lung, colorectal, breast, prostate, bladder and pancreatic cancer
- Promising prospective clinical studies
- Elimination of cancer stem cell features, inhibition of proliferation, metastasis and NF-κB signaling, sensitization to chemotherapy
- Anti-inflammatory properties in several models

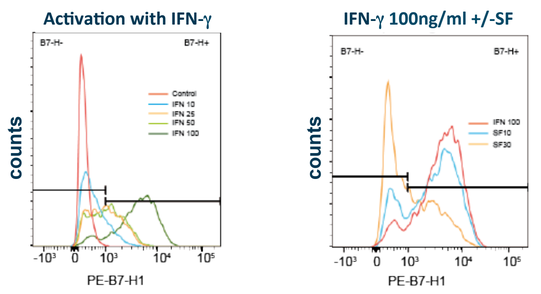
Immunoregulation in pancreatic cancer?

Immunoregulatory properties of SF in PDA?



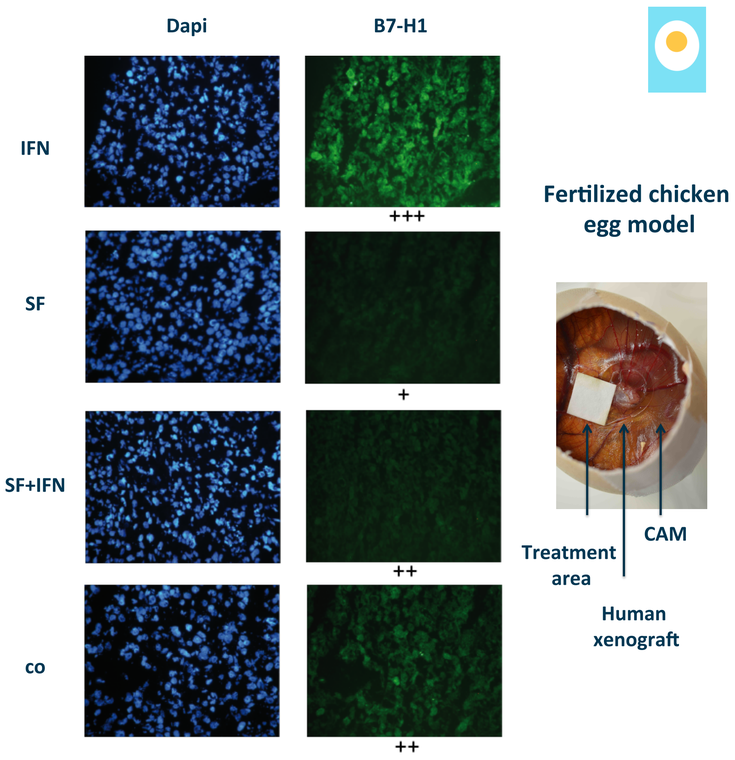
SF reduces the IFN-γ induced expression of B7-H1 in PDA tumor cells

in vitro



Human PDA cells (ASAN-PaCa) were treated with IFN-γ (IFN, ng/ml) and sulforaphane (SF, μM) for 24 hours. The expression of B7-H1 molecule was investigated by flow cytometry. Representative pictures from 3 independent experiments are shown.

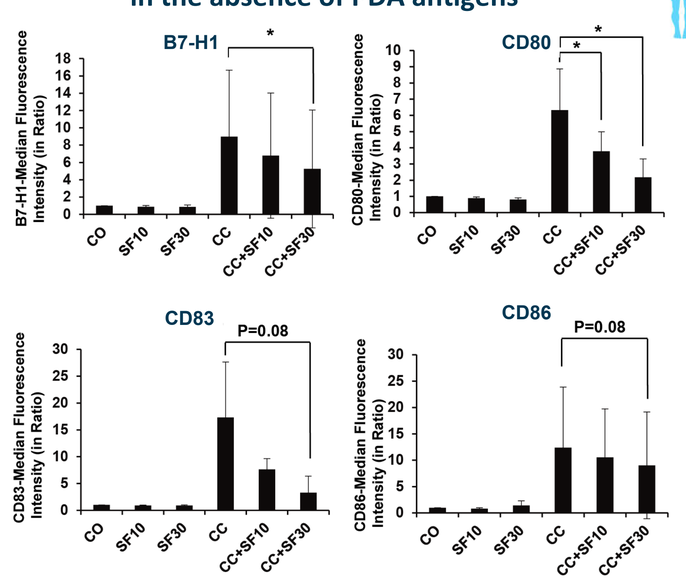
in ovo



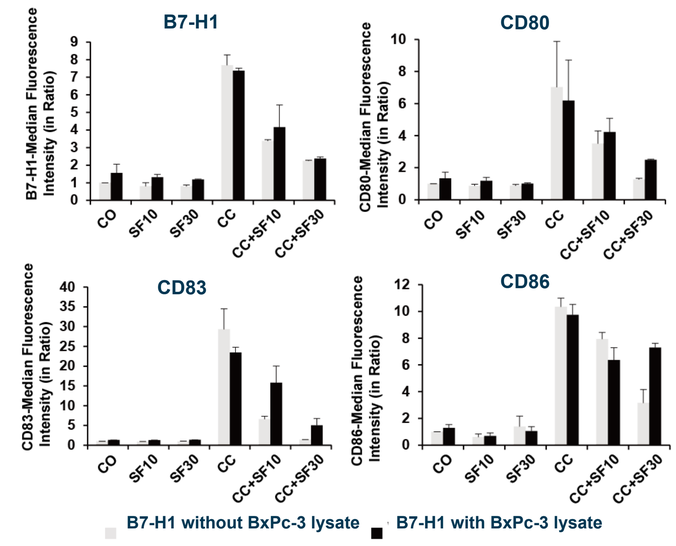
Human PDA cells (ASAN-PaCa) were transplanted into the CAM of fertilized chicken eggs at developmental day 9 and treated with IFN-γ (IFN), sulforaphane (SF) or vehicle control (co). The expression of B7-H1 molecule in human xenografts was investigated by IHC. Representative pictures from 3 independent experiments are shown.

SF modulates the expression of regulatory molecules on human MoDC

in the absence of PDA antigens

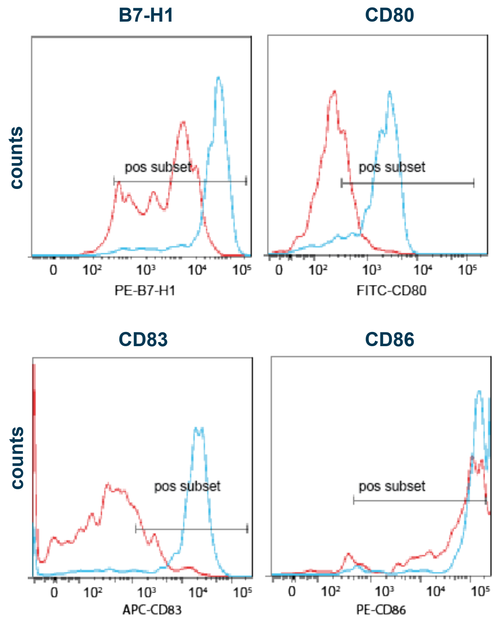


in the presence of PDA antigens



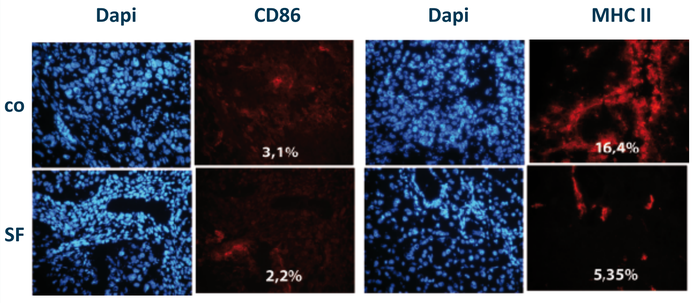
Human monocyte derived dendritic cells were treated with classical maturation cytokine cocktail (CC), sulforaphane (SF, μM) and vehicle control (co). Where indicated, DC were additionally pulsed with PDA tumor lysates (BxPc-3). The expression of different regulatory molecules was investigated by flow cytometry.

SF modulates the expression of regulatory molecules on human mDC



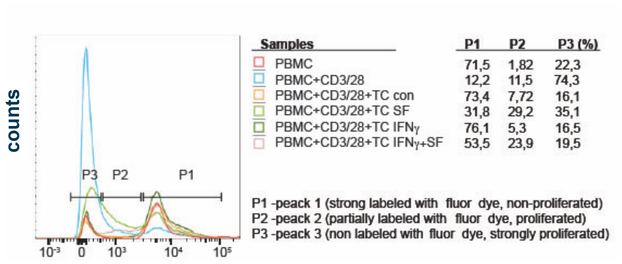
Human myeloid blood DC (mDC) were isolated from blood of healthy donors and treated with classical maturation cytokine cocktail (CC) and with sulforaphane (CC+SF) for 24 hours. The expression of regulatory molecules was investigated by flow cytometry. Representative pictures from 3 independent experiments are shown.

SF modulates the expression of regulatory molecules on mouse myeloid cells in human PDA xenograft



Mice were transplanted with human PDA cells (BxPc-3) and treated with sulforaphane (SF) or vehicle control (co). The expression of regulatory molecules on mouse immune myeloid cells in human tumor xenografts was investigated by IHC. Representative pictures are shown.

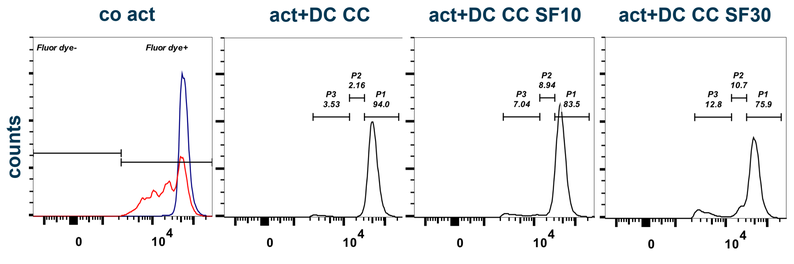
SF treated PDA cells are less suppressive for T cell proliferation



Samples	P1	P2	P3 (%)
PBMC	71,5	1,82	22,3
PBMC+CD3/28	12,2	11,5	74,3
PBMC+CD3/28+TC con	73,4	7,72	16,1
PBMC+CD3/28+TC SF	31,8	29,2	35,1
PBMC+CD3/28+TC IFNγ	76,1	5,3	16,5
PBMC+CD3/28+TC IFNγ+SF	53,5	23,9	19,5

PDA tumor cells (TC, ASAN-PaCa) were treated with vehicle control (con), IFN-γ and SF for 24 hours and co-cultured with activated (CD3/28) human immune cells, pre-labeled with proliferation dye Fluor 680. Proliferation of T cells was investigated by flow cytometry. Representative pictures are shown.

SF treated PDA-antigen-pulsed DC have different activating ability



Human monocyte derived dendritic cells (DC) were pulsed with PDA tumor lysates (BxPc-3) and treated with classical maturation cytokine cocktail (CC) and sulforaphane (SF, μM). Afterwards they were co-cultured with activated (co act, anti-CD3/28) human immune cells, pre-labeled with proliferation dye Fluor 680. Proliferation of T cells was investigated by flow cytometry. Representative pictures are shown.

Conclusions:

Sulforaphane favorably reduces the expression of B7-H1 molecules in PDA cells *in vitro* and *in ovo* and decreases their immune suppressive potential.

Sulforaphane modulates the expression of regulatory molecules on human DC in the presence and absence of PDA tumor antigens and affects the activation ability of PDA-antigen-pulsed DC.

Immunomodulatory functions of sulforaphane are of clinical relevance in PDA therapy and should be investigated in further depth.

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