

Curcuminoids and omega-3 lipidic mediators with NK cells are cytotoxic against pancreatic adenocarcinoma



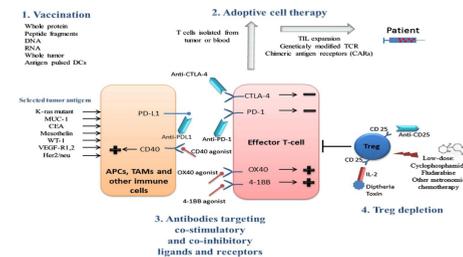
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Introduction:

Proinflammatory mechanisms in cancer

1. **Chronic inflammation** is a precursor of cancer.
2. Cancer is **immunosuppressive** to NK cells and CD8 T cells through regulatory T cells (**Tregs**), myeloid derived suppressor cells (**MDSCs**), and tumor associated macrophages (**TAMs**) via **TNF-alpha**, **TGF-beta** and **IL-6** induce **cytokines**, **chemokines**, **adhesion molecules** and **metalloproteinases** promoting cancer spread;
3. **Proinflammatory eicosanoids**: cyclooxygenases (**COX-1**, **COX-2**) leading to prostaglandins: **PGE₂** pro-cancer; **PGD₂** pro- and anti-cancer. Therefore, therapy of familial adenomatous polyposis with **celecoxib**, and colon cancer chemoprevention with **aspirin**

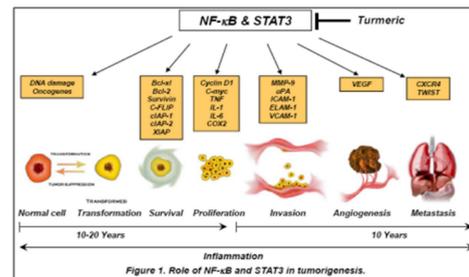
Diagrammatic representation of immune treatment strategies under investigation in pancreatic cancer (from Sideras et al)



Turmeric and curcuminoids

- 1) Curcumin is derived from turmeric (*Curcuma longa*) and is a natural polyphenol.
- 2) Turmeric is safe as it has been used as a food, coloring agent, and traditional medicine for centuries. Curcuminoids can be administered safely at doses of up to 12 g/day.
- 3) Curcuminoids are the most active turmeric components against cancer cells.
- 4) Curcuminoids inhibit cell proliferation through suppression of NFκB, Akt and anti-apoptotic gene activation (Aggarwal, Ichikawa et al. 2006).
- 5) Curcuminoids were tested in patients with gemcitabine-unresponsive pancreatic cancer (PC) with improved survival time after initiation of curcumin continuation therapy compared to patients without curcumin (Kanai, Yoshimura et al. 2011).

Turmeric inhibits tumorigenesis through inhibition of multiple processes and pathways



Approach

Anti-inflammatory strategies against pancreatic cancer in the labs of M.Fiala and A. Jewett

- 1) Omega-3 (DHA and EPA) are precursors of anti-inflammatory and anti-cancer resolvins: resolvins D1 and D2, and resolvins E1 and E2;
- 2) Curcuminoids inhibit inflammation and cell proliferation through suppression of NFκB, Akt and anti-apoptotic gene activation (Aggarwal, Ichikawa et al. 2006);
- 3) NK cells are deactivated by tumor cells;
- 4) Curcuminoids in Smartfish potentiate cytotoxic activity of NK cells
- 5) Hypothesis: Antioxidants in Smartfish may protect resolvins

Methods

1) Cells and Reagents

MP2 and L36 immortalized pancreatic adenocarcinoma cells
 Natural killer (NK) (IL-2 or IL-2/CD16 treated) cells
 omega-3 emulsion (DHA and EPA 10 µg/ml) (Smartfish)
 Turmeric curcuminoids (1–10 µg/ml).

2) Procedures

Overnight co-culture

Experimental treatments:

Curcuminoids, omega-3 fatty acids (DHA and EPA), and RvD1 (the lipidic mediator from omega-3), and methanol solvent (for Curc)

Experimental design of apoptosis testing:

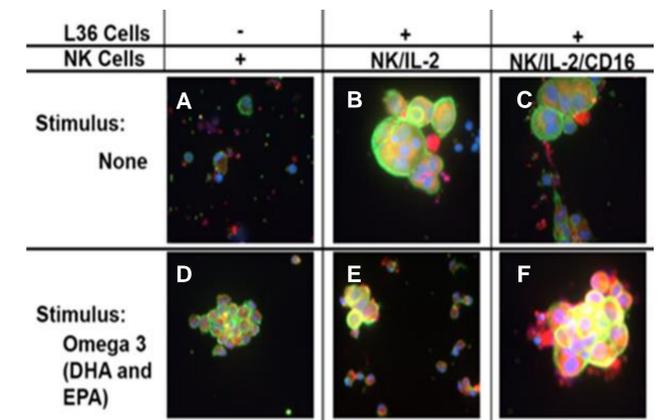
Caspase-3 – assay of PDAC MP2 either alone or in co-culture with natural killer (NK) cells by Promega caspase-3 enzyme assay

Apoptosis (caspase-3) testing by: (a) immunofluorescence, (b) apoptosis enzyme assay

3) Smartfish with or without curcuminoids

- 1) The oil phase of Smartfish: The marine oil is from fish caught in the Arctic and is oxidation protected by botanicals rich in antioxidants, including polyphenols, pectin and whey proteins, tocopherols and rosemary extract. The oxidation levels and the environmental toxin levels are extremely low.
- 2) The curcumin ingredient (Turmipure) in Smartfish is ground turmeric (*Curcuma longa* L.) root which is extracted with acetone and is dispersed into the water phase. The water phase with Turmipure and the oil phase are mixed, emulsified and homogenized in the final product.

2. Immunofluorescence assay of caspase-3



Induction of apoptosis (caspase-3-positive =red) in cultures of L36 cancer cells (=large green cells) co-cultured overnight with NK (IL-2) or NK (IL-2/CD16) cells (=small green or shriveled cells).

NK cells alone in (A) are shriveled whereas in (D) they have green cytoplasm (protected by Smartfish)

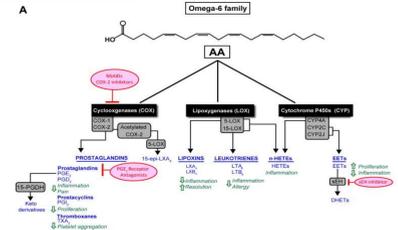
(B) With NK (IL-2) cells without Smartfish, L36 cancer cells have mostly green cytoplasm indicating that they are surviving, whereas with NK (IL2) cells with Smartfish in (E) only few cancer cells are surviving and displaying yellow or orange cytoplasm indicating their apoptosis;

(C) With NK (IL-2/CD16) cells without Smartfish, L36 cancer cells have mostly green cytoplasm indicating that they are surviving, whereas in (F) cancer cells are in a clump displaying apoptosis (yellow or red).

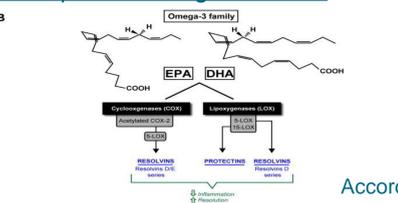
NK cells in (B), (C), (E), (F) are apoptotic (showing either only nuclei or red cytoplasm). Thus cancer cells and NK cells both die in the fight but more cancer cells die in presence of omega-3.

Lipidic mediators

Pro-inflammatory eicosanoids



Anti-inflammatory and pro-resolving mediators



According to C. Serhan

Results

1. Caspase-3 enzyme assay

Curcuminoids in emulsion

with omega-3/antioxidants

increase apoptosis of MP2

cancer cells compared to

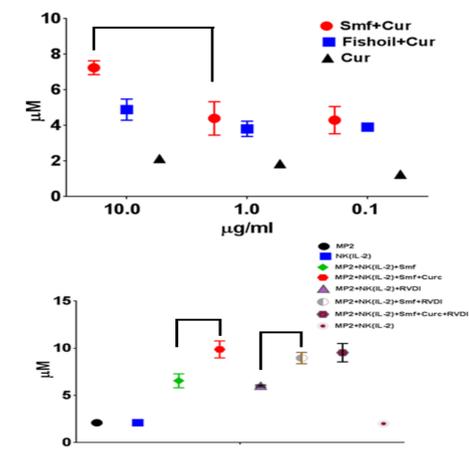
curcuminoids in fish oil

Curcuminoids with Smartfish

potentiate cytotoxic effects of

NK cells on MP2 cells. RvD1 affect

resembles the effect of curcuminoids



Conclusions

- The caspase-3 enzyme assay showed greater induction of caspase-3 in cancer cells treated:
- (a) with curcuminoids (10 µg/ml) in omega-3/botanical emulsion (Smartfish) vs. regular fish oil (P<0.0001),
- (b) with curcuminoids (10 µg/ml) in presence of NK cells and Smartfish (N.S).
- The immunofluorescence microscopy showed greater induction of caspase-3 in cancer cells in co culture with NK cells in Smartfish. Both cancer cells and NK cells suffer apoptosis.
- The potentiation of cancer cell apoptosis by Smartfish could be related to stabilization of the lipidic mediator RvD1 by botanical antioxidants.