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Free fatty acid receptors as new pharmacological targets in the treatment of colorectal cancer: validating the hypothesis and designing new interventions based on dietary supplementation

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Introduction

Colorectal cancer (CRC) is the third most diagnosed cancer in the world. Recent studies suggest the involvement of free fatty acids (FFAs) and FFA receptors (FFARs) in the pathophysiology of CRC. FFARs divide into 4 types: FFAR2 and FFAR3 are activated by short chain fatty acids (SCFAs), while FFAR1 and FFAR4 are activated by long chain fatty acids (LCFAs). A decrease in FFAR2 and FFAR4 expression are observed in patients with CRC. Moreover, there is evidence that FFAR2 ligands, such as butyrate and propionate are considered as anti-tumor agents, which induce growth arrest and apoptosis of CRC cells.

Aim and Methodology

The objective of this study was to evaluate the effect of synthetic FFAR2 agonist, 4-CMTB and FFAR4 agonist, GSK137647 as well as combinations of natural SCFAs (acetate and butyrate at the concentrations of 50 and 2.5 mM, respectively) and LCFAs (stearate and palmitate, both at the concentrations of 25 µM) on cell viability of human colonic epithelial (CCD-841 CoN) and adenocarcinoma (HT-29) cell lines. Cell viability was determined after 48h incubation with tested compounds using MTT assay. qPCR was used to identify alterations in *FFAR2* and *FFAR4* expression. Migration was investigated by commercially available test. Colitis-associated colorectal cancer was induced by a single intraperitoneal injection of azoxymethane (AOM, 10 mg/kg b.w.) and subsequent addition of DSS into drinking water (week 2, 5, 8; 1.5%) to balb/c mice.

Results

- The synthetic FFAR2 agonist 4-CMTB significantly reduced the viability of HT-29 cells compared to non-cancer cells CCD-841 CoN, while the synthetic FFAR4 agonist GSK137647 at the concentration of 25 µM significantly reduced the viability of non-cancer CCD-841 CoN cells compared to HT-29 cells. Both synthetic FFAR2 and FFAR4 agonists increased *FFAR2* expression in non-cancer CCD-841 CoN cells and cancer SW-480 cells. On the other hand, FFAR4 agonist increased the expression of *FFAR4* in both cancer cell lines, HT-29 and SW-480. Furthermore, both synthetic ligands significanlty reduced SW-480 cell migration by approximately 20%.

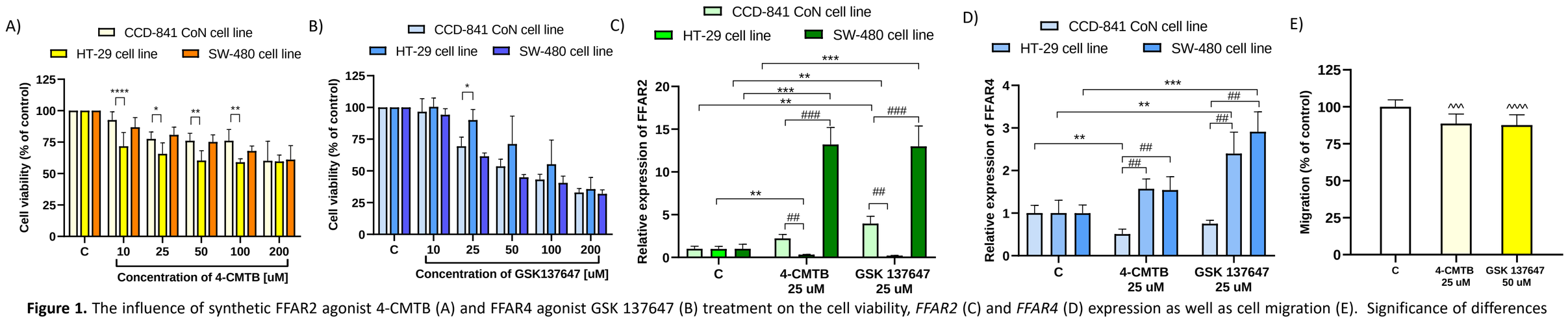

Figure 1. The influence of synthetic FFAR2 agonist 4-CMTB (A) and FFAR4 agonist GSK 137647 (B) treatment on the cell viability, *FFAR2* (C) and *FFAR4* (D) expression as well as cell migration (E). Significance of differences between means: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 cancer cells, HT-29; ##P < 0.01, ###P < 0.001, ####P < 0.0001 cancer cells, SW-480 versus non-cancer cells treated the same way; ^^P < 0.01, ^^^^P < 0.0001 versus untreated cells . Each value represents the mean value ± S.D., n=3 independent experiments (each experiment was carried out in six repetitions).

- All combinations of natural compounds significantly decreased the viability of HT-29 cells as compared to non-cancer cells. All selected natural FFARs ligands separately as well as in combination increased *FFAR2* and *FFAR4* expression in cancer cells as compared to untreted cells. Interestingly, only combination SCFAs+LCFAs significantly decreased SW-480 migration.

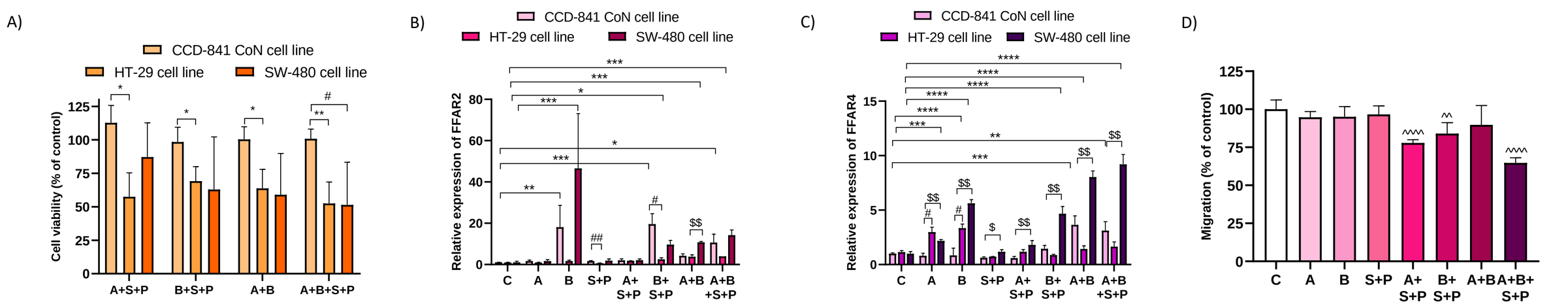

Figure 2. The influence of combination of various FAs (A) treatment on the cell viability, *FFAR2* (B) and *FFAR4* (C) expression as well as cell migration (D). Significance of differences between means: *P < 0.05, **P < 0.01 cancer cells, HT-29 versus non-cancer cells treated the same way; #P < 0.05, ##P< 0.01 and , $P < 0.05, $$P < 0.01 cancer cells, SW-480 versus non-cancer cells; ^^ P < 0.01, ^^^^ P < 0.0001 versus untreated cells. Each value represents the mean value ± S.D., n=3 independent experiments (each experiment was carried out in six repetitions). Abbreviations: A-acetate, B-butyrate, S-stearate, P-palmitate.

- Both synthetic and natural FFAR2 and FFAR4 agonists did not influence the number of tumors in mouse model of CRC, however 4-CMTB significantly increased the FFAR2 expresion whereas diet with 7% lard elevated the FFAR4 expression.

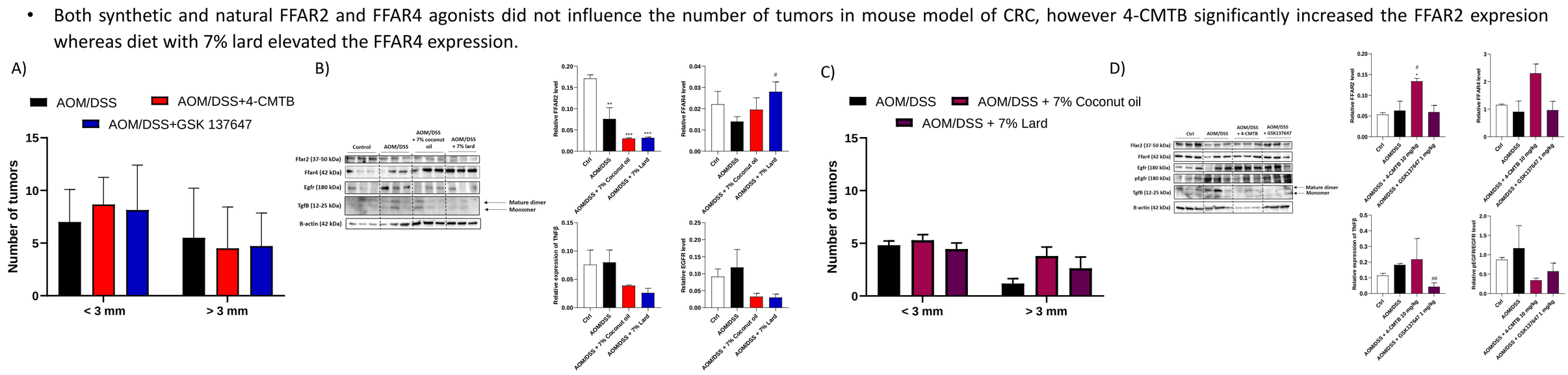

Figure 3. The influence of FFAR2 agonist 4-CMTB and FFAR4 agonist GSK137647 treatment on the number of tumors (A) and representative blots and densytometry of Ffar2, Ffar4, Egfr, TgfB and B-actin for control, AOM/DSS and AOM/DSS + 4-CMTB or + GSK137647 (B) in mouse model of CRC induced by AOM/DSS. As well, the influence of diet enriched with cocuonut oil or lard on the number of tumors (C) and representative blots and densytometry of Ffar2, Ffar4, Egfr, TgfB and B-actin for control, AOM/DSS and AOM/DSS + 7% coconut and 7% lard (D).

Conclusions

- Natural and synthetic FFAR2 agonists significantly reduced the survival of cancer cells and inhibited their migration.
- FFAR4 agonists reduced cells survival of both non-cancer and cancer cell lines to a similar extent.
- Both synthetic compounds as well as natural FFARs ligands increased *FFAR2* and *FFAR4* expression in SW-480 cells.
- Number of tumors were not altered in *in vivo* studies after FFARs agonists treatment, however 4-CTMB significantly increased the expression of FFAR2, while a diet rich in long-chain fatty acids elevated the expression of FFAR4.

Acknowledgment

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