

# Effects of *trans*-fatty acids on the microRNA expression in human colon cancer cells

Solveigh Köpke, Thorsten Buhrke, Alfonso Lampen

Trans fatty acids (TFAs) are fatty acids with at least one double bond in *trans*-configuration. They are generated during industrial oil hardening and refinement processes at high temperatures or are formed by bacterial metabolism in the rumen of ruminant animals. TFAs can be contained in high amounts in food products such as bakery products, french fries, margarines and different snack products. While there is a clear correlation between the consumption of TFAs and an increased risk of coronary heart disease, a potential role in the development of colorectal cancer (CRC) is still under discussion. MicroRNAs (miRNAs) are small (~22 nt), non coding RNAs that regulate the gene expression on a posttranscriptional level. At the moment miRNAs are assumed to regulate ~ 30 % of human genes. Specific miRNA-expression profiles are described for a multitude of cancer types. However, it is not known if TFAs may have an impact on coloncarcinogenesis by epigenetic effects. Therefore, this project aimed to investigate the question if *trans*-fatty acids can influence the miRNA-expression of human colon cancer cells and thereby contribute to coloncarcinogenesis.

## Material and Methods

To identify adequate concentrations for substance exposition, proliferating Caco-2 cells (human colorectal adenocarcinoma cell line) were incubated with eight different fatty acid concentrations for 24 h. Subsequently the viability of the cells was determined using the Celltiter Blue Assay (Promega) (Fig. 1 A-D). To investigate potential fatty acid-induced miRNA-expression changes, the qRT-PCR Array miScript Human Cancer PathwayFinder (Qiagen) was used. The cells were treated for 24 h with 125  $\mu$ M (C 18:0; C 18:1; C 18:2 Isomers), or respectively 25  $\mu$ M (C 18:3 Isomers) fatty acids. The RNA was isolated using a commercial Kit especially designed for the isolation of small RNAs (miRNeasy Kit; Qiagen). For cDNA synthesis a system enabling the specific transcription and polyadenylation of small RNAs was used (miScript II RT Kit; Qiagen). Afterwards the qRT-PCR Array was performed (three biological replicates for each fatty acid). The array contains 84 miRNA specific primer assays for miRNAs associated with carcinogenesis. The data were normalized against four small RNAs as reference genes (small nuclear RNAs/small nucleolar RNAs) that are stably expressed in the used Caco-2 cell model. The fold change was determined in comparison to three biological replicates of untreated Caco-2 cells using the  $\Delta\Delta$ CT-method for relative quantification.

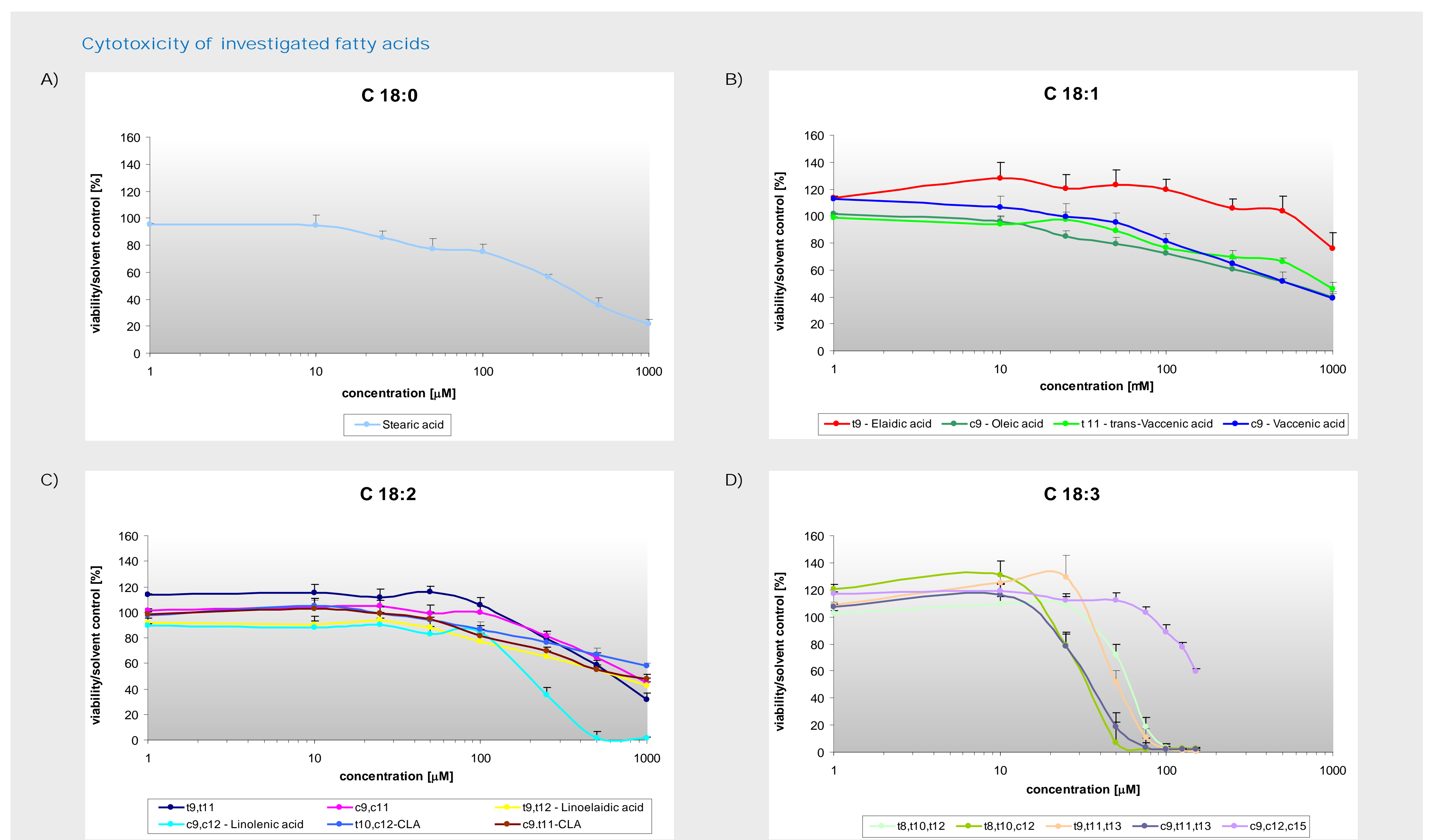


Fig. 1: Determination of the metabolic capacity with the Cell Titer Blue Assay (A-D). Viable cells reduce the indicator dye resazurin to the highly fluorescent reaction product resafurin. The fluorescence was determined after 24 h fatty acid incubation with a plate reader (Berthold Mithras). The data were normalized against the solvent control; 0,005% Triton-X was used as a positive control. The cytotoxicity of the fatty acids rises with increasing number of double bonds.

## Fatty acid-induced miRNA expression changes C 18:2 c9,c11; C 18:2 t9,t11/control (Blank 1-3)

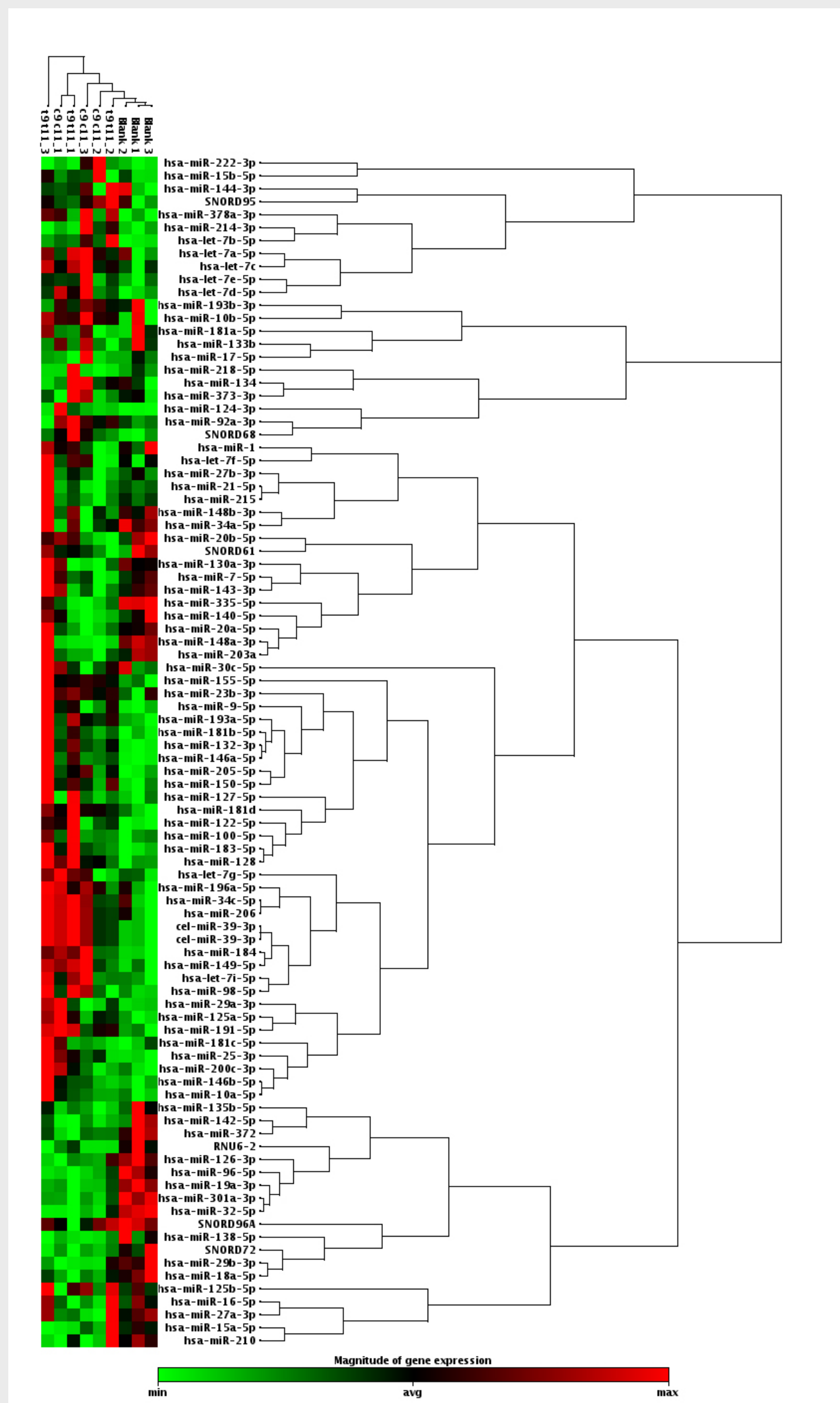


Fig. 2: Regulations of the cancer-associated miRNAs of the qRT-PCR Arrays for C 18:2 c9,c11 and C 18:2 t9,t11 compared to untreated cells (Blank 1-3). *Cis* and *trans*-Isomer of the different fatty acids show an aligned regulation of the miRNA-Expression either *Up* oder *Down* in most cases.

## Multiply regulated miRNAs

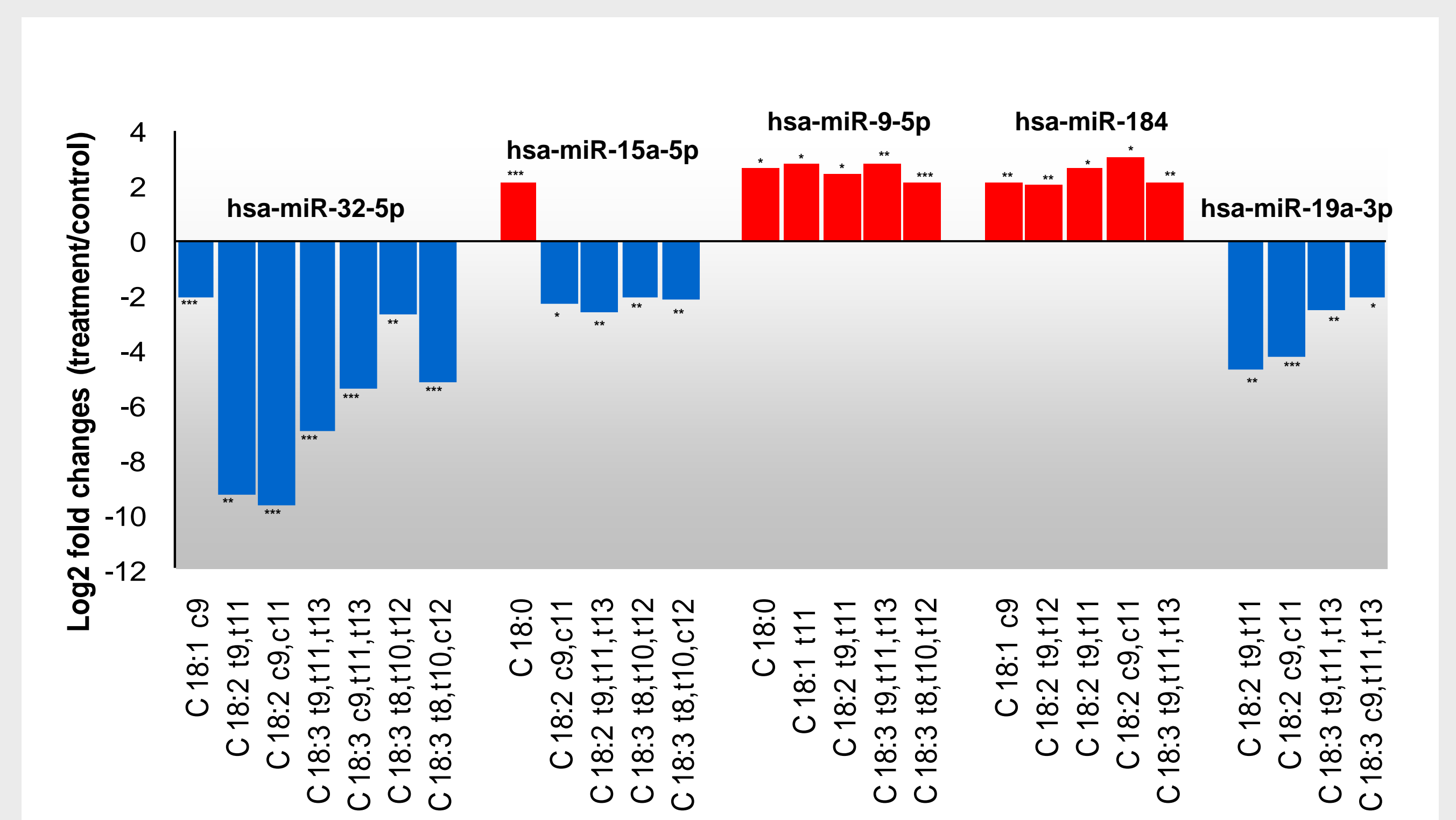


Fig. 3: miRNAs regulated by more than 3 fatty acids. The  $\Delta\Delta$ CT-method for relative quantification was used to determine the fold change; p-values were determined with the Student's t-Test. Except for the stearic acid-induced regulation of hsa-miR-15a-5p, the shown miRNAs were either up- or downregulated in an aligned manner.

## Summary

The investigated fatty acids have an impact on miRNA expression in human colon cancer cells. *Trans* and *cis*-isomers do not show significant differences but regulate the miRNA expression in an aligned manner. The *cis* and *trans*-isomer of C 18:2 showed the strongest regulation effect on miRNA expression. Five of the 84 investigated miRNAs were regulated by more than three fatty acids (Fig. 3). There was no indication of fatty acid-induced regulations of miRNAs directly linked to coloncarcinogenesis. Important points for further considerations based on the current literature are:

- miR-32 (downregulated after fatty acid exposure) targets the proapoptotic BH3-only protein bim (BCL2L11) directly in human acute myeloid leukemia (AML) cells; substances with the potential to downregulate this miRNA were proposed as **sensitizer** for chemotherapeutic drugs during chemotherapy (1)

- miR-32 (downregulated after fatty acid exposure) modulates the synthesis of long chain fatty acids by targeting SLC45A3 in oligodendrocytes (2)

- miR-9 (upregulated after fatty acid exposure) was identified as tumor-suppressor-like miRNA in CRC and is downregulated in colorectal tumors and adenomas (3)

➤ **Next aim: Study of potential molecular targets of the fatty acid regulated miRNAs in the cellular context of human colorectal cancer (different tumorigenic colon cells)**

[1] E. Gocek, X. N. Wang, X. P. Liu, C. G. Liu, G. P. Studzinski, MicroRNA-32 Upregulation by 1,25-Dihydroxyvitamin D-3 in Human Myeloid Leukemia Cells Leads to Bim Targeting and Inhibition of AraC-Induced Apoptosis. *Cancer Res.* 71, 6230 (Oct, 2011).  
[2] D. Shin, S. Y. B. Hwang, L. J. Ptacek, Y. H. Fu, Mir-32 and Its Target SLC45a3 Regulate the Lipid Metabolism of Oligodendrocytes and Myelin. *Neuroscience* 213, 29 (Jun, 2012).  
[3] L. Cekaite et al., miR-9, -31, and -182 Deregulation Promote Proliferation and Tumor Cell Survival in Colon Cancer. *Neoplasia* (New York, N.Y.) 14, 868 (Sept, 2012).