IMPACT of METABOLIC SHIFT TOWARD OXIDATIVE PHOSPHORYLATION (OXPHOS) on METASTATIC PROCESS and CYTOTOXICITY of NATURAL KILLER CELLS AGAINST TUMORS

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ABSTRACT

As the majority of cancer deaths originate from secondary tumors, they are more and more targeted in anti-cancer therapies, also immunotherapies. We have discovered that the pyruvate dehydrogenase kinase 1 (PDK1) inhibitor dichloroacetic acid (DCA) stimulates increase of fatty acid oxidation (FAO) in tumoral cells and that this involves kinase MAP ERK5/MEF2 pathway. Our data show that DCA also increases the cancer cells motility. We have showed that the switch towards FAO in motile (mesenchymal) tumoral cells involves an increase of expression of fatty acid translocase CD36. We have demonstrated that DCA boosts the cytotoxicity of natural killer (NK) cells towards tumoral cells in 2-dimensional models. We hypothesize that this may be due to the increased expression of membrane stress markers, such as MICB and ULBP1, and EMT markers as their expression is augmented upon DCA treatment in our data. Membrane stress markers are recognized by activating receptors of NK cells, such as NKG2D. Our 3-dimensional spheroid model for investigation of the sensitization of metastatic cancer cells towards NK cells cytotoxicity is up-to-date a unique approach.

RESULTS

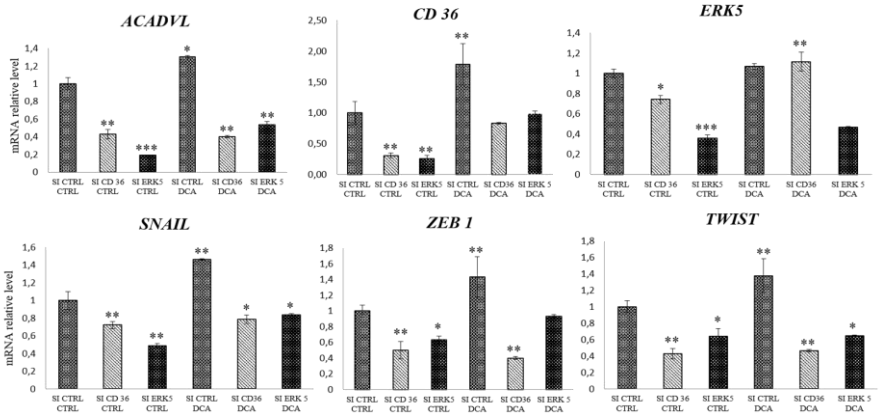
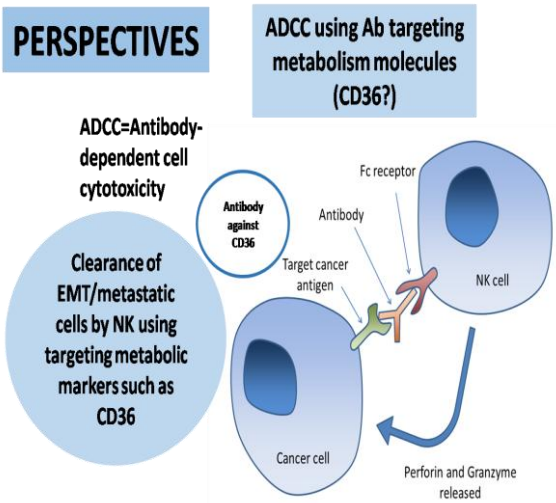


Fig. 1 HCT116 breast cancer cell line treated with DCA 5 mM for 48 h increased the expression of fatty acid translocase CD36 and very long-chain specific acyl-CoA dehydrogenase, essential in lipid metabolism, as well as expression of EMT markers; SNAIL, ZEB1 and TWIST by comparison to non-treated cells (SI CTRL DCA vs. SI CTRL CTRL). The expression of mentioned genes was reduced upon siERK5 and siCD36 transfection. Data from 2 independent experiments.



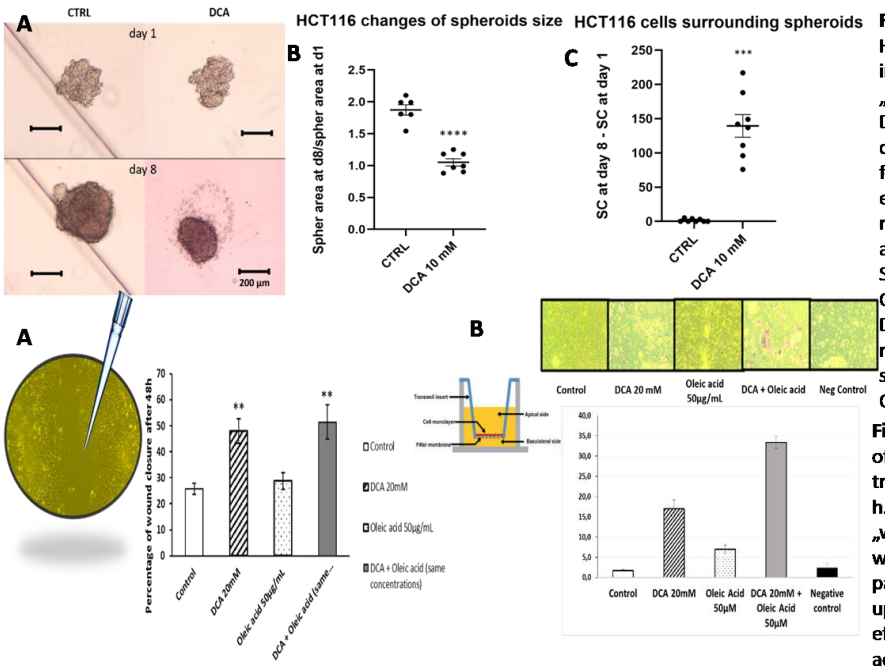


Fig. 2 3D model of motility. HCT116 cells were plated at day 0 in „spheroid medium" and on „spheroid plates" and treated with DCA 10 mM / water (CTRL) for 6 days (day 2 to day 7). **A.** Spheroids formed after 24h (day 1) and at the end of DCA treatment. Same representative spheroids in CTRL and DCA condition are shown. **B.** Spheroids increased their size in CTRL and stayed the same upon DCA treatment. **C.** At the day 8 more cells (SC) surounded spheroids in DCA condition than in CTRL.

Fig. 3 Two-dimensional models of motility. HCT116 cells were treated with DCA 20 mM for 48 h. **A.** DCA treatment accelerated „wound healing" (also combined with fatty acid). **B.** More cells passed through the membrane upon DCA treatment and this effect was enhanced with fatty acid.

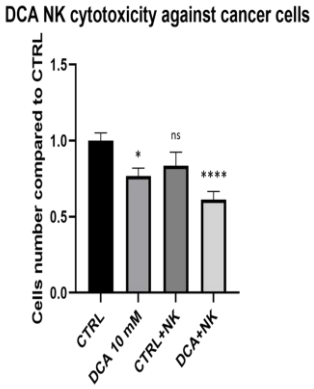


Fig. 4 Cytotoxicity of NK combined with DCA treatment against cancer cells. Data represent results from 3 independent experiments. HCT116 cells were pretreated with DCA 10 mM for 48 h and incubated with NK cells for 2 h.

CONCLUSIONS

Our data indicate that EMT process is related to a lipid metabolism shift through OXPHOS up-regulation. This sensitizes NK cells and favour their action against EMT/metastatic cells. We need complementary experiments using our 3D model to study NK effects on co-cultured tumoral cells combined with DCA treatment. We also need to further characterize CD36 involvement in the process in order to employ it in a targeted therapy using eNK cells and a specific anti-CD36 antibody.