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The knockout of fatty acid synthase decreases the proliferation but increases fatty acid uptake in A431 cells

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The characteristics of lipid metabolism and its important role in cancer cell biology have been intensively investigated by many scientists. Fatty acid synthase (FASN) is the key enzyme involved in endogenous fatty acid synthesis, which is the most important pathway for the supply of fatty acids to various types of cancer cells. Fatty acids are active by long-chain acyl-CoA synthetase (ACSL) before entering different metabolic pathways: synthesis of either triglycerides (TG) or cholesterol esters (CE) for storage in lipid droplets (LDs), phospholipids (PLs) for membrane synthesis or ß oxidation for energy generation. Increased accumulation of LDs in cancer cells is a hallmark of cancer invasiveness and LDs gradually became the hot topic in cancer research. How FASN and ACSL3 correlation with LDs accumulation in cancer cells remain unclear.

In this study, to better understand the role of FASN in the proliferation of cancer cells and its relevance in lipid metabolism, also for exploring new strategy by target lipid metabolism for cancer therapy, FASN and ACSL3 KO A431 cells were established, either alone or in combination (double knockout, DKO) using the CRISPR Cas9 technique. And stable ectopic expression of FASN.A431 cell line was also successfully generated for the gain function of FASN study.

Results showed FASN KO decreased proliferation and anchorage independent growth ability of A431 cells. And overexpression FASN contributed to the proliferation of A431 cells. These data from loss and gain of function experiments demonstrated that FASN

might play an important role in proliferation and anchorage independent growth of A431 cells. ACSL3 knockout slightly reduced proliferation and anchorage independent growth. After the simultaneous knockout of FASN and ACSL3, both the proliferation and the adherence-independent growth were reduced in A431 cells: This decrease was more pronounced in the double knockout compared to the single knockouts, but did not reach statistical significance.

Lipid metabolism was examined by using a radioactive tracer. FASN KO resulted in decreased C14 acetate uptake and C14 acetate incorporation into phospholipids was strongly reduced. This decreased phospholipid synthesis after FASN knockout revealed that FASN might relevant in membrane lipid synthesis. And more surprising, FASN KO up-regulated exogenous oleic acid uptake and incorporation into triglyceride in A431 cells when C14 oleic acid was used as radioactive tracer, which indicated intracellular metabolism altered by FASN knockout can drive fatty acid uptake, also suggested cancer cells are versatile in acquiring nutrients from their environment. Thus, FASN inhibition alone might not be the best option in limitation fatty acids supply in cancer cells. ACSL3 KO resulted in decreased C14 oleic acid uptake and incorporated less into triglyceride suggesting that ACSL3 not only activates fatty acids with CoA, might also increases fatty acid uptake likely by metabolic trapping of fatty acids as their CoA derivations.

In this study, lipid droplets accumulation was observed by Nile Red staining neutral lipids. A431 cells had fewer LDs accumulated when growth in medium with reduced FCS. And the proliferation of A431 cell lines in low glucose low FCS condition was positively correlated to the amounts of lipid droplets in cells, which hinted the sufficient storage and usage of lipid precursors from lipid droplets would be necessary for the proliferation. Both FASN and ACSL3 KO reduced LDs accumulation in A431 cells, and FASN rather than ACSL3 conferred cells with LDs accumulation to adapt to stressful condition (when cells cultured in FCS free medium). No matter how complicated the oncogenic factors regulating the metabolism of all alternative fuels, targeting FASN and ACSL3 (or other up-regulated ACSLs) in combination could be a promising novel

strategy to treat a wide spectrum of cancers, irrespective of the source of fatty acid supply. However, it needs further validation in more cell lines, and also in vivo confirmation is necessary.