Elaidic acid promotes cell proliferation and migration via LSR in epithelial ovarian cancer

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Background and Purpose

・Previously, we identified lipolysis-stimulated lipoprotein receptor (LSR), which is a single-pass membrane protein originally identified in liver, as a new therapeutic target of epithelial ovarian cancer (EOC), and we reported anti-cancer effect of our newly developed monoclonal antibody (mAb) against LSR-positive EOC *in vitro* and *in vivo* (Hiramatsu K, Naka T *et al*. Cancer Res 2018).

・We also demonstrated that LSR took in triglyceride-rich protein and contributed cell proliferation, however, lipid metabolic pathway *via* LSR is still unclear. Thus, we aimed to reveal the function of LSR on lipid metabolism in EOC cells.

Method

・We investigated the activation of beta-oxidation, ATP production, cell proliferation and cell migration *via* LSR after administration of fatty acid (FA) using LSR-positive EOC cells (RMG-I) and LSR-knockout EOC cells (LSR siRNA transfected RMG-I). Moreover, we also investigated anti-cancer effect of our anti-LSR mAb against lipid metabolism *via* LSR.

・Production of ATP was measured using CellTiter-Glo®.
Cell proliferation was analyzed by WST-8 assay. Migration assay was performed using CytoSelect®.

・In all experiment, we used low glucose medium (50% decreased) with 1% BSA (FBS was not added).

Results

In LSR-positive EOC cells, Elaidic acid promoted cell proliferation *via* LSR

To investigate the ligand of LSR, we administrated five fatty acids (myristic acid (MA), palmitic acid (PA), arachidonic acid (AA), oleic acid (OA) and elaidic acid (EA)) to LSR-positive cells (RMG-I). After 72hrs, we performed cell proliferation assay.

		MA			PA			AA			OA			EA		
LSR	(μM)	0	50	100	0	50	100	0	50	100	0	50	100	0	50	100
(+)	RMG-I	1.000	0.772	0.753	1.000	0.591	0.503	1.000	0.769	0.789	1.000	1.366	1.408	1.000	1.364	1.368
(-)	SKOV3	1.000	0.934	0.914	1.000	0.566	0.425	1.000	0.533	0.310	1.000	0.953	0.865	1.000	1.079	0.881

Relative ratio of viable cells compared to no treatment (0μM)

OA and EA promoted cell proliferation of LSR-positive cells (RMG-I) dose-dependently.

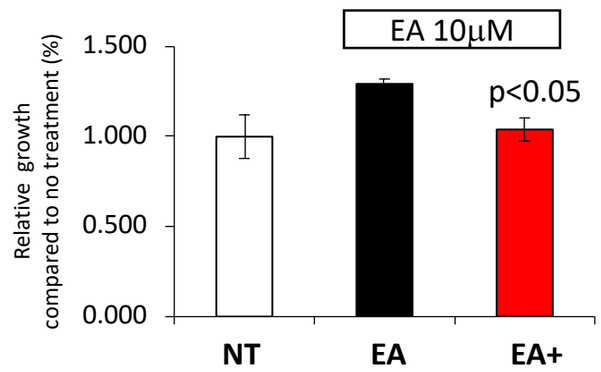
However, MA, PA and AA did not. In addition, OA and EA did not promoted cell proliferation of LSR-negative cells (SKOV3).

In LSR-positive EOC cells, anti-LSR mAb inhibited cell proliferative effect of EA

To demonstrate specific uptake of EA *via* LSR, we inhibited the function of LSR by transfection of LSR siRNA or administration of anti-LSR mAb.
In LSR siRNA transfected cells, cell proliferative effect of EA was decreased, however, that of OA was not.

		OA			EA		
	(μM)	0	5	10	0	10	25
in LSR-siRNA transfected RMG-I	72h	1.000	1.274	1.763	1.000	1.034	1.026

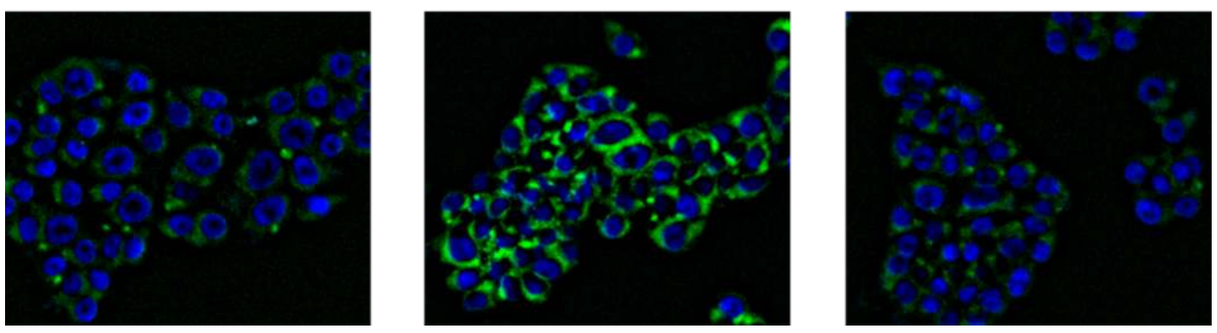
Moreover, anti-LSR mAb also inhibited cell proliferative effect of EA in LSR-positive cells. These results suggest that uptake of EA is dependent on LSR.



In LSR-positive EOC cells, EA administration increased intracellular lipid accumulation

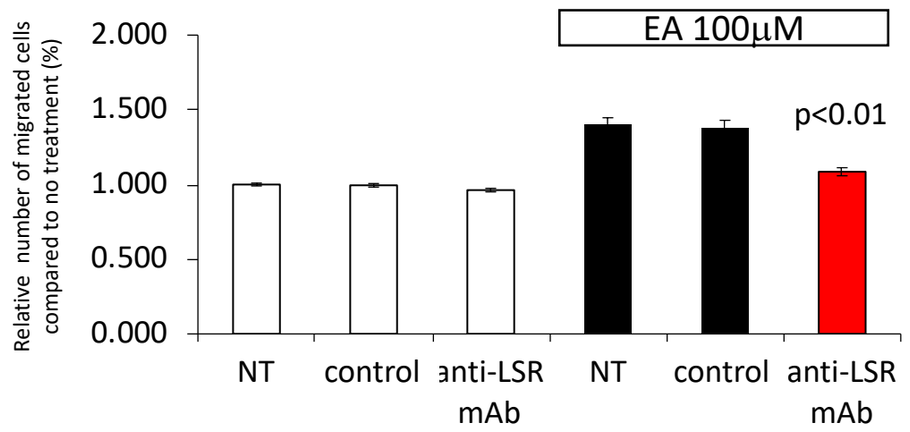
To investigate intracellular lipid accumulation after administration of EA, we stained intracellular lipid by BODIPY® after 3hrs of EA administration.

EA administration increased intracellular lipid accumulation, and anti-LSR mAb inhibited this process.



EA promoted cell migration in LSR-positive EOC cells

We gynecologist usually observe ovarian cancer metastasis to omentum (omentum cake). Therefore, we investigated EA effect for cell migration.
EA promoted cell migration of LSR-positive cells and anti-LSR mAb inhibited this activity.

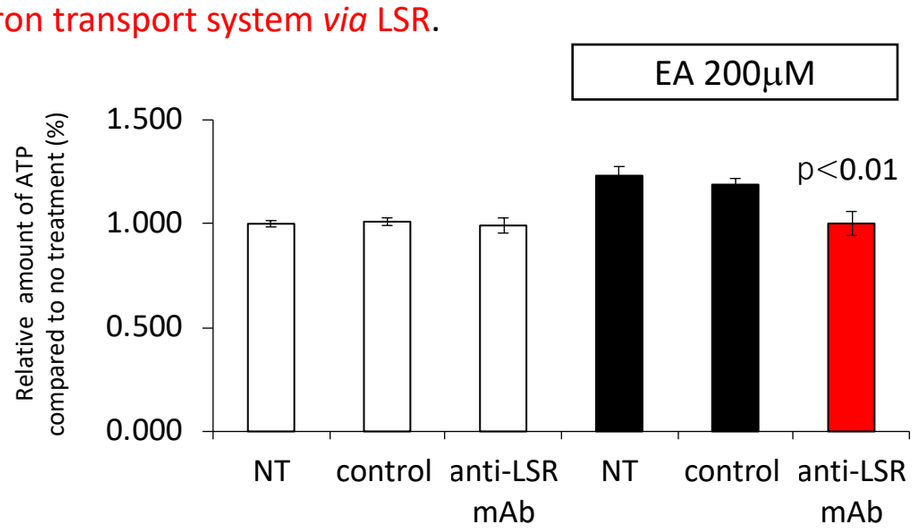


LSR promoted EA uptake and ATP production

To confirm the use of EA in LSR-positive cells, we measured intracellular ATP content after administration of EA.

EA promoted ATP synthesis in LSR-positive cells.

Moreover, anti-LSR mAb treatment showed significant inhibition of ATP synthesis. These results suggest that EA promoted β oxidation, TCA cycle and electron transport system *via* LSR.



Discussion

・Our research showed that LSR captured EA and took it into cell. Moreover, we revealed that in glucose restricted environment, EA administration activated beta-oxidation, TCA cycle, electron transport system and ATP synthesis, and promoted cell proliferation and migration. Meanwhile, anti-LSR mAb inhibited tumor growth by inhibition of lipid metabolism.

・These results suggest that LSR takes in EA in nutrition restricted environment to survive and spread.

・However, it is not unclear which proliferative pathway is activated by EA *via* LSR. In addition, the effect of glucose restricted environment to LSR is also unclear.

・Further investigation is required to reveal lipid metabolism in EOC.

Conclusion

・Elaidic acid uptake *via* LSR in glucose restricted environment contributed to cell proliferation and migration subsequent to activation of lipid metabolism, and anti-LSR mAb inhibited these processes.

・LSR might contribute to cancer spread and metastasis of EOC.