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Mitotane inhibits Sterol-O-acyltransferase leading to lipid-mediated Endoplasmic Reticulum stress and apoptosis of Adrenocortical Carcinoma Cells

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CONTEXT

Mitotane is the only drug approved for treatment of adrenocortical carcinoma (ACC) and in clinical use for more than 50 years. Mitotane counteracts both tumor growth and tumoral steroid hormone production but treatment is associated with severe side effects. The molecular mechanism of mitotane is still unknown, which hampers progress in treatment of ACC.

OBJECTIVE

To identify the mechanism of action and molecular target of mitotane.

METHODS

We combined expression genomics and lipidomics in an in vitro study using the NCI-H295 ACC reference cell line and four non-steroidogenic cell lines.

RESULTS

Pathway analysis of gene expression data demonstrated activation of endoplasmic reticulum (ER) stress and altered lipid metabolism.

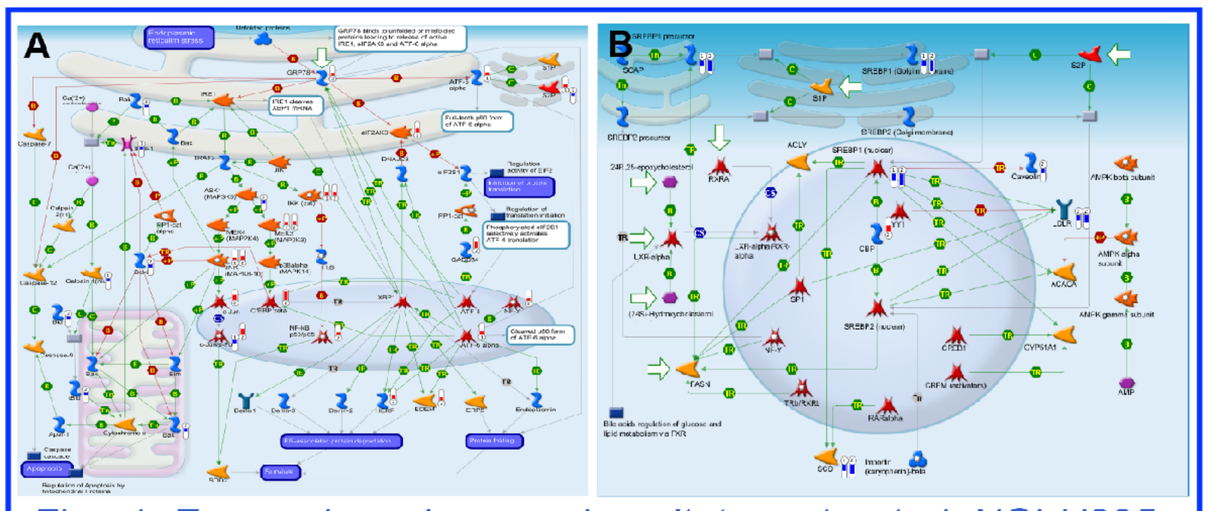

Fig. 1 Expression changes in mitotane treated NCI-H295 cells of ER-stress genes (A) and lipid metabolism (B).

Real-time PCR confirmed that ER-stress markers CHOP and activated XBP1-mRNA splicing were strongly upregulated by mitotane in the ACC cells in sharp contrast to weak ER-stress activation in the non-steroidogenic cell lines. ER-stress inducer thapsigargin mimicked and enhanced the mitotane effects on CHOP expression and steroidogenesis.

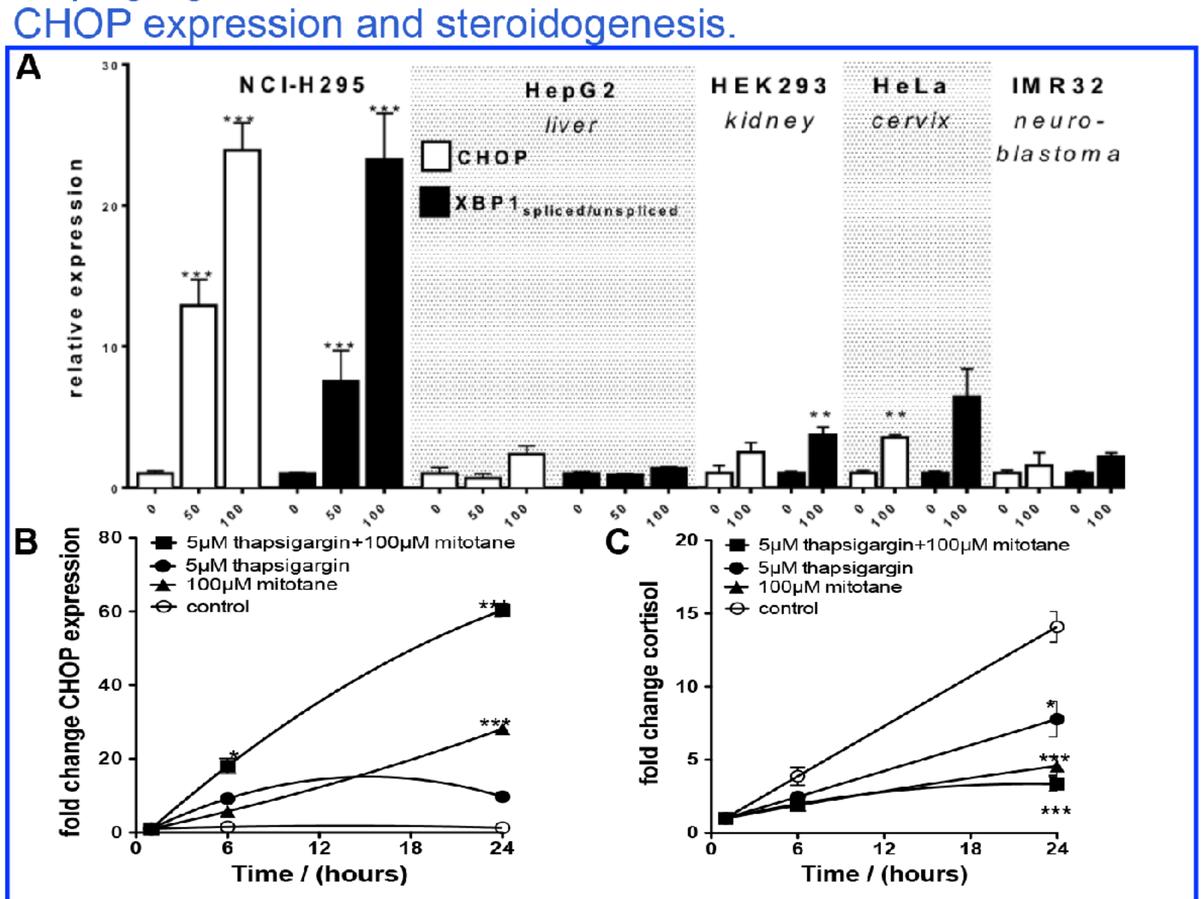

Fig. 2 *ER-stress induction by mitotane and thapsigargin. (A) Mitotane induces ER-stress specifically in ACC cells. (B) ER-stress inducer thapsigargin enhances mitotane effect and supresses cortisol production.*

Mass spectrometry revealed specific mitotane-induced lipid alterations with elevated free cholesterol, oxysterols and fatty acids, paralleled by a decrease in cholesterol esters in NCI-H295 cells but not in other cell lines.

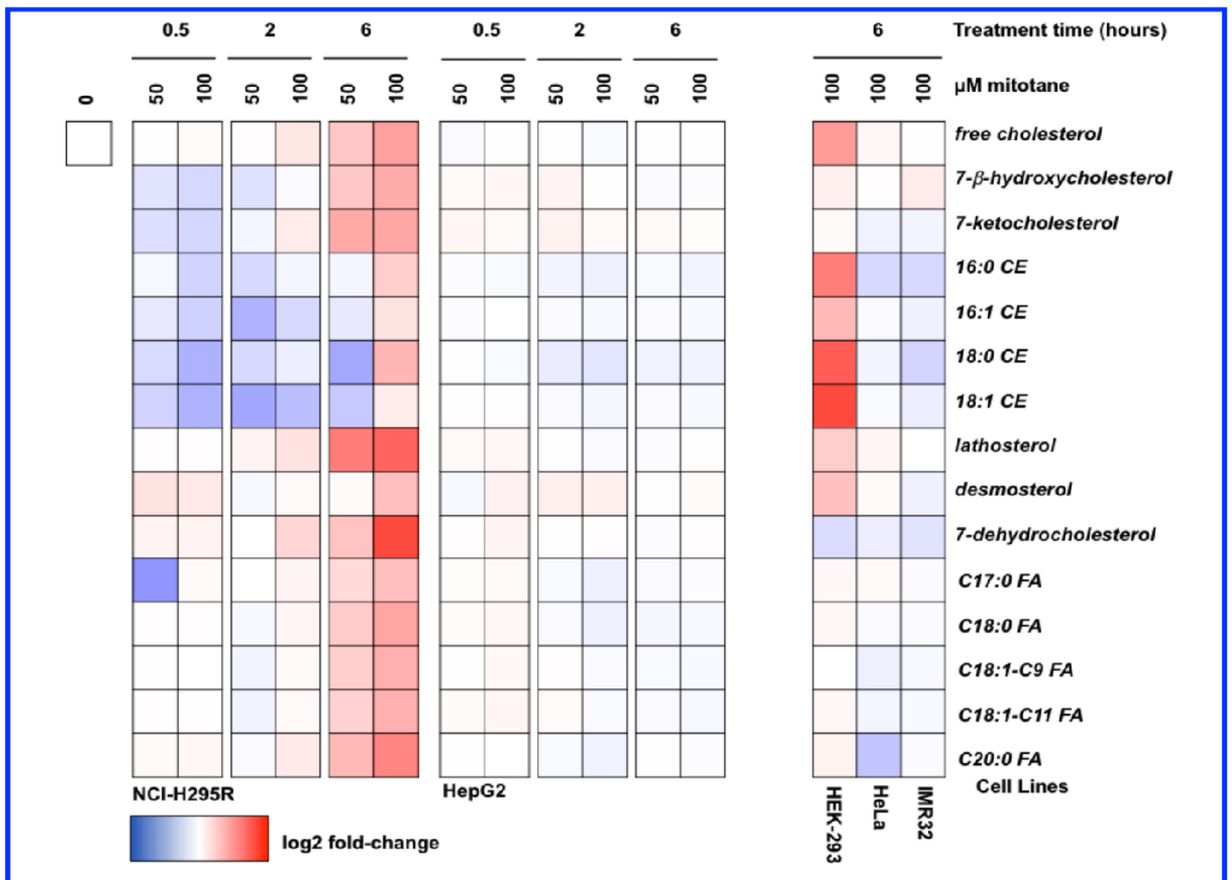

Fig. 3 *Heatmap of selected lipids altered by mitotane in several cell lines*

Inhibition by mitotane of Sterol-O-acyl-transferase (SOAT1), an ER located enzyme responsible for cholesterol esterification was identified as the mechanism underlying these events.

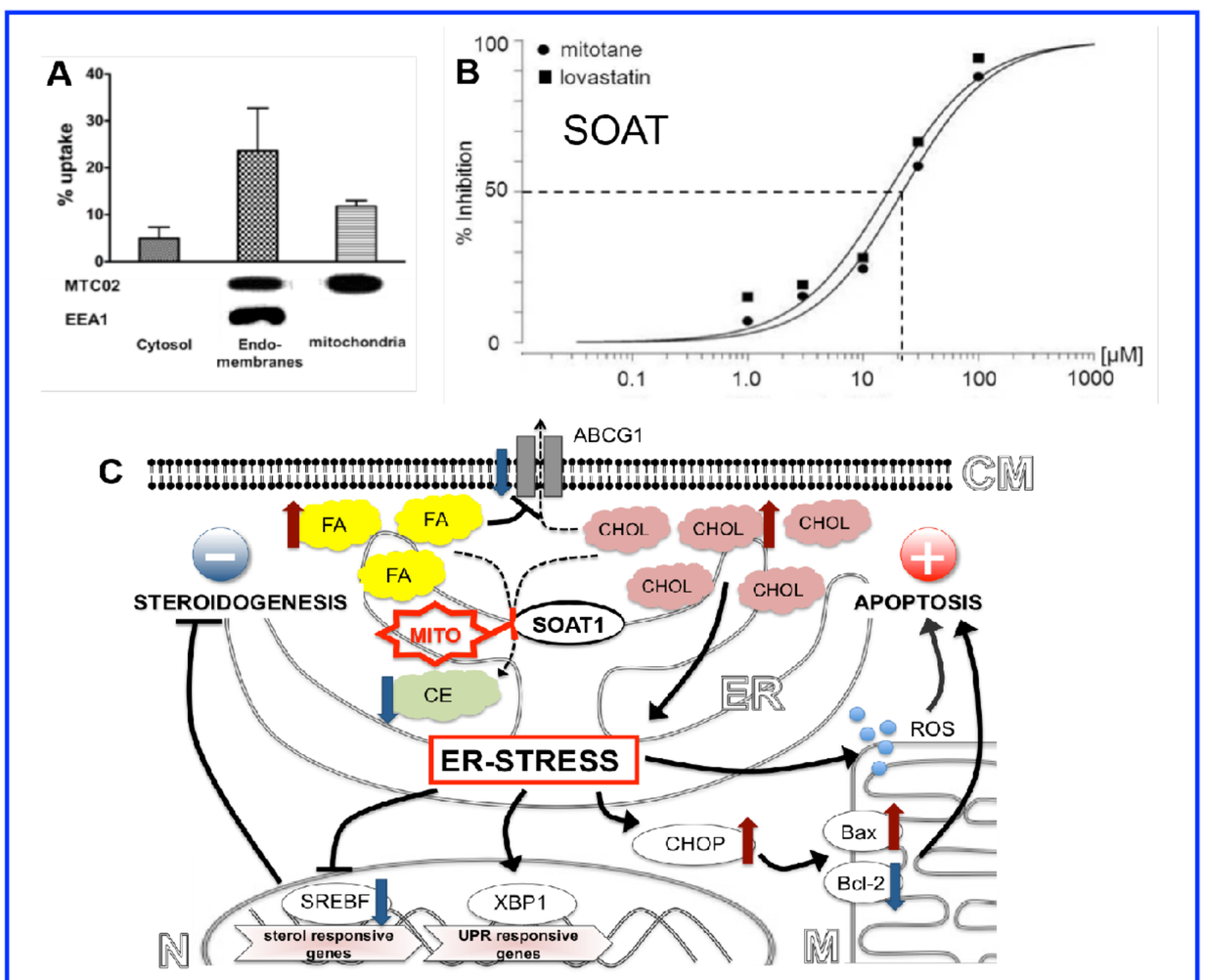

Fig. 4 *Mitotane accumulates in endo-membranes (A) and inhibits SOAT (B) leading to lipid-induced ER-stress, increased apoptosis and reduced steroidogenesis (C).*

CONCLUSIONS

Mitotane confers adrenal specific cytotoxicity and down-regulation of steroidogenesis by lipid-induced ER-stress through inhibition of SOAT-activity. This finding opens new avenues for improved ACC treatment. Cancer specific lipid metabolism may be a treatment target in other types of cancer.

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