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

S. Sbiera¹, M. Fassnacht¹, E. Leich², G. Liebisch³, A. Schirbel¹, L. Wiemer¹, S. Matysik³, J. T. Vanselow², F. Gardill¹, A. Gehl¹, S. Kendl¹, M. König¹, M. Balá³, C. Ronchi¹, T. Deutschbein¹, A. Schlosser², G. Schmitz³, A. Rosenwald², B. Alolio¹, M. Kroiss¹

¹ University Hospital Würzburg, ²University of Würzburg, ³University Hospital Regensburg

GP-29-01

CONTEX
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.

Real-time PCR confirmed that ER-stress markers CHOP and activated XBPl-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.

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.

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.

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.

This study was supported by grants of the DFG (grant FA 466/4-1 to M.F. and KR 4377/1-1 to M.K.), CCC Mainfranken to M.K., DZK Würzburg (grant B-281 to M.F.), and the ERA-NET “E-Rare” (grant 01GM1427 to M.F.)