EBERHARD KARLS UNIVERSITÄT TÜBINGEN

@Laim_Ziz
linkedin.com/in/Laimdota/

Department of Preclinical Imaging and Radiopharmacy
Core Facility Metabolomics, Faculty of Medicine, University of Tübingen

Deciphering the Spatial Metabolome of Senescent Tumors by MALDI Imaging Mass Spectrometry

Laimdota Zizmare[1,2,3,4], Francisco J. Reche-Pérez[3,4], Jonathan Cotton[3,4], Corinna Henkel[5], Bernd J. Pichler[3,4,6,*], Christoph Trautwein[1,2,3,4,*]

[1] Core Facility Metabolomics, Faculty of Medicine, University of Tübingen, 72076, Germany. [2] The M3 Research Center for Malignome, Metabolome and Microbiome, University of Tübingen, 72076, Germany. [3] Cluster of Excellence iFIT "Image Guided and Functionally Instructed Tumor Therapies "(EXC 2180), University of Tübingen, 72076, Germany.
[4] Werner Siemens Imaging Center, Department of Preclinical Imaging and Radiopharmacy, University of Tübingen, 72076, Germany. [5] Bruker Daltonics GmbH & Co.KG, 28359 Bremen, Germany.
[6] German Cancer Research Center, German Cancer Consortium DKTK, Partner Site Tübingen, Germany. * equal contribution

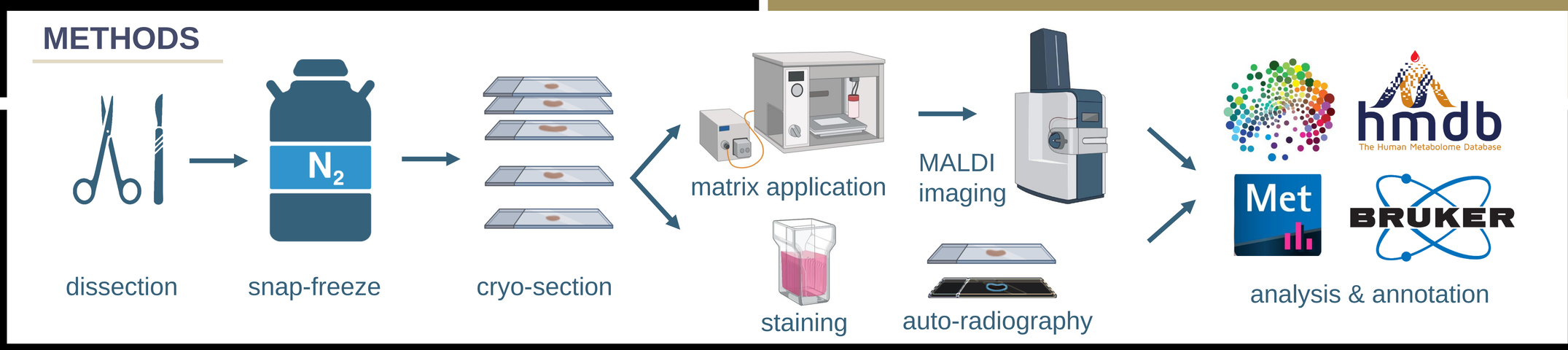
INTRODUCTION

- Cellular senescence, a double-edged sword, initially causes the inhibitory cell-cycle arrest, yet further also initiates the cellular adaptation of the senescence-associated secretory phenotype (SASP), transforming fibroblasts into tumor progression-favorable pro-inflammatory cells, and ultimately aiding the therapy resistance.
- Aim: unravel the metabolic role of senescence in tumor microenvironment heterogeneity by spatial metabolomic characterization of senescent tumors.
- Subcutaneous liver carcinoma cell senescent and control tumor metabolomics was analyzed by ion mobility time-of-flight spectrometry (timsTOF fleX MALDI-2 imaging) data was acquired at 10 µm pixel resolution,100 – 1000 m/z.

SUMMARY

- The tumor microenvironment is highly heterogenous and therefore needs to be studied spatially.
- We demonstrate how **spatial metabolomics** aid the elucidation of the metabolomic and lipid alterations in response to **senescence** induction and in the areas of senescence tracer localization.
- Bioactive lipids, including sphingolipids, oxidized phospholipids, arachidonic acid, lipid droplets, and free fatty acids contribute to chronic **inflammatory** state, regulation and amplification of SASP, and **cellular membrane remodeling**. Hence these are important study targets for potential therapeutic interventions and progression monitoring.

METHODS



RESULTS

Fig 1. Staining of Ki67, p21, and p53 confirms the senescent phenotype in Cx-5461 compared to Na_2HPO_4 control, while hematoxylin and eosin (H&E) staining demonstrates the general tissue morphology.

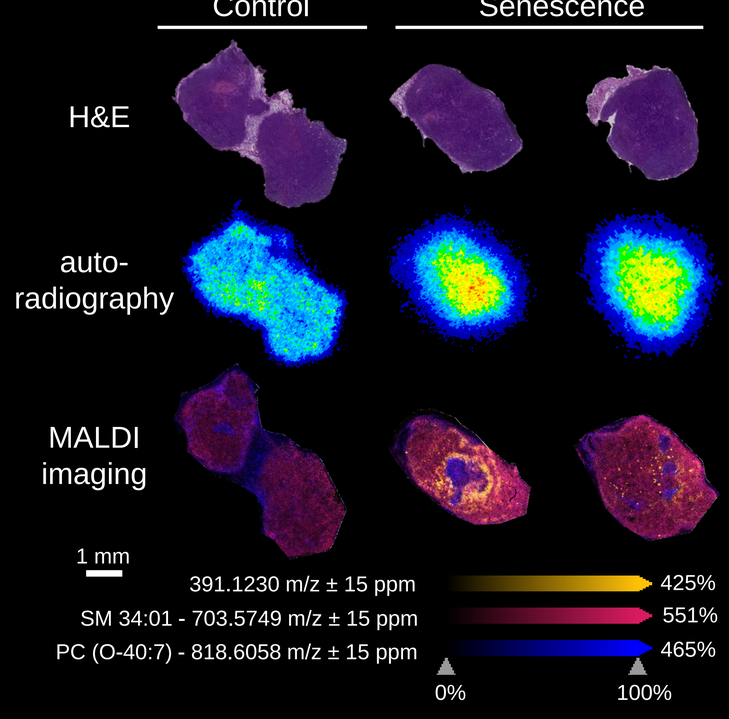


Fig 2. While H&E does not visually characterize differences between senescent and control tumors, senescence tracer uptake spatial distribution shows specific uptake peak regions (in red) by autoradiography that can be correlated with adjacent tissue MALDI-2 imaging positively ionized example feature spatial distribution. *SM = sphingomyelin, O-PC = ether phosphatidylcholine*

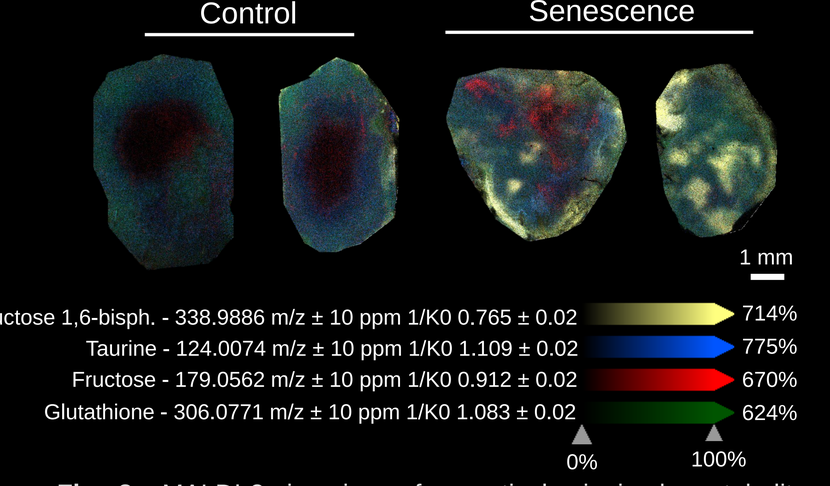


Fig 3. MALDI-2 imaging of negatively ionized metabolites. Example overlapping metabolite spatial distribution illustrates the spatial variability of metabolite distribution and intensity, signifying control tumor core. Senescent tumors visually present increased intensity for energy/sugar metabolism and reactive oxygen/nitrogen species scavenger-related metabolites. Annotations confirmed with accurate mass, ion mobility and cross collision section (ccs) value match.
Fructose 1,6-bisph. = D-fructose 1,6-bisphosphate

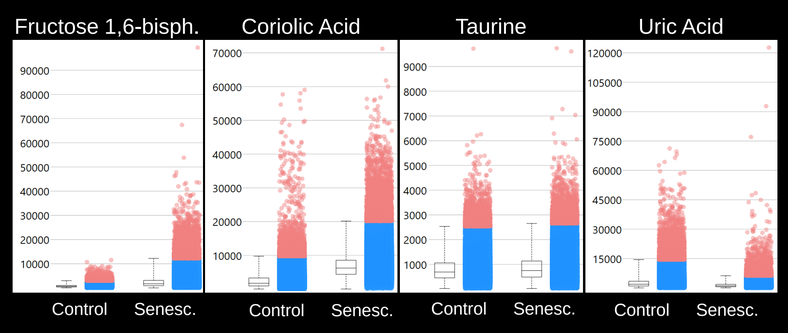


Fig 4. Example ion intensity box plots and scatter plots showing the increased intensity of D-fructose 1,6-bisphosphate and coriolic acid (13-HODE), and reduced uric acid. Taurine stresses the importance of spatial visualization alongside overall intensity estimation.

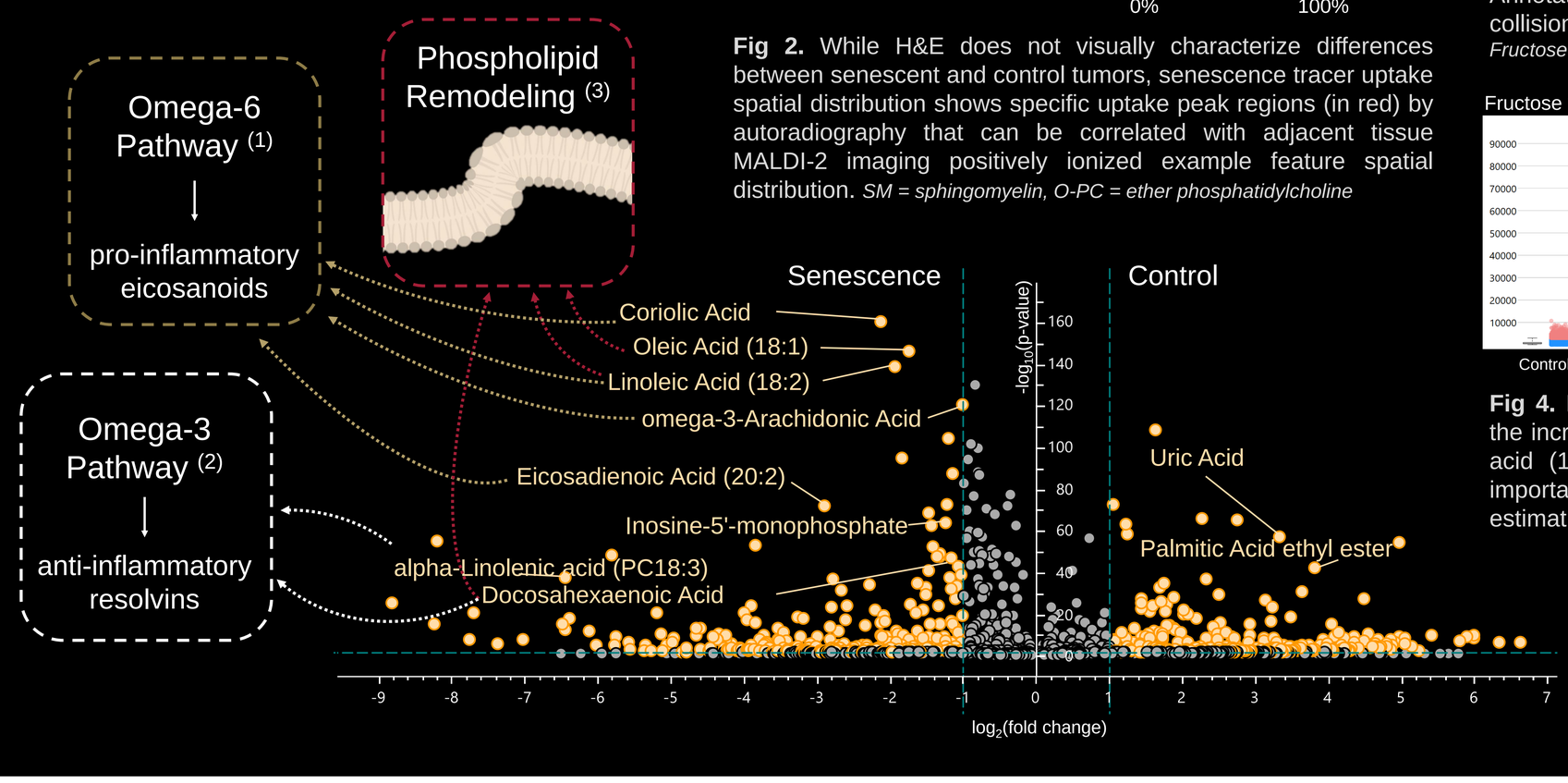


Fig 5. (left) Volcano plot of one negatively ionized MALDI-2 imaging slide run of metabolites and lipids highlights altered omega 3 and 6 fatty acid metabolic pathways in senescent tumors. Phospholipid remodeling was observed in line with the previous quantitative metabolomics findings on cellular composition modifications upon senescence induction (4).

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WSIC Werner Siemens Imaging Center
M3 MALIGNOME. METABOLOME. MICROBIOME.

Contact: **Dr. Laimdota Zizmare**
Core Facility Metabolomics
Otfried-Müller-Straße 37, 72076 Tübingen, Germany
E-mail: **Laimdota.Zizmare@med.uni-tuebingen.de**

iFIT Visualizing and Targeting Cancer Stress
Universitätsklinikum Tübingen