Altered metabolism in prostate cancer and stroma detected through MALDI-TOF MSI

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Aim of study:

To identify the distribution of metabolites and lipids within the different tissue types (benign epithelium, stroma, cancer) of prostate cancer samples using MALDI-TOF MSI in both ion modes.

Material:

- **45 prostate tissue** samples from **15 patients**
- **Histopathology** (uropathologist): benign epithelium, stroma, cancer

Methods:

- MALDI-TOF MSI (rapifleX)
- Metabolites/ lipids (*m/z* 100-1000)
- Spatial resolution: 30 µm
- Matrices: DHB (+), NEDC (-)
- MS/MS on selected masses

Data analysis

- Total ion count normalization
- Pair-wise comparisons with OPLS-DA (leave-one patient-out crossvalidation)
- Verified with permutation testing (1000 iterations)

Results and discussion

All six OPLS-DA models were significant (p<0.001):

BE=benign epithelium Spec. = specificity Sens. = sensitivity	Negative ion mode			Positive ion mode		
	Accuracy	Spec.	Sens.	Accuracy	Spec.	Sens.
BE vs Stroma	77.6%	85.8%	65.4%	78.8%	88.7%	69.0%
BE vs Cancer	74.7%	67.9%	81.5%	77.9%	74.7%	81.1%
Stroma vs Cancer	83.7%	75.8%	91.6%	86.1%	81.0%	91.3%

Several metabolites were differently expressed between the tissue types, which were defined by a variable importance on projection (VIP) score threshold ≥ 1:

- **Citrate** (*m/z* 191.02, −) and **spermine** (*m/z* 203.27, +) had reduced levels in stroma and in cancer compared to benign tissue (Figure 1 and 2), confirming previous findings [3].
- The antioxidant **taurine** (*m/z* 124.02, −) had higher levels in stroma compared to benign epithelium and cancer. We recently reported higher levels of taurine in reactive stroma and significant correlation with the expression of genes involved in immune processes [4].
- **Lipids** measured in negative ion mode were found to have higher levels in cancer (Figure 1), supporting lipids as key players in cancer development [5].

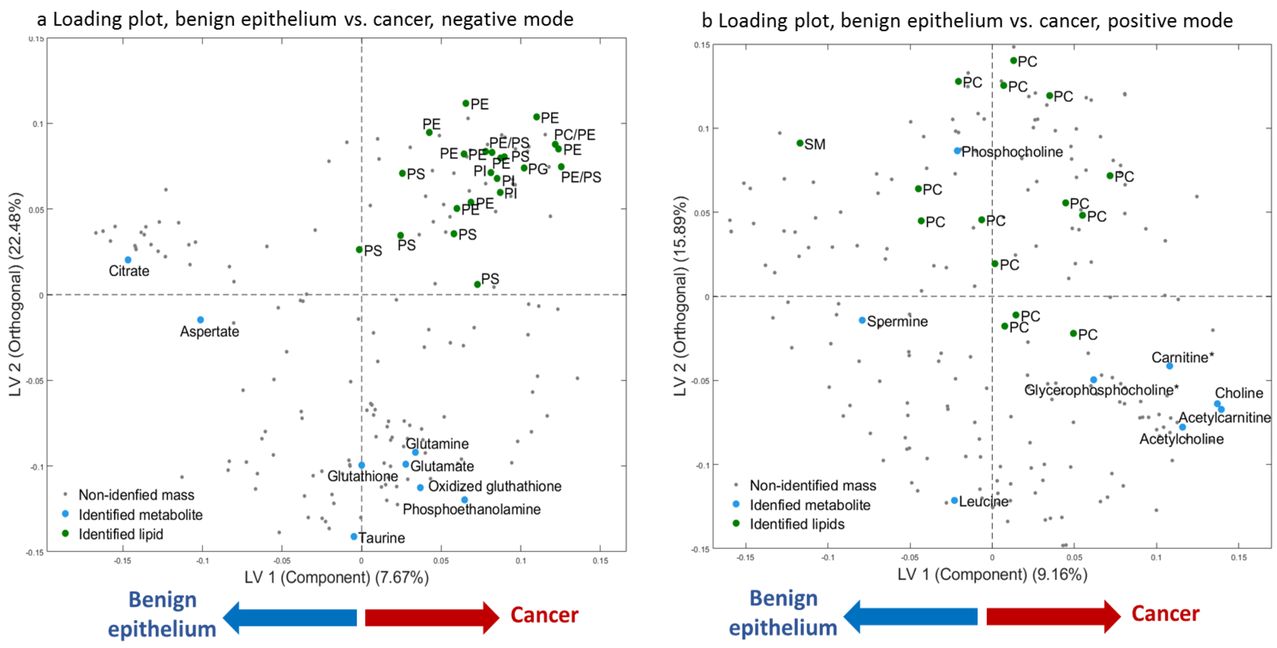

Figure 1: OPLS-DA models comparing benign epithelium and cancer: (a) Loading plot for (−) OPLS-DA model, and **(b)** Loading plot for (+) OPLS-DA model. *Suspected identity, not verified with MS/MS, PE=phosphoethanolamines, PC=phosphatidyl-cholines, PG=phosphatidyl-glycerols, PI=phosphatidyl-inositols and PS=phosphatidylserine, SM= sphingomyelin

- **Carnitine** (m/z 162.10, +) and **acetylcarnitine** (*m/z* 204.17, +), parts of the carnitine shuttle, had higher levels in cancer compared to benign epithelium and stroma, but no observed differences between stroma and benign epithelium. To our knowledge, this is the first time the metabolites carnitine and acetylcarnitine are reported at higher levels in prostate cancer tissue. The carnitine shuttle is important for regulation of β-oxidation vs. lipid synthesis balance [6] (Figure 3).

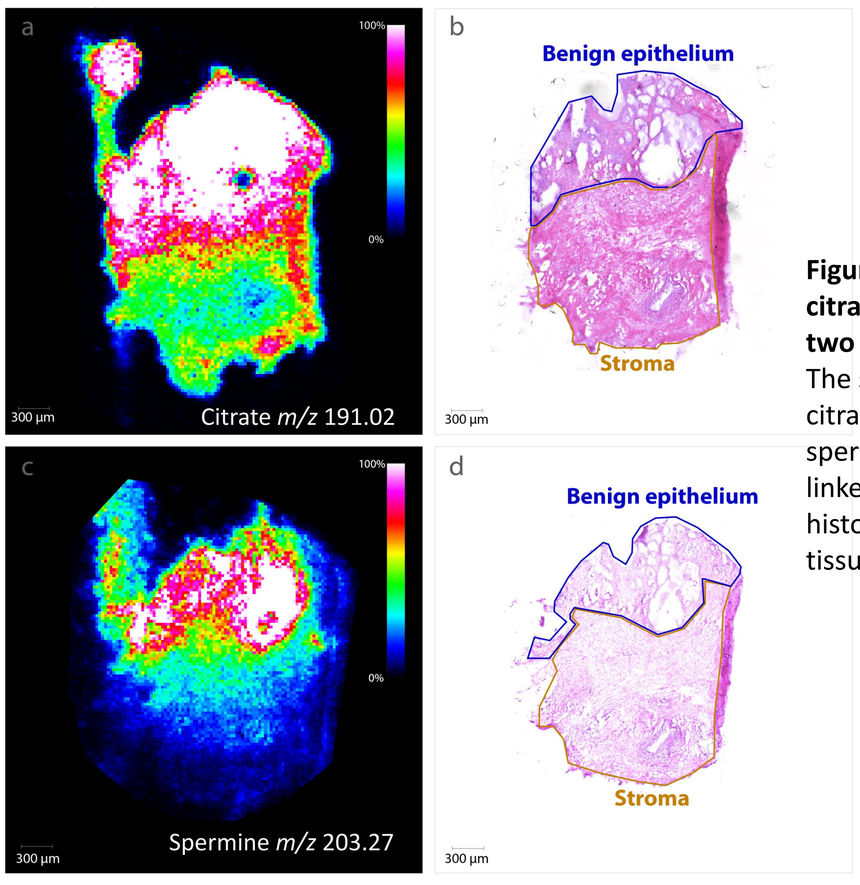

Figure 2: Spatial distribution of citrate (−) and spermine (+) on two consecutive tissue sections. The spatial distribution of **(a)** citrate (*m/z* 191.02) and **(c)** spermine (*m/z* 203.27) were linked to the **(b, d)** histopathology of the same tissue sections.

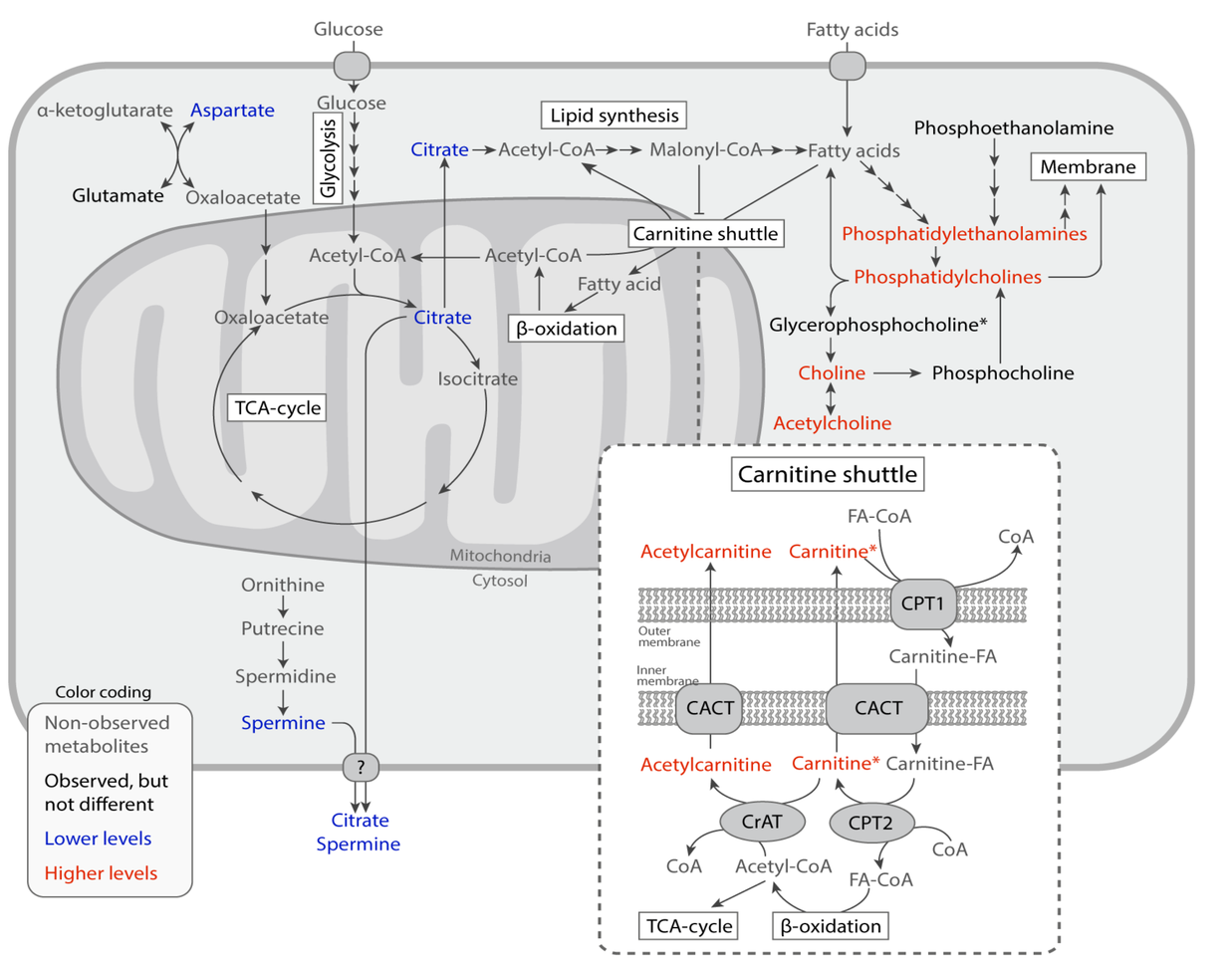

Figure 3: Altered metabolic pathways in cancer compared to benign epithelium: Metabolites and lipids with VIP > 1 in the OPLS-DA models comparing benign to cancer are colored as red or blue for higher or lower levels in cancer, respectively. *Mass is not identified with MS/MS, CPT1= Carnitine palmitoyltransferase 1, CACT = carnitine-acetylcarnitine transferase (CACT) and CrAT = carnitine O-acetyl-transferase.

Conclusion

The elevated levels of **carnitine** and **acetylcarnitine** in prostate cancer tissue compared to benign epithelium and stroma is a novel finding and should be further investigated as potential biomarkers for patient stratification in larger cohorts. We identified alterations in lipid synthesis, β-oxidation, prostatic secretory function and inflammation in prostate tumors compared to stroma and benign epithelium. The differences between the defined tissue structures pinpoint the importance of the spatial information obtained by MALDI imaging.

Acknowledgement: This project was funded by the European Research Council (ERC) under the Horizon 2020 program (grant agreement No 758306), the Norwegian Cancer Society, the LINK program of the Dutch province of Limburg and the Liason Committee between the Central Norway Regional Health Authority (RHA) and NTNU.

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