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.

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

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<th>Material:</th>
<th>Methods:</th>
<th>Data analysis:</th>
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<td>- 45 prostate tissue samples from 15 patients</td>
<td>- MALDI-TOF MSI (rapiflex)</td>
<td>- Total ion count normalization</td>
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<tr>
<td>- Histopathology (uropathologist): benign epithelium, stroma, cancer</td>
<td>- Metabolites/ lipids (m/z: 100-1000)</td>
<td>- Pair-wise comparisons with OPLS-DA</td>
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<td></td>
<td>- Spatial resolution: 30 µm</td>
<td>(leave-one patient-out crossvalidation)</td>
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<td>- Matrices: DHB (+), NEDC (-)</td>
<td>- Verified with permutation testing</td>
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<td>- MS/MS on selected masses</td>
<td>(1000 iterations)</td>
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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].
- **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 1:** OPLS-DA models comparing benign epithelium and cancer: (a) Loading plot for (–) OPLS-DA model, and (b) Loading plot for (+) OPLS-DA model. *Expected identity, not verified with MS/MS, PE=phosphoethanolamines, PC=phosphatidylcholines, PG=phosphatidylglycerols, Ps=phosphatidylinositol and Ps=phosphatidylserine, SM=sphingomyelin.

**Figure 2:** Spatial distribution of citrate (–) and spermine (+) on two consecutive tissue sections. The spatial distribution of (a) citrate (m/z 191.02) and (d) spermine (m/z 203.27) were linked to (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 cancer to colorectal 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-acetyl carnitine transerase (CACT) and C1AT= carnitine O-acetyl-transerase.

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.

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