High-throughput non-targeted metabolic profiling with hybrid stationary phase LC column coupled to Q-TOF-MS in cancer research

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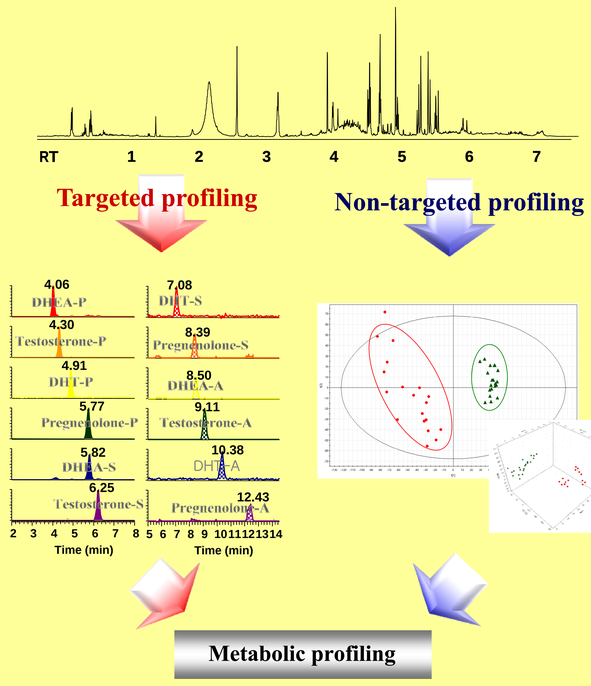
Introduction

Metabolomics is a suitable approach in monitoring early changes of metabolic pathways and these metabolic perturbations represent a biomarker of cancer, which is one of highly complex diseases. Although gas chromatography-mass spectrometry based approaches have been primarily used in metabolomics, difficulties in detection of polar compounds with high molecular weight or hydrophilicity limit this technique. In contrast targeted analysis, non-targeted metabolic profiling involves a large number of different metabolites with the objective of identifying a specific metabolite responsible for biological changes. A rapid and reproducible sample preparation is necessary in non-targeted metabolomics because it may affect reproducibility as a result of the heterogeneity of metabolites derived from cell populations. Here, we introduce a non-targeted metabolic profiling technique using hybrid stationary phase LC column coupled to a quadrupole-time-of-flight mass spectrometer (Q-TOF-MS) to identify altered metabolites and to find potential biomarkers, which may indicate progress of cervical and prostate cancers.

Metabolomic approaches



Experimental procedures

Sample pretreatment steps

200 µL Urine

Filteration

Inject 5 µL into Q-TOF-MS

Instrumental conditions

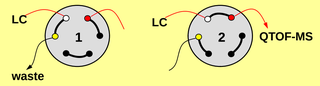
LC parameters

Instrument: ACQUITYTM Ultra Performance Liquid Chromatography system
Column: Cadenza HS-C18 (2.0 × 100 mm, 3 µm)
Flow rate: 0.4 mL/min
Injection volume: 5 µL
Column temperature: 40 ºC
Mobile phase:
A: 0.1 % formic acid in 5 % ACN
B: 0.1 % formic acid in 95 % ACN
Gradient:
100 % A (4 min) —10 min→ 10 % A —2 min→ 100 % A

MS parameters

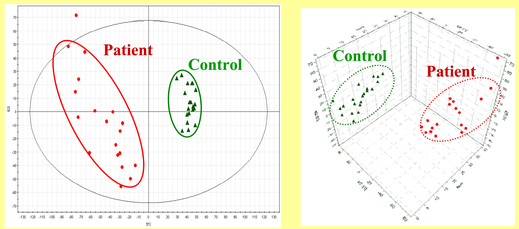
Instrument: Waters Q-TOF micro MS
Ionization: ESI+ ion modes
Acquisition mode: Scan (*m/z* 50-1,200)
Nebulization gas: 600 L/h
Cone gas: 60 L/h
Source temperature: 105 ºC
Capillary voltage: 3,100
Cone voltage: 40 V

Column switching

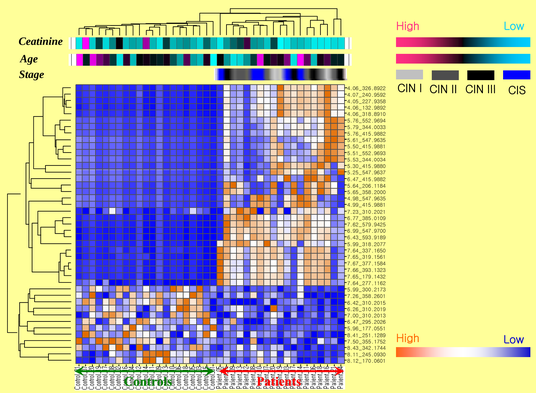


Statistical analysis

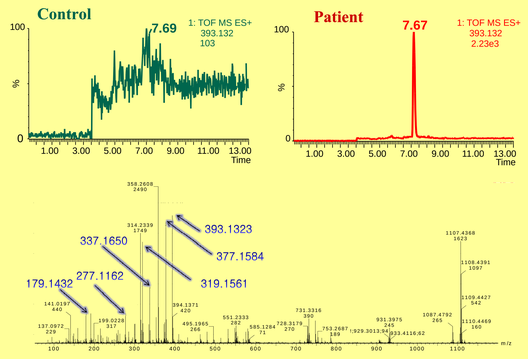
PLS-DA score plot



Hierarchical clustering analysis



Identification of potent biomarker



Search of Datasbase

319.1561

- Ubiquinone Q2
- Leukotriene A4
- 5 HEPE
- Epipregnanolone
- Alloepipregnanolone
- Allopregnanolone
- Melanin

377.1584

- Resolvin D1
- Resolvin D2
- 18-Oxocortisol
- 7a-OH-5b-cholanic acid
- 12b-OH-5b-cholanic acid
- Lithocholic aicd
- Isolithocholic acid
- Isoallolithocholic acid
- Allolithocholic acid

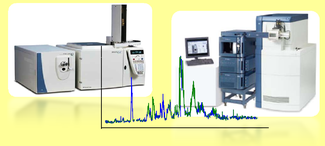
393.1323

- Allodeoxycholic acid
- Isodeoxycholic acid
- Deoxycholic acid
- Ursodeoxycholic acid
- Murocholic aicd
- Hyodeoxycholic acid
- 3a,7a-Dihydroxycholanoic acid
- 3b,7a-Dihydroxy-5b-cholanoic acid
- 3b,12a-Dihydroxy-5b-cholanoic acid
- 3a,12b-Dihydroxy-5b-cholanoic acid
- 3b,12a-Dihydroxy-5a-cholanoic acid
- 3b,12b-Dihydroxy-5b-cholanoic aicd
- Allochenodeoxycholic acid
- Chenodeoxycholic acid
- Isoursodeoxycholic acid
- Isohyodeoxycholic acid
- 7b,12a-Dihydroxycholanoic acid

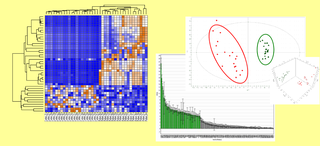
Procedure for Non-targeted profiling

Sampling

- Solid-phase extraction
- Liquid-liquid extraction
- Column switching

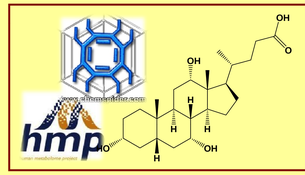


GC-Ms or LC-MS analysis



Statistical analysis

- PLS-DA with SIMCA-P®
- Hierarchical clustering with Decision Site®



Database search

Potent biomarker

"Targeted metabolic profiling"

Conclusion

Using the present non-targeted metabolic profiling technique combined with hybrid stationary phase LC column, two potential biomarkers which may indicate progress of cervical dysplasia was identified and they might be correlated with lipid or energy metabolism.