Imaging Mass Spectrometry to differentiate between pancreatic adenocarcinoma and cholangiocarcinoma

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Pancreatobiliary Neoplasias

Pancreatic ductal adenocarcinoma versus choriocarcinoma

Difficult Diagnostic Problems in Pancreatobiliary Neoplasia

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Immunohistochemical distinction between intrahepatic cholangiocarcinoma and pancreatic ductal adenocarcinoma
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Immunohistochemical Markers Distinguishing Cholangiocellular Carcinoma (CCC) from Pancreatic Ductal Adenocarcinoma (PDAC) Discovered by Proteomic Analysis of Microdissected Cells\textsuperscript{b}
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Annexin A10 optimally differentiates between intrahepatic cholangiocarcinoma and hepatic metastases of pancreatic ductal adenocarcinoma: a comparative study of immunohistochemical markers and panels
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The Novel Monoclonal Antibody HPC2 and N-cadherin Distinguish Metastatic Pancreatic Ductal Adenocarcinoma from Cholangiocarcinoma
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MALDI TOF MS

Matrix assisted laser desorption ionization time of flight mass spectrometry
Why Imaging Mass Spectrometry?

- Data can be obtained directly from intact tissue sections
  - no microdissection or tissue homogenization
- **Correlation with histology**
- Maps of molecular distribution throughout the tissue section
- Assessment of hundreds of analytes (e.g. proteins) in parallel
- **No need for target specific reagents** → ideal discovery tool
- Capable of high throughput
Imaging Mass Spectrometry (IMS)

- **Tissue slide**
- **Matrix application**
- **Laser ablation**
- **Tandem MS**
- **MS**
  - **MS/MS spectrum**
  - **Mass spectra for each x,y coordinate**
  - **Single m/z values**
  - **Biocomputational analysis**

- **Peptide fragments**
  - **Database search**
  - **Protein ID**
  - **Protein images**
  - **Classification images**

Modified from: Schwamborn and Caprioli, Nature Reviews Cancer 2010
Previous Study

- 33 pancreatic ductal adenocarcinomas (PDAC)
- 22 cholangiocarcinomas (CCC)
  
  (all duplicates)

Sensitivity CCC – 81.82%
Sensitivity PDAC – 71.05%
Tissue Microarrays

Cholangiocarcinoma (CCC) (3 TMAs, $N_{\text{total}} = 122$) triplicates

Pancreatic ductal adenocarcinoma (PDAC) (3 TMAs, $N_{\text{total}} = 107$) triplicates
Paraffin removal and antigen retrieval

FFPE tissue slide

Pneumatic sprayer
TM Sprayer, HTX Technologies

Trypsin application
0.025 µg/µl

Incubation @ 50ºC for 2h

Matrix application
10 mg/ml CHCA

Mass spectrometer
RapifleX, Bruker

Protocol: Ly et al., Proteomics Clin Appl. 2019
Tissue Microarrays – Histological Annotation

CCC

PDAC
Overall Sum Spectra
Methodology of Classification

60%  
Training

20%  
Validation

20%  
Test

LDA  
(linear discriminant analysis)
Results of Classification – PDAC

Pancreatic ductal adenocarcinoma:

Accuracy 90.48 %
(19/21 patients)

○ Correctly classified
○ Wrongly classified
Results of Classification – CCC

Choalangio-carcinoma:
Accuracy 100 % (22/22 patients)

Correctly classified
Wrongly classified
Conclusion
➢ Imaging Mass Spectrometry is an efficient and reliable tool for the classification of pancreatic ductal adenocarcinoma and cholangiocarcinoma
➢ Results can be achieved utilizing only a single tissue section

Outlook
➢ Identification of differentially peptides/proteins by MS/MS
➢ Validation of candidate proteins by IHC
➢ Applying the algorithm to samples from different institutions
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