Identification of preoperative diagnostic markers for discrimination between benign and malignant thyroid cyst by a proteomic approach

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Conflict of interest disclosure

Nothing to disclose
Outline

- Introduction
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- Results
- Conclusions
- Acknowledgment
Introduction

- Cystic PTC – 4-13% of all PTC
- Developed as mural nodule within a thyroid cyst
Fine needle aspiration biopsy (FNAB)

Cystic fluid only...

Insufficient amount of follicular cells
Cyst fluid

- Liquid component of thyroid cyst
- Accumulating various compounds of epithelial products
- Discarded in routine FNAB
- Little is known about protein content of fluid from benign and malignant thyroid cysts
Aim

To identify and evaluate proteins expressed by the cystic variant of PTC by protein profiling of fluid accumulated in benign and malignant thyroid cysts
Material

Fresh frozen cyst fluid and FFPE tissues samples

- Cystic PTC (n = 20)
- Benign thyroid cysts (n = 56)
Methods

- Depletion of high abundant proteins
- iTRAQ labeling
- Liquid chromatography tandem mass spectrometry (LC-MS/MS)
- Western blot
- Immunohistochemistry
- ELISA
Proteomic profiling data analyses

1581 proteins in total

1467 proteins in both iTRAQ pools

841 overlapped proteins

41 proteins with different expression levels (p<0.05)

59 proteins with a good predictive power
OPLS model of 59 proteins

- Gap junction beta-2 protein
- Isoform 2 of Immunoglobulin superfamily member 1
- Serum amyloid P-component
- Apolipoprotein A-IV
- Isoform 3 of ATP-binding cassette sub-family B member 9
- Fibrinogen beta chain
- Centromal protein of 135 kDa
- Isoform Gamma-A of Fibrinogen gamma chain
- Fibrinogen alpha chain
- Protein S100-A13
- Protein S100-A1
- Keratin, type I cyrtelatal 19
- Peptidyl-prolyl cis-trans isomerase B
- Carboxymethylbetanoclastic homolog
- Plastin-3
- Tubulin-folding cofactor B
- Phosphatidylinositol transfer protein beta isoform
- Glycogen phosphorylase, brain form
- Annexin A3
- Isoform 2 of Transmembrane glycoprotein NMB
- Ig mu chain C region
- Ig delta chain C region
- Isoform 2 of Prothymin alpha
- Translin
- Calpain small subunit 1
- Isoform 2 of Proteasome subunit alpha type-3
- Migration and invasion enhancer 1
- Proteasome subunit alpha type-6
- Proteasome subunit beta type-6
- Proteasome subunit beta type-7
- Proteasome subunit alpha type-2
- Isoform 2 of STE20-like serine/threonine-protein kinase
- Vimentin
- Isoform 2 of Nuclear mitotic apparatus protein 1
- Isoform Cytoplasmic of Glutathione reductase, mitochondrial
- Isoform 3 of Cullin-3
- Actin, cytoplasmic 1
- Heat shock protein HSP 90-alpha
- Phosphoglycerate mutase 1
- X-ray repair ccs-complementing protein 5
- 60 KDa heat shock protein, mitochondrial
- Isoform 2 of Protein diaphanous homolog 1
- Serpin B6
- NADP-dependent malic enzyme
- Tissue alpha-L-lucidase
- Isoform 7 of Plectin
- Twinfilin-2
- 14-3-3 protein gamma
- Leukotriene A-4 hydrolase
- Alpha-actinin-4
- Isoform 4 of Splicing factor 1
- 26S proteasome non-ATPase regulatory subunit 6
- Synaptic vesicle membrane protein VAT-1 homolog
- Beta-hexaminidase subunit alpha
- Eukaryotic translation initiation factor 3 subunit A
- Coatomer subunit epsilon
# Top 5 proteins

<table>
<thead>
<tr>
<th>Accession ID</th>
<th>Protein name</th>
<th>Gene symbol</th>
<th>No of peptides</th>
<th>Expression in cPTC vs. Benign</th>
<th>p-value</th>
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<td>cPTC-6</td>
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<td>cPTC-2</td>
<td>Benign-2</td>
<td>Benign-4</td>
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</table>

**ANXA3**

\[ \approx 36 \text{ kDa} \]

**CMBL**

\[ \approx 28 \text{ kDa} \]
Deregulation of S100A13

Cystic PTC

Colloid goiter
Deregulation of Cytokeratin 19

Cystic PTC

Colloid goiter
**Validation by ELISA and ROC curve analyses**

<table>
<thead>
<tr>
<th></th>
<th>CK-19</th>
<th>S100A13</th>
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<tbody>
<tr>
<td><strong>Protein concentrations by ELISA</strong></td>
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<tr>
<td>cPTC: mean (range)</td>
<td>59 ng/ml</td>
<td>244 pg/ml</td>
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<td>Benign cystic lesions: mean (range)</td>
<td>36 ng/ml</td>
<td>230 pg/ml</td>
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<td><strong>Receiver operating characteristics (ROC)</strong></td>
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<tr>
<td>Cut-off</td>
<td>55 ng/ml</td>
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<tr>
<td>Sensitivity</td>
<td>82%</td>
<td>94%</td>
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<tr>
<td>Specificity</td>
<td>82%</td>
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<tr>
<td>Area under ROC curve (AUC)</td>
<td>0.86</td>
<td>0.69</td>
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<td>Diagnostic accuracy</td>
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<td>Diagnostic odds ratio (DOR)</td>
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Conclusions

The proteins CK-19 and S100A13 are potential diagnostic markers in the cystic variant of PTC, which can be applied to cystic fluid in addition to regular FNAC.
Acknowledgment

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