A study of the expression of miR185, miR29a and their targets in IPF and lung cancer: a BALF study.

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- Common risk factors
- Poor response to treatment
- Poor prognosis
- Similar anatomical distribution

The Impact of Lung Cancer on Survival of Idiopathic Pulmonary Fibrosis
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• Genetic changes (microsatellite instability, telomere shortening, telomerase impairment).
• Epigenetic changes (promoter hypermethylation/global hypomethylation).
• Abnormal expression of micro RNAs.
• Cellular and molecular aberrances:
  ✓ Altered cell-cell communication
  ✓ EMT
  ✓ Delayed apoptosis
  ✓ Pathways (Wnt/b-catenin, PI3K/AKT, JAK/STAT, Tyrosine kinases and others....)

## Idiopathic pulmonary fibrosis and lung cancer: a clinical and pathogenesis update

*Katerina M. Antoniou*, Sara Tomassetti, Eliza Tsitoura, and Carlo Vancheri

<table>
<thead>
<tr>
<th>Epigenetic mechanism</th>
<th>IPF</th>
<th>Cancer</th>
<th>Effect</th>
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</thead>
<tbody>
<tr>
<td><strong>Noncoding RNAs</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>miR-21</td>
<td>Upregulated [71,72]</td>
<td>Upregulated [43,60]</td>
<td>Oncomir and promising serum biomarker for IPF</td>
</tr>
<tr>
<td>Let7d</td>
<td>Downregulated [74*]</td>
<td>Upregulated or downregulated [73]</td>
<td>Targets tumor suppressors including PTEN. Amplifies the TGF-b signaling pathway by targeting SMAD7</td>
</tr>
<tr>
<td>miR-210</td>
<td>Upregulated [78*]</td>
<td>Upregulated [79]</td>
<td>Hypoxia inducible, promotes fibroblast hypertrophy</td>
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<tr>
<td>miR-17–92 family</td>
<td>Downregulated [88]</td>
<td>Upregulated [86,87]</td>
<td>Targets tumor suppressors including PTEN, CDKN1A/ p21&lt;sup&gt;waf1&lt;/sup&gt;/cip1 causing increased cell proliferation, inhibition of apoptosis, angiogenesis, EMT transformation</td>
</tr>
<tr>
<td>miR-29 family</td>
<td>Downregulated [81]</td>
<td>Downregulated [82,85*]</td>
<td>Targets ECM proteins-like collagens targets DNMT3A/B leading to accumulation of aberrant epigenetic marks</td>
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</tbody>
</table>
65% of the CPG islands with altered methylation in IPF are also modified in LC.

The methylation profile of IPF shared similarities with both groups, but were more similar to LC group.
We have previously identified significant decrease of miR185 and mir29a in IPF BAL consistent to the profibrotic profile of lung macrophages in IPF. However there are few data published regarding LC. Is there common markers in the BALF of LC?

miR185:
• down-regulated both in IPF and LC
• associated with deregulation of cell cycle and cell proliferation
• alleviate TGFb-induced EMT and col(v) overexpression.
• Inhibits the PI3K-AKT pathway by targeting DNMT1 which activates PTEN through methylation of its promoter.

miR29a:
• down-regulated both in IPF and LC
• Targets DNMTs
• Reduces the expression of COL1a1 through inhibiting the phosphorylation of AKT.
METHODS

• BALF cells from: 57 IPF and 32 LC patients.

• MiR-185, miR29a and corresponding mRNA targets (DNMT1, DNMT3b, AKT1, AKT2) were analysed by RT-PCR.

• Expression levels were evaluated according to:
  • Disease
  • Age
  • smoking status
  • PFTs
  • Side of endobrochial lesion
  • Cytology
  • pathology results

<table>
<thead>
<tr>
<th>Demographics</th>
<th>LC (32)</th>
<th>IPF (n=57)</th>
</tr>
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<tbody>
<tr>
<td><strong>Age (p=0.01)</strong></td>
<td>67.7</td>
<td>72</td>
</tr>
<tr>
<td>**Gender (m/f)</td>
<td>26/6</td>
<td>46/11</td>
</tr>
<tr>
<td><strong>Pyrs (p=0.001)</strong></td>
<td>70,2</td>
<td>37,4</td>
</tr>
<tr>
<td>**Non smoker/smoker</td>
<td>2/26</td>
<td>13/43</td>
</tr>
<tr>
<td><strong>PFTs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC%</td>
<td>ND</td>
<td>78.6</td>
</tr>
<tr>
<td>FEV1%</td>
<td>ND</td>
<td>84.8</td>
</tr>
<tr>
<td>%</td>
<td>ND</td>
<td>83.8</td>
</tr>
<tr>
<td>TLC</td>
<td>ND</td>
<td>73.6</td>
</tr>
<tr>
<td>TLCO/SB</td>
<td>ND</td>
<td>52.3</td>
</tr>
<tr>
<td>KCO</td>
<td>ND</td>
<td>88.5</td>
</tr>
<tr>
<td><strong>BALF %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>81,3</td>
<td>76,6</td>
</tr>
<tr>
<td>Lympocytes</td>
<td>10,2</td>
<td>11,5</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>8</td>
<td>9,3</td>
</tr>
<tr>
<td>Eosinophils (p=0.01)</td>
<td>1,3</td>
<td>1,8</td>
</tr>
</tbody>
</table>
**RESULTS**

Mir185: inverse correlation with age ($R = -0.5$ $p=0.009$) and PYrs ($R = -0.45$ $p=0.02$)

DNMT3b

AKT1

AKT2
RESULTS

402 CpG islands

**IPF**

**LC**
RESULTS
Conclusion

• Similarities between IPF and LC were noticed at the level of miRNAs (miR-185 and miR-29a) with known implication in both diseases and their targets.

• However, DNMT1 seems to be downregulated in LC and further reduced in the presence of malignant burden in BALF.

• More targets of the two miRNAs at the level of mRNA and protein are planned to be estimated, such as col1a1 and Cox2a.

• Moreover, further evaluation of BALF cells profile are going to be estimated between the two diseases using flow cytometry.
Collaborators

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