Insights from transcriptomic and proteomic analysis of human aneurysmal cells

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Disclosure of Interest

Speaker name: Dr Florence PINET
• I do not have any potential conflict of interest
Objectives

• Identification without any *a priori* new biomarkers of abdominal aorta aneurysm

  • Deciphering the key cells in aneurysmal and control aorta

  • Isolate the cells in aortic biopsies by laser-microdissection

  • Screen specific cellular targets by proteomic and transcriptomic (a new class of regulatory RNAs: microRNA)

  • Validate the targets for diagnostic and prognostic purposes in dedicated clinical studies and in population samples
**Distribution of cells in aneurysmal and control aorta**

<table>
<thead>
<tr>
<th>Aorta</th>
<th>Aneurysmal aorta</th>
<th>Smooth muscle cells (α-SMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aneurysmal</td>
<td>M2 subtype</td>
<td>control</td>
</tr>
<tr>
<td>normal</td>
<td>M1 subtype</td>
<td>AAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Images:**

- **a, b, c:** Images of aneurysmal aorta showing different cellular distributions.
- **d:** Image of normal aorta.
- **e, f:** Images of smooth muscle cells (α-SMA) in control and AAA conditions.

**Immunohistochemistry:**

- **CD68:** M2 subtype macrophages are mainly located in the adventitia and M1 in the luminal side.
- **CD206:** In aneurysmal aorta, smooth muscle cells are only present in the media.

**Notes:**

- Aorta: complex tissue.
Laser microdissection and proteomic analysis of isolated cells from AAA biopsies

LMD isolation and characterization of M1 and M2 macrophages

M1 macrophages express TNF and IL1β

M2 macrophages express MRC1 and CCL18

Validation in cultured M1 and M2 macrophages

Laser microdissection allows specific isolation of M1 and M2 macrophages

Boytard et al, ATVB (2013)
Laser microdissection and proteomic analysis of isolated cells from AAA biopsies

Proteomic analysis of isolated M1 and M2 macrophages by LMD identify peroxiredoxin 1 as specific marker of M1 macrophage

The upregulation of PRDX-1, previously found in the plasma of AAA patients (Martinez-Pinna et al, 2011) may originate from proinflammatory M1 macrophages in the aneurysmal tissue.
microRNAs screening of isolated cells from AAA biopsies

LOCALISATION for MICRODISSECTION

- Immunostained
- Unlabeled adjacent section
- Surface microdissected 9.8 mm² [4.7-16.2]

SCREENING of 850 miRNAs

- 850 miRNAs screened
- 92 miRNAs detected
  - SMC: 54 miRNAs
  - Macrophages: 87 miRNAs
  - Control SMC: 40 miRNAs
  - AAA SMC: 49 miRNAs
  - M1: 50 miRNAs
  - M2: 75 miRNAs

Threshold of miR-29b value in aneurysmal cells

10 miRNAs selected for RT-qPCR analysis
miRNAs expression in isolated cells from aneurysmal and control aortas

- **miR 24**
  - M1 macrophages: **AAA** vs. **non-AAA**
  - M2 macrophages: **AAA** vs. **non-AAA**
  - SMC: **AAA** vs. **non-AAA**
  - Aorta: **AAA** vs. **non-AAA**

- **miR 199a - 3p**
  - M1 macrophages: **AAA** vs. **non-AAA**
  - M2 macrophages: **AAA** vs. **non-AAA**
  - SMC: **AAA** vs. **non-AAA**
  - Aorta: **AAA** vs. **non-AAA**

- **miR 1207-5p**
  - M1 macrophages: **AAA** vs. **non-AAA**
  - M2 macrophages: **AAA** vs. **non-AAA**
  - SMC: **AAA** vs. **non-AAA**
  - Aorta: **AAA** vs. **non-AAA**

- **miR 21**
  - M1 macrophages: **AAA** vs. **non-AAA**
  - M2 macrophages: **AAA** vs. **non-AAA**
  - SMC: **AAA** vs. **non-AAA**
  - Aorta: **AAA** vs. **non-AAA**

- **miR 34a**
  - M1 macrophages: **AAA** vs. **non-AAA**
  - M2 macrophages: **AAA** vs. **non-AAA**
  - SMC: **AAA** vs. **non-AAA**
  - Aorta: **AAA** vs. **non-AAA**

- **miR 29a**
  - M1 macrophages: **AAA** vs. **non-AAA**
  - M2 macrophages: **AAA** vs. **non-AAA**
  - SMC: **AAA** vs. **non-AAA**
  - Aorta: **AAA** vs. **non-AAA**

- **miR 29b**
  - M1 macrophages: **AAA** vs. **non-AAA**
  - M2 macrophages: **AAA** vs. **non-AAA**
  - SMC: **AAA** vs. **non-AAA**
  - Aorta: **AAA** vs. **non-AAA**

- **miR 29c**
  - M1 macrophages: **AAA** vs. **non-AAA**
  - M2 macrophages: **AAA** vs. **non-AAA**
  - SMC: **AAA** vs. **non-AAA**
  - Aorta: **AAA** vs. **non-AAA**

- **miR 451**
  - M1 macrophages: **AAA** vs. **non-AAA**
  - M2 macrophages: **AAA** vs. **non-AAA**
  - SMC: **AAA** vs. **non-AAA**
  - Aorta: **AAA** vs. **non-AAA**
miRNAs expression in isolated cells from aneurysmal and control aortas
## LILAS Study

<table>
<thead>
<tr>
<th></th>
<th>AAA (n=24)</th>
<th>PAD (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>68.0 ± 6.1</td>
<td>62.3 ± 6.6</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m2</strong></td>
<td>27.4 ± 3.7</td>
<td>26.3 ± 4.1</td>
</tr>
<tr>
<td><strong>Aortic diameter</strong></td>
<td>56.1 ± 2.3</td>
<td></td>
</tr>
<tr>
<td><strong>Cardiovascular risk factors, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>4 (17)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>Past smoking</td>
<td>17 (71)</td>
<td>13 (72)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>14 (58)</td>
<td>12 (67)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (63)</td>
<td>10 (56)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (17)</td>
<td>5 (28)</td>
</tr>
</tbody>
</table>

# LILAS study
Lamblin et al, *J Prot Res* 2010
Boytard et al, *ATVB* 2013
Let-7f a new potential biomarker of AAA
Conclusions

• Utility of profiling isolated aneurysmal cells to identify miRNAs and proteins

• Let-7f:
  – down-regulated in aneurysmal cells compared to control SMCs
  – A significant lower expression in M1 compared to M2 expression
  – A significant down-regulation in whole aneurysmal aorta compared to control aorta
  – Significant levels of circulating let-7f in AAA patients compared to PAD patients
Future directions

miRs identified in ATLOs
Human: miR-15a-3p, 30a-5p, 489-3p

miRs deregulated in VSMCs from aneurysmal tissue
Human: miR-516a-5p, 1260

miRs deregulated in endothelium of aneurysmal aorta
Animal: miR-1, 21, 133b, 205, 207, 378, 712, 1249, 1952, 1957

miRs deregulated in whole aneurysmal aortic tissue
Human: miR-20a, 21, 24, 27a, 27b, 28, 29b, 30a-3p, 30c-2*, 92a, 93, 99a, 124a, 126, 133a, 133b, 146a, 150, 155, 204, 205, 221, 222, 223, 302b*, 331-3p, 378, let-7f
Animal: miR-19a, 19b, 21, 24, 26a, 29b, 132, 195, 221

Fibroblast
Immune cell
Vascular smooth muscle cell
Endothelial cell

Future directions

HEAL: a machine learning framework
Future directions

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ATLOs

B lymphocytes

Mast cells

ATLOs (n = 4)

Whole tissue samples (n = 3)

A

miR-15a-3p

p = 0.0354

2-dCt

PAD

AAA

B

miR-30a-5p

p = 0.0381

2-dCt

PAD

AAA