Erythroid-Specific Transcriptional Changes in PBMCs from Pulmonary Hypertension Patients

Background I

- Pulmonary arterial hypertension (PAH) defined as mean pulmonary artery pressure (PAP) of > 25 mmHg (European Society of Cardiology et al., 2009).
- PAH can be a result of a wide array of underlying diseases
Etiology:

1. iPAH
2. CTEPH
3. Cardiac malformations
4. Pulmonary disease (e.g. Scleroderma $\rightarrow$ 10-15% develop PAH (Steen et al., 2005))
5. Others
Hypothesis

• Chronic exposure of PBMCs to a hypertensive pulmonary environment will manifest in specific transcriptional changes and reveal a difference between PH of various etiologies, Scleroderma (SSc) and healthy controls.
• Transcript profiles of PBMCs from:
  – 42 SSc associated PAH patients
  – 30 iPAH patients
  – 19 SSc patients
  – 8 patients with SSc complicated by interstitial lung disease and PH (SSc-PH-ILD)
  – 41 healthy controls
were compared to each other by Microarray analysis.
Microarray

- Allows genome wide analysis of gene expression data
- Either cDNA or synthetic Oligonucleotides used
- RNA is rewritten into cDNA and hybridized with Probes on the Microarray
- Fluorescent-labeled RNA is then analyzed by a laser
Results

- 118 genes were significantly up-regulated in the PH groups in which
- 7 genes were highly enriched for blood gas transport
- Subgroup of those 118 genes was involved in platelet biology

Venn diagrams illustrating the distribution of statistically significant disease-specific changes in gene expression.
Heat map illustration of the distribution of gene expression among all samples for genes selected by pathways.
Signature Identification

- Clustering of 296 genes showed a clear trend of cluster formation of correlated gene expression
- Clustering revealed 6 major patterns of related genes which largely overlap with groups mentioned above
• IR-DR, IR-UR and IN signatures were of less interest → non-specificity or inability to distinguish between disease groups

• Gene expression of Erythroid Differentiation Signature (EDS) and Platelet derived (PI) signature are specific mostly to the included disease groups of patients with PH (iPAH, SSc-PAH, SSc-PH-ILD)
Characterisation of gene expression by using disease specific gene sets derived from the *Mouse Genome Informatics*

Patterns of disease specific gene set expression were detected which mapped to mouse gene sets involved in multiple phenotypes of blood disorders.
• Marked elevation of blood-disorder specific gene expression in the PH groups, but not for SSc patients

• Foremost the difference between SSc-PAH and SSc patients is tremendous
• Those findings suggest that the afflicted PH patients might be identified by a gene expression shift in PBMCs which might be induced by tissue hypoxia as seen in PH
Tissue specificity?

- EDS expression is restricted only to a distinctive group of cells of the hematopoietic lineage (especially reticulocytes and bone marrow).
- Especially reticulocytes from cord blood (less mature) were highly enriched in comparison to reticulocytes from circulation.
• **ALAS2** and **ERAF** were highly increased → essential for terminal erythroid differentiation
• ALAS2 $\rightarrow$ hemoglobin production
• ERAF $\rightarrow$ nascent alpha globin incorporation into hemoglobin-A
• Conditions of hypoxia have shown to directly result in elevated levels of ALAS 2 (Kaneko et al., 2009)
Overexpression of ALAS 2 and ERAF in hypertension samples was confirmed by RT-PCR.

Expression levels of these two genes tracks very well with the EDS signature expression in general.

Those genes are mostly expressed in the fetal liver, bone marrow and in CD71+ early erythroid cells in the circulation.

External validation of Data: Risbano et al., 2010, Pendergrass et al., 2010.
Correlation of EDS with hemodynamic measurements

A. ALAS2 expression vs. RAmean
   IPAH
   \[ r = 0.78 \]

B. ALAS2 expression vs. CI
   IPAH
   \[ r = -0.45 \]

C. ALAS2 expression vs. PVRI
   IPAH
   \[ r = 0.75 \]

D. ALAS2 expression vs. PA Sat
   IPAH
   \[ r = -0.71 \]
• ALAS2 is significantly overexpressed in SSc-PAH relative to SSc patients.

• EDS signature is associated with erythrocyte development and is present in PH patients.

• EDS signature may be an indicator for increased red blood cell recruitment as a response to chronic local hypoxia.
• Therefore an increase in RBC trafficking may constitute a useful marker of PH disease and also of increased disease severity specifically in iPAH patients.
• EDS lacks correlation with hemodynamic measurements in SSc-PAH patients, maybe because of different etiologies of these two types of PH.
• Cell specific source of the EDS has not been identified yet
• ALAS2 and ERAF overexpression is mainly found exclusively in CD71+ erythroid progenitor cells
• EDS gene expression signature might be derived from a population of nucleated reticulocytes which co-sediment with lymphocytes and monocytes in the PBMC fraction
• EDS might be an important new marker in chronic diseases, foremost associated with hypertension/hypoxia

• At least in hypertension the expansion of immature precursor cells may actually constitute an active biological response to increasingly severe disease conditions