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Erythroid-Specific Transcriptional Changes in PBMCs from Pulmonary Hypertension Patients

Cheadle C, Berger AE, Mathai SC, Grigoryev DN, et al.(2012) PLoS ONE 7(4): e34951.

Philipp Hacker, March 2014

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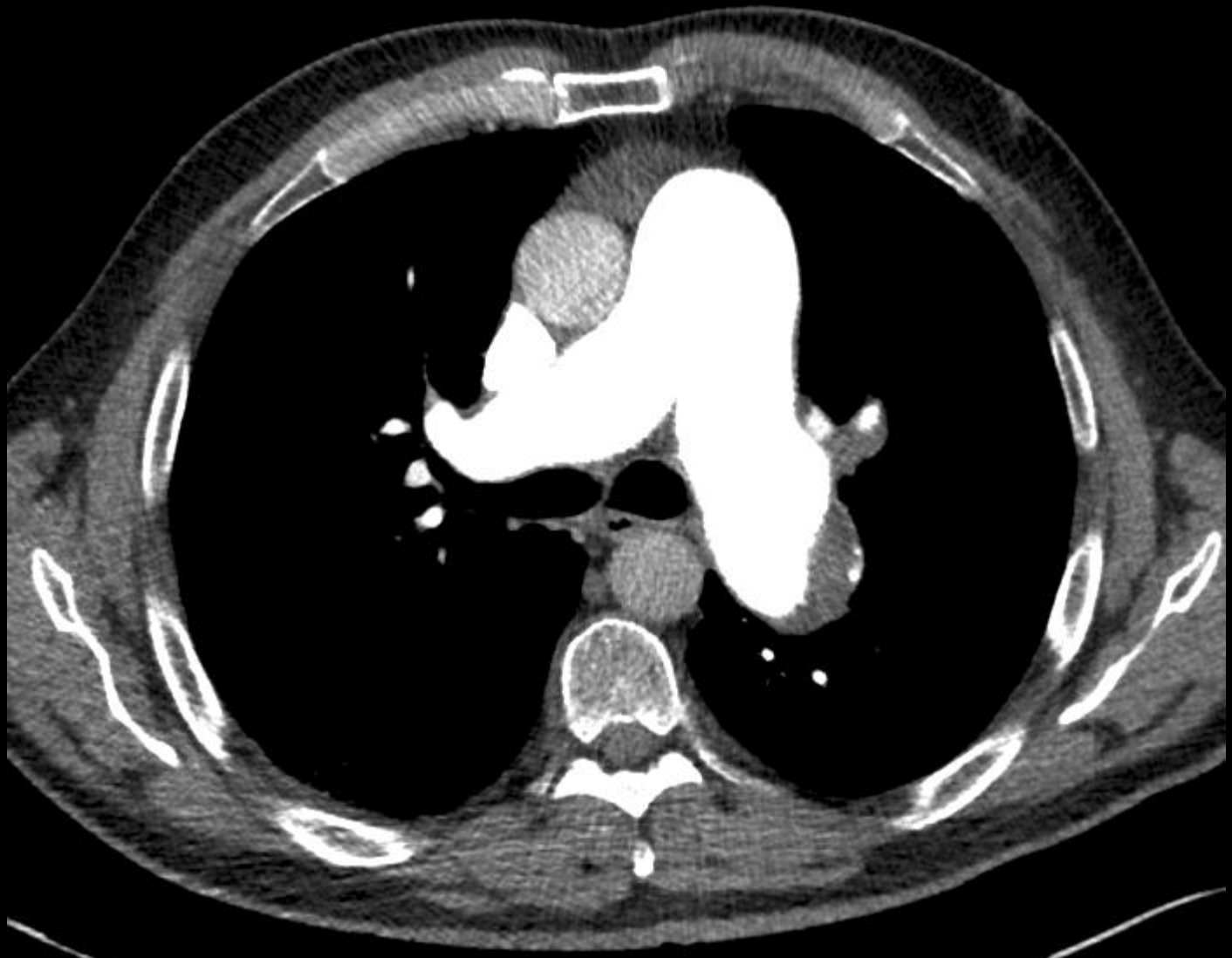
Background I

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



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Background II

Etiology:

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

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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.

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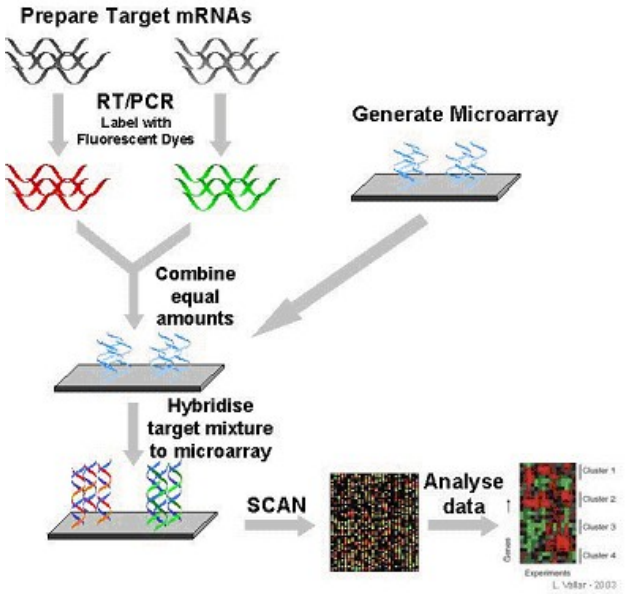
Microarray

- 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 Microarry
- Fluorescent-labeled RNA is then analyzed by a laser



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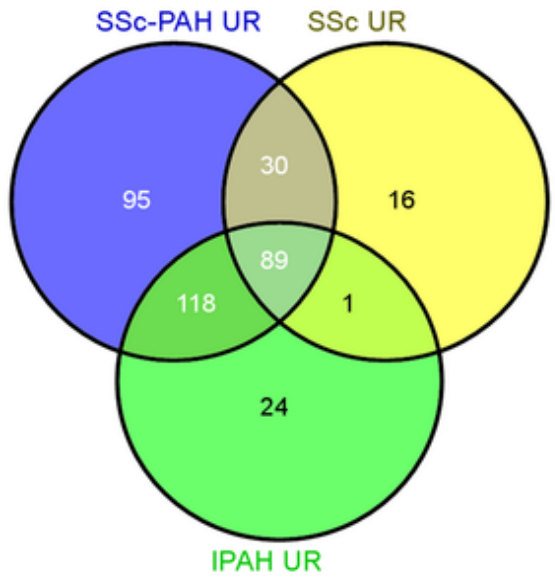
Results



SSc-PAH UR 332
SSc UR 136
IPAH UR 232

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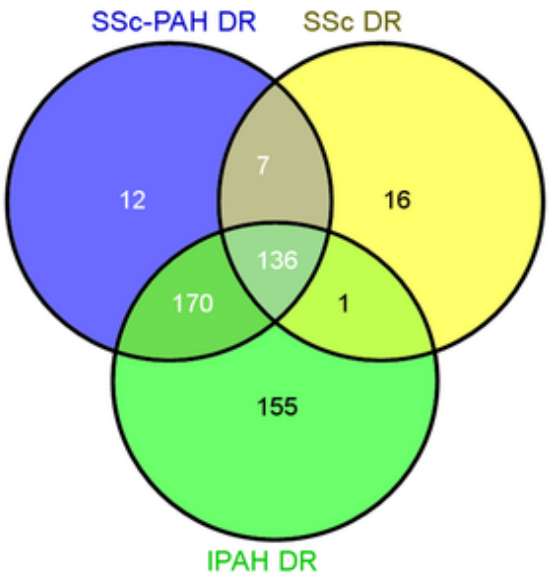

SSc-PAH DR 325
SSc DR 160
IPAH DR 462

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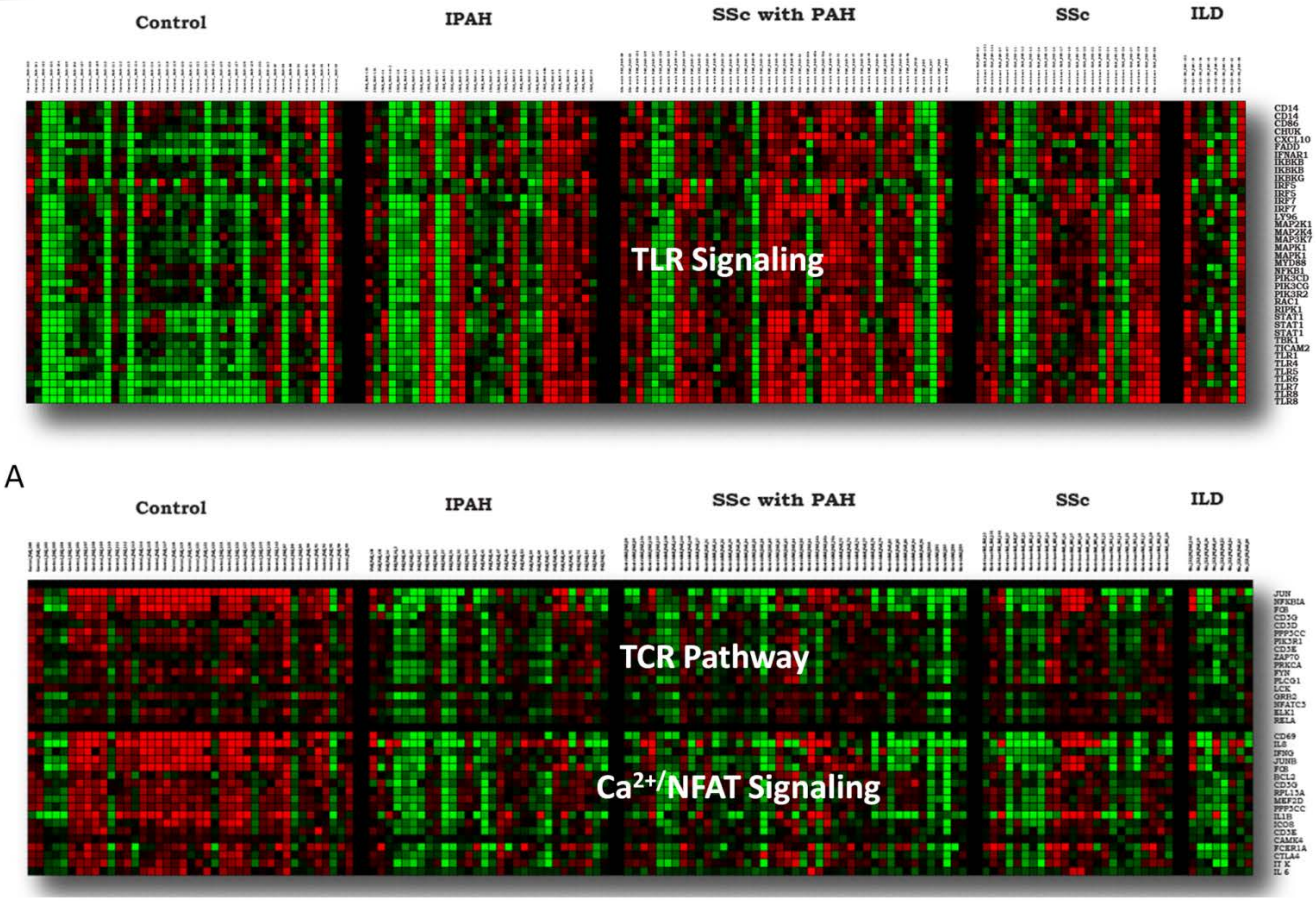
- 118 genes were siginificantly up-regulated in the PH groups in which
- 7 genes were highly enriched for blood gas transport
- Subgroup of those 118 genes was involved in platelet biology

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.

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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

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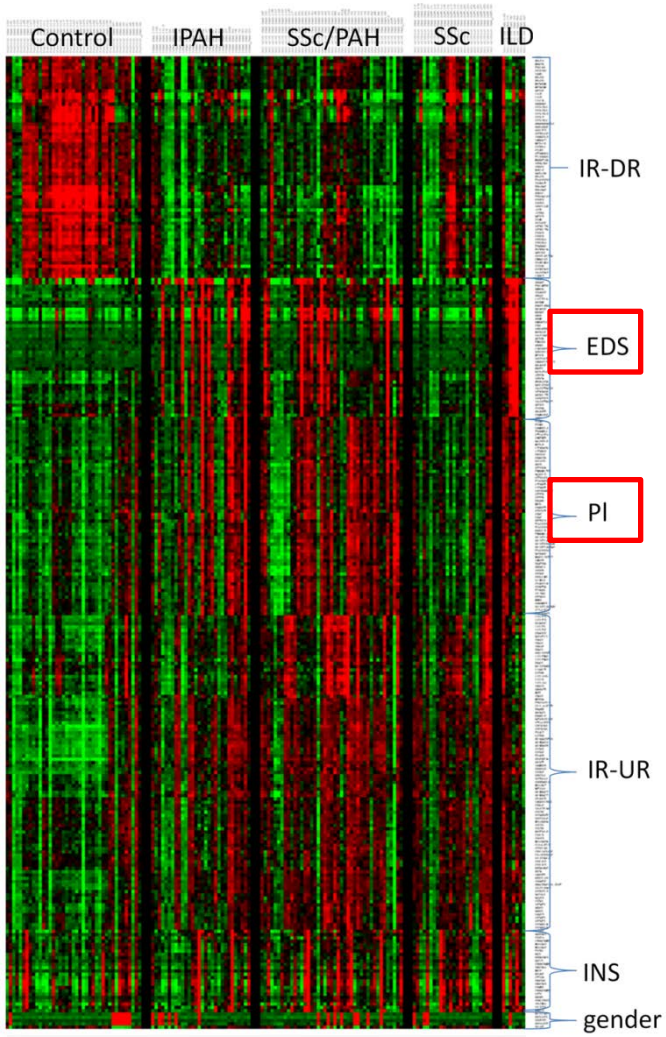
Signature Identification



- IR-DR, IR-UR and IN signatures were of less interest → non-specifity 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)**

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Signature Characterization

- 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

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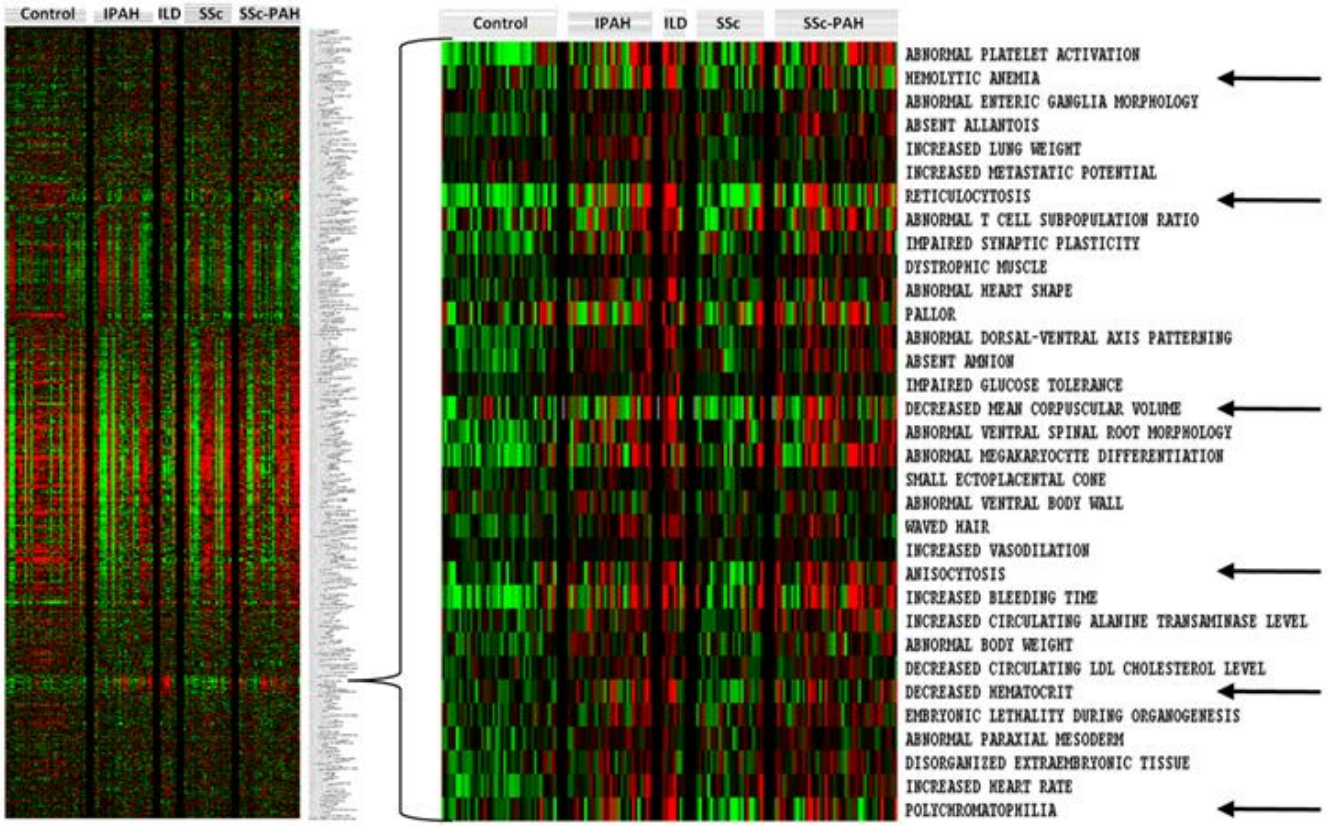

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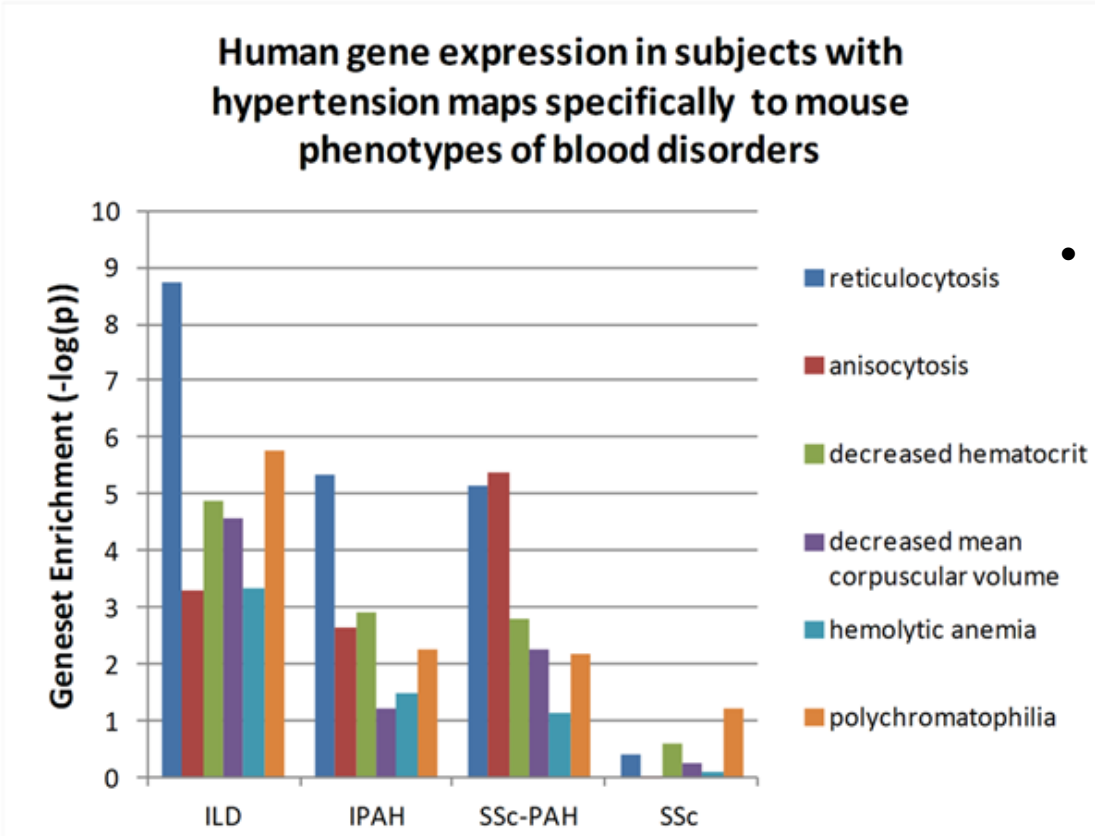

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- Marked elevation of blood-disorder specific gene expression in the PH groups, but not for SSc patients
- Foremost the difference between SSc-PAH and SSc patients is tremendous

- 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

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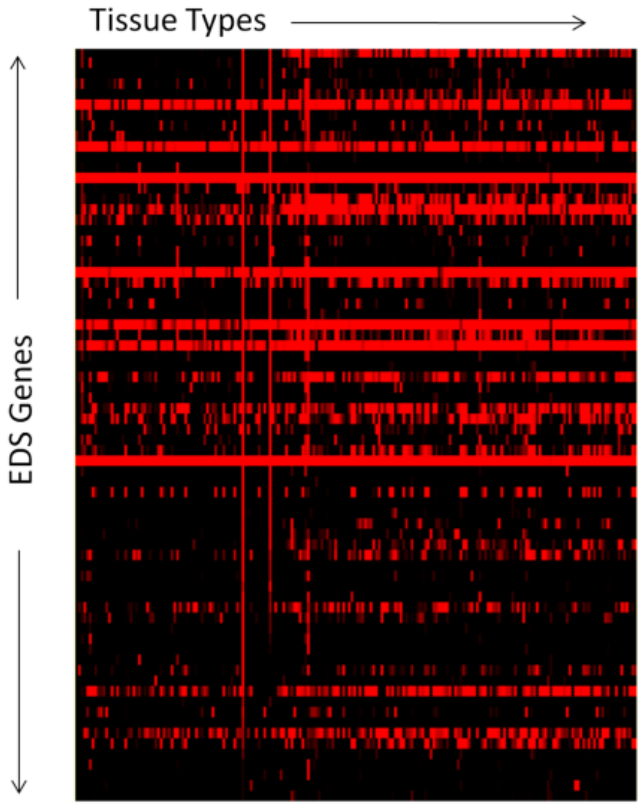
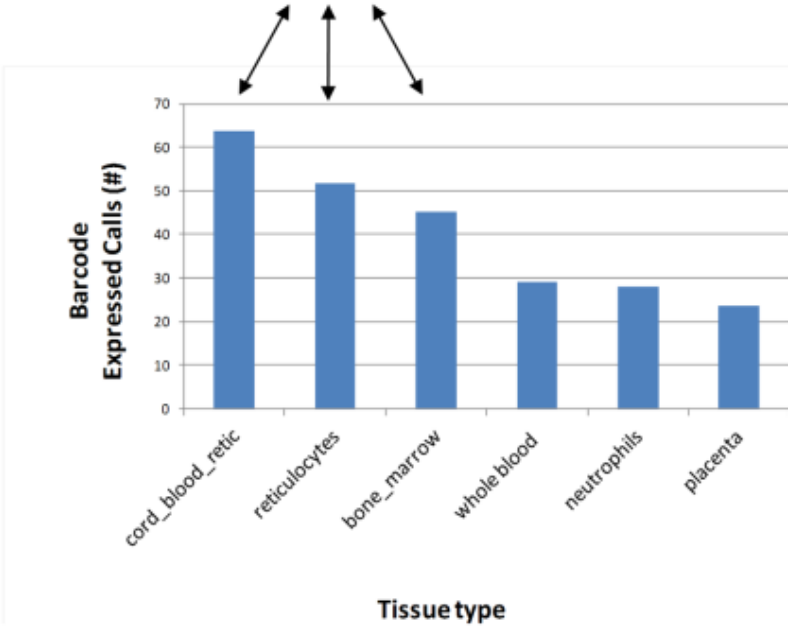
Tissue specifity?





- 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

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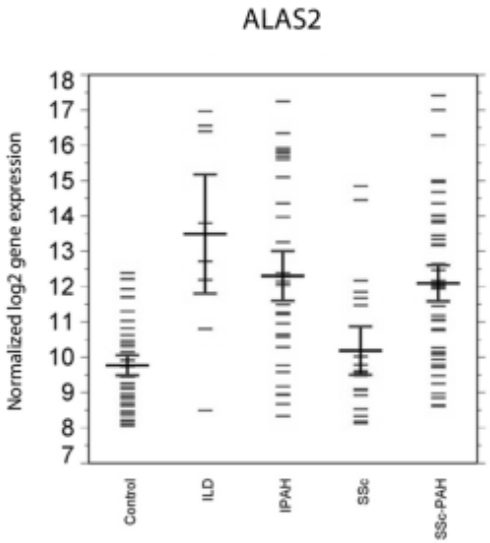

Comparison	p-Value
ILD-Control	0.0103
IPAH-Control	<0.0001
SSc/PAH-Control	<0.0001
SSc-Control	0.4215

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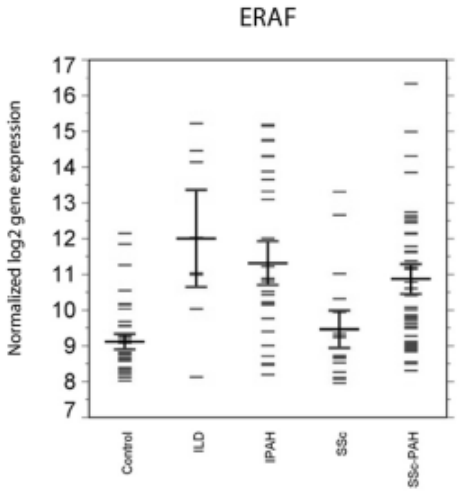

Comparison	p-Value
ILD-Control	0.0122
IPAH-Control	<0.0001
SSc/PAH-Control	<0.0001
SSc-Control	0.3771

- ALAS2→hemoglobin production
- ERAF →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

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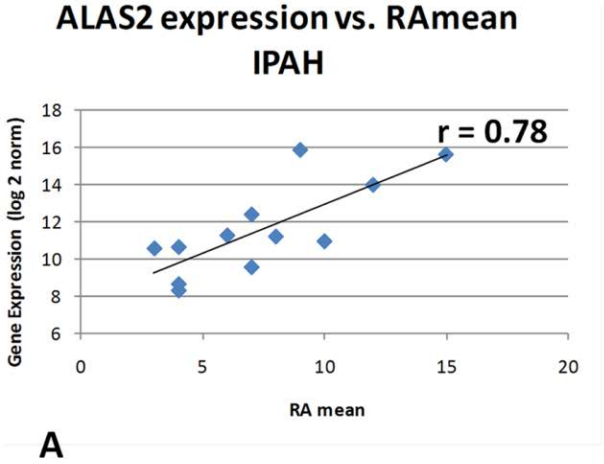
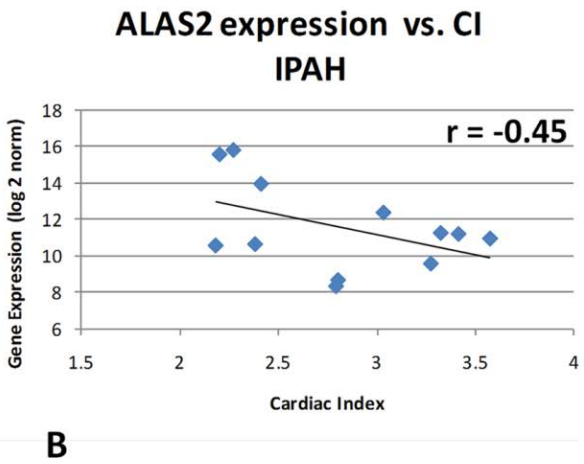
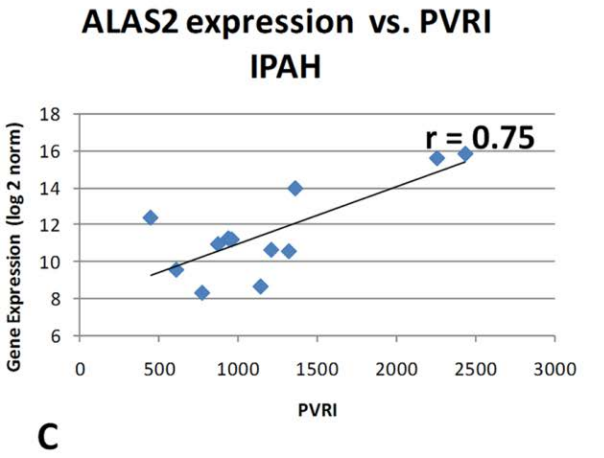
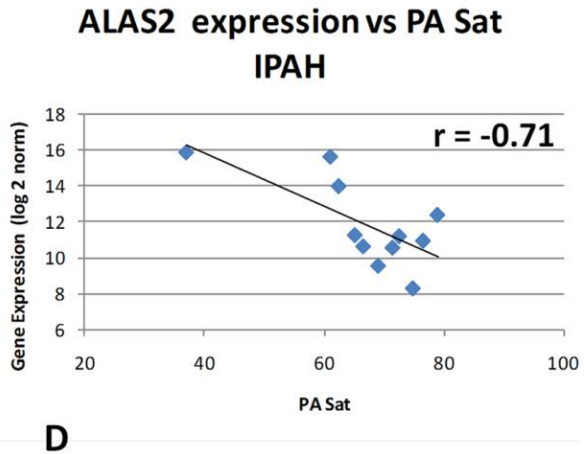
Correlation of EDS with hemodynamic measurements









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Discussion/ Summary

- 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 becausec 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