



Osteopontin lung expression is a marker of disease severity in pulmonary arterial hypertension



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Rationale. Proliferation of smooth muscle cells (SMCs) and pulmonary arterial remodelling are key mechanisms in the pathogenesis of pulmonary arterial hypertension (PAH). Osteopontin (OPN) is a pleiotropic cytokine involved in the proliferation of pulmonary vascular smooth muscle cells (PASMCS). We recently discovered that OPN is upregulated in the lungs of patients with PAH associated with pulmonary fibrosis, suggesting that the lung tissue is a source of OPN. We hypothesized that OPN lung expression is elevated in PAH and is correlated with hemodynamics.

Hypothesis. OPN lung expression is elevated in PAH and is correlated with pulmonary hemodynamics.

Methods.

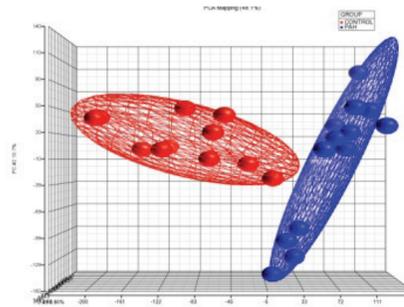
Microarray analysis (Affymetrix[®]) was performed after RNA was extracted from explanted lungs in 15 patients with PAH (m/f 2:13, age 41±12 years, 6 idiopathic PAH, 4 connective tissue disease, 4 congenital heart disease and 1 chronic thromboembolic PAH) who underwent lung transplantation (LTx) and 11 normal controls (normal lung tissue surrounding lung cancer resections). Pulmonary artery pressures (PAPs) were recorded intra-operatively immediately before starting LTx: average mPAP was 39±11 mmHg, wedge pressure 7±5 mmHg, pulmonary vascular resistance 701±208 dym.sec.cm⁻⁵.

Patients' characteristics

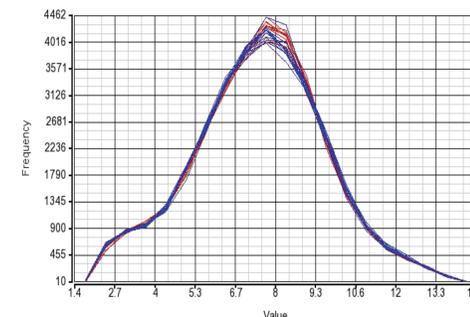
Variable	PAH (n=15)
Age (years)	41 ± 12
Gender (male/female) (%)	3/12 (20%)
BMI (Kg/m ²)	23 ± 4
RVSP (mmHg)	58 ± 19
DLCO (% pred)	57 ± 26
6-min walking distance (m)	271 ± 122
mPAP (mmHg)	56 ± 12
PVR (dym.sec.cm ⁻⁵)	1197 ± 595

BMI: body mass index
RVSP: right ventricle systolic pressure (2D echocardiogram)
DLCO: diffusing lung capacity
mPAP: mean Pulmonary Artery pressure
PVR: pulmonary vascular resistance

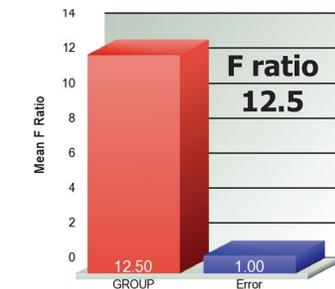
Distribution of Gene Expression shows distinct separation between normal and PAH Lung



Signal histogram showed no outliers in the analysis

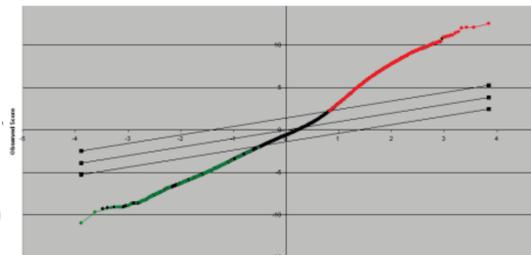


Sources of variation (all genes): signal-to-noise ratio is very high

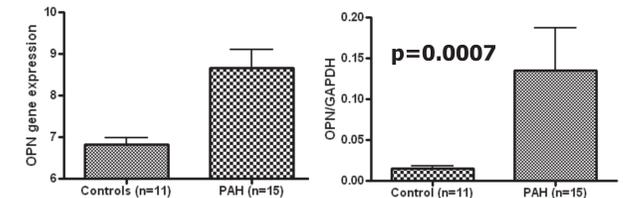


Significance analysis of microarray:

760 differentially expressed genes
636 upregulated in PAH
124 downregulated in PAH
Criteria: q value (false discovery ratio) <0.0001 and fold change ≥2.0



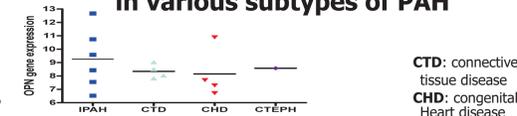
Lung gene expression level of OPN is significantly higher in PAH vs. ctrl



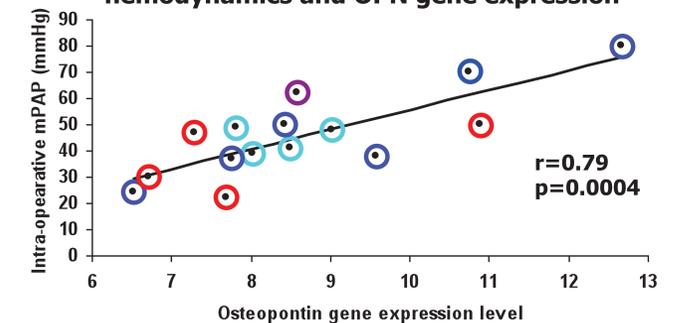
Top 5 upregulated genes in PAH (among 33,297 genes)

1. Periostin fold change 5.3
2. Solute carrier family 7-A11 f.c. 5.1
3. Peptidase inhibitor-15 f.c. 4.3
4. Tryptophan 2,3-dioxygenase f.c. 3.8
5. **Osteopontin f.c. 3.6**

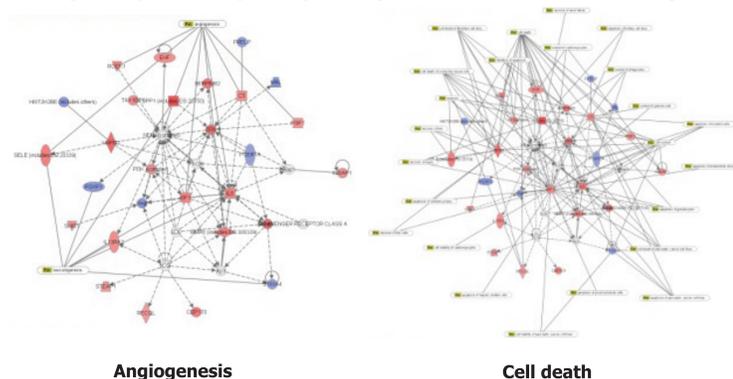
OPN gene expression is no different in various subtypes of PAH



Positive and significant relationship between hemodynamics and OPN gene expression



Ingenuity Pathway Analysis: significant networks involving OPN

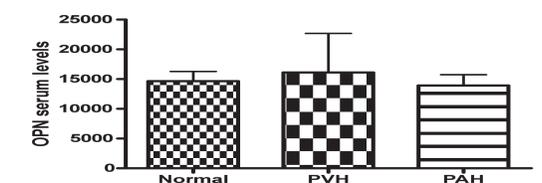


IPA: upregulated functions in PAH

Top Bio Functions		
Name	p-value	# Mo
Diseases and Disorders		
Cancer	1.10E-06 - 3.68E-02	177
Reproductive System Disease	4.34E-05 - 3.95E-02	102
Dermatological Diseases and Conditions	4.37E-05 - 3.34E-02	55
Genetic Disorder	4.37E-05 - 3.84E-02	95
Gastrointestinal Disease	9.12E-05 - 3.55E-02	76
Molecular and Cellular Functions		
Cell Cycle	1.56E-07 - 3.73E-02	63
Cellular Movement	1.37E-05 - 3.98E-02	58
Cellular Assembly and Organization	1.80E-05 - 3.42E-02	69
Cellular Function and Maintenance	1.80E-05 - 3.36E-02	46
RNA Post-Transcriptional Modification	1.08E-04 - 2.02E-02	14
Physiological System Development and Function		
Embryonic Development	5.67E-04 - 3.42E-02	25
Hair and Skin Development and Function	1.17E-03 - 2.72E-02	15
Tissue Development	1.17E-03 - 3.42E-02	39
Renal and Urological System Development and Function	2.42E-03 - 3.98E-02	12
Hematological System Development and Function	3.26E-03 - 3.98E-02	22

- PAH:
 - Cell growth
 - Cell death
 - Angiogenesis
 - Cell proliferation

OPN serum levels do not distinguish PAH from normal



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Conclusions. In the lungs of patients with severe PAH who failed medical therapy and underwent LTx, OPN is highly expressed and the level of expression is significantly correlated with disease severity. OPN may play an important role in the pathogenesis of PAH, driving an uncontrolled proliferation of PASMCS.