Exercise benefits in pulmonary hypertension: insights from systems biology.

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Abbreviations
ANN, artificial neural networks
NCAM1, neural cell adhesion molecule 1
PAH, pulmonary arterial hypertension
RCT, randomized controlled trial
SPARCL1, SPARC-like protein-1

An adjuvant therapy for pulmonary arterial hypertension (PAH), a condition characterized by complex vascular remodeling ultimately culminating in occlusive arteriopathy and right heart failure, is regular exercise (1). Yet, whether/how exercise impacts PAH pathobiology is unknown. Systems biology can help reveal the protein networks involved in disease conditions and how they are affected by treatment.

Using this approach, we studied the plasma proteome of a sub-group of PAH patients participating in a randomized controlled trial (RCT) where the intervention group performed an 8-week exercise program, which significantly improved a PAH-mortality predictor, cardiorespiratory fitness (*vs* no changes in inactive controls) (1). To minimize risk of bias given the multifactorial etiology of PAH, resting samples for proteomic analyses were collected before and after the RCT only in those participants (n=9 [5 from intervention group, 1 male, 35–53yrs] and 4 controls [1 male; 40–56yrs]) presenting with homogeneous pathophysiology (idiopathic, hereditary or connective tissue disease-associated PAH) and treatment (oral anticoagulants+phosphodiesterase-5 inhibitors+endothelin-receptor antagonists).

We determined protein expression with tandem-mass tag-based quantitative proteomics (2), and then used two statistical approaches to identify relevant proteins potentially associated with exercise-training effects on the pathobiology of PAH: Wilcoxon's test, to identify proteins that were differentially expressed as a result of the 8-week exercise training intervention (*vs* the control group); and threshold-based cross-validation methods, to identify proteins with the best between-group classifier potential (with 'good classifiers' allowing assignment of a given plasma sample to one of the two patient groups, exercise or control [*P*-value of accuracy-cross-validation<0.05, applying leave-one-out analysis]). For this second approach, we employed a

data-mining strategy developed by Anaxomics Biotech (www.anaxomics.com) using artificial intelligence-based methods.

From the 1,251 proteins identified, only 25 showed changes in their expression after the training program that were potentially associated with the pathobiology of PAH: 10 differentially-expressed, 12 good classifiers, and 3 accomplishing both criteria. To assess their biological role in PAH, we performed two different systems biology-based analyses. We initially studied the relationship of the identified proteins with those known to be relevant in PAH ('effector proteins') using protein-protein networks generated on the basis of a molecular definition of PAH that considered five main disease 'effector motifs' (including 81 effector proteins in total): *(i)* pulmonary vascular remodeling *(ii)* inflammation and *(iii)* vasoconstriction, *(iv)* vascular extracellular matrix remodeling, and *(v)* endothelial-to-mesenchymal transition. Public databases were consulted for PAH molecular characterization and protein-network generation (2). The direct physical/functional interactions between the differentially-expressed proteins and PAH effectors were evaluated, unveiling protein-protein links through which the 25 identified proteins could play a role in the pathobiology of PAH.

The potential molecular relationships between the identified proteins and PAH pathobiology were evaluated using artificial neural networks (ANN), through the application of 'Therapeutic Performance Mapping System' technology (http://www.anaxomics.com/ourtechnology/tpms/#tpms), which applies supervised machine learning methods based on human protein functional networks to infer clinical and protein level knowledge. We used ANN to determine relationships between proteins and clinical elements of the network, assigning a 0–100 ANN score to each protein according to its functional link to

PAH (globally or for each effector motif): strong (>76, P<0.05), medium-strong (40–76, P=0.05–0.25) or weak (<40, P>0.25).

The best candidates were cathepsin-D, NCAM1, neuropilin-1, profilin-1, and SPARC-like protein-1 (SPARCL1) (Table). Next, we studied the effects of *acute* exercise on these proteins in all the patients (n=19) of the RCT intervention group by measuring their plasma concentration before and after a typical RCT session (cycle-ergometer+resistance exercise) using enzyme-linked immunosorbent assays. Only neuropilin-1 concentration changed significantly (+6.7%, 3 replications average; Wilcoxon's P<0.034), a finding that was replicated in another cohort (n=9 healthy individuals [6 female, 40–55yrs]) (+15.5%, 3 replications average; P<0.029).

These latter results are in agreement with previous research showing increased skeletal-muscle neuropilin-1 mRNA levels following acute resistance exercise (3). This glycoprotein is involved in systemic/lung vascular development, and loss of semaphorin-3/neuropilin-1 signaling is associated with hypertensive changes in fetal lung arteriolar walls (4). Further research might determine whether neuropilin-1 acts, as our preliminary results suggest, as a muscle-derived factor (*i.e.*, myokine) with potential beneficial effects at the pulmonary vessel level (by attenuating vascular remodeling).

It remains to be established whether the benefits of regular exercise on PAH are mediated by *chronic* effects on protein networks (*e.g.*, NCAM1 down-regulation [Table]) and/or cumulative *acute* effects (*e.g.*, neuropilin-1 increases).

References

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Table 1. Candidate proteins linked to exercise training benefits in pulmonary arterial hypertension.

	Statistical strategy		Systems biology analysis		Bibliography
Protein (full name and abbreviation)	**Classifier (*P*-value)**	**Differentially expressed with exercise training *vs* control (Wilcoxon-test *P*-value)**	**Interactions with pulmonary arterial hypertension (PAH) effectors (identified by gene name)**	**Artificial neural network scores (0–100) for the corresponding effector motif**	**Main process (effector motif) in which the candidate protein is involved**
Cathepsin-D	✖ 0.357	✔ **0.032 (↓)**	✔ elastase, neutrophil expressed (ELANE), fibronectin-1(FN1), mitogen-activated protein kinase (MAPK)1	✔ (PAH, 59; pulmonary vascular remodeling, 50)	Pulmonary vascular remodeling
Neural cell adhesion molecule 1 (**NCAM1**)	✔ **0.040**	✔ **0.016 (↓)**	✔ angiopoietin- 1, epidermal growth receptor factor (EGRF), fibroblast growth factor 2 (FGF2), platelet-derived growth factor (PDGF) subunit A (PDGFA)and B (PDGFB), and receptor A (PDGFRA) and B (PDGFRB)r B, tyrosine kinase endothelial (TEK)	✔ (PAH, 72; pulmonary vascular remodeling, 64; pulmonary vasoconstriction, 70)	Pulmonary vascular remodeling
Neuropilin-1	✔ **0.048**	✖ 0.190	✔ FGF2, integrin α-5 (ITGA5), PDGFB, transforming growth factor β receptor 1 (TGFBR1), tumor necrosis (TNF)α	✔ (PAH, 71; pulmonary vascular remodeling, 46; endothelial-to-mesenchymal transition, 65)	Pulmonary vascular remodeling
Profilin-1	✔ 0.405	✔ **0.032 (↓)**	✔ FN1, hypoxia-inducible factor 1-alpha (HIF1A), MAPK3, vasoactive intestinal polypeptide receptor 1 (VIPR1)	✔ (PAH, 69; pulmonary vasoconstriction, 67)	Pulmonary vasoconstriction
SPARC-like protein 1 (**SPARCL1**)	✖ 0.405	✔ **0.032 (↑)**	✔ EGRF, TNFα	✔ (PAH, 72; pulmonary inflammation, 60)	Pulmonary vascular remodeling, vascular extracellular matrix remodeling

↑ increases (↓, decreases) with exercise training; ✔ fulfills (✖, does not) the corresponding condition.