DOI: 10.1002/pul2.12434

RESEARCH ARTICLE

Pulmonary Circulation Open Access | PVRI | WILEY

Transcriptome analyses reveal common immune system dysregulation in PAH patients and *Kcnk3*-deficient rats

Grégoire Ruffenach[1] | **Hélène Le Ribeuz**[2,3] | **Mary Dutheil**[2,3,4] | **Kristell El Jekmek**[2,3] | **Florent Dumont**[2,5] | **Anaïs Saint-Martin Willer**[2,3] | **Marc Humbert**[2,3,6] | **Véronique Capuano**[2,3,4] | **Lejla Medzikovic**[1] | **Mansoureh Eghbali**[1] | **David Montani**[2,3,4,6] | **Fabrice Antigny**[2,3]

[1]Department of Anesthesiology and Perioperative Medicine, Division of Molecular Medicine, David Geffen School of Medicine, University of California, Los Angeles, USA

[2]Faculté de Médecine, Le Kremlin-Bicêtre, Université Paris-Saclay, Le Kremlin-Bicêtre, France

[3]INSERM UMR_S 999 "Hypertension Pulmonaire: Physiopathologie et Innovation Thérapeutique", Hôpital Marie Lannelongue, Le Plessis-Robinson, France

[4]Groupe Hospitalier Paris Saint-Joseph, Hôpital Marie Lannelongue, Le Plessis-Robinson, France

[5]UMS Ingénierie et Plateformes au Service de l'Innovation Thérapeutique, Université Paris-Saclay, Orsay, France

[6]Assistance Publique—Hôpitaux de Paris (AP-HP), Service de Pneumologie et Soins Intensifs Respiratoires, Centre de Référence de l'Hypertension Pulmonaire, Hôpital Bicêtre, Le Kremlin-Bicêtre, Le Kremlin-Bicêtre, France

Correspondence
Fabrice Antigny, INSERM UMR_S 999, Hôpital Marie Lannelongue, 133, Ave de la Résistance, F-92350 Le Plessis Robinson, France.
Email: fabrice.antigny@inserm.fr

Funding information
Agence Nationale de la Recherche, Grant/Award Number: ANR-18-CE14-0023; French National Program "Investissement d'Avenir", Grant/Award Number: ANR-10-INBS-09; French National Institute for Health and Medical Research (INSERM); Université Paris-Saclay, the Marie Lannelongue Hospital; Federation Française de Cardiologie, and the French National Agency for Research (ANR); Fondation du Souffle et Fonds de Dotation Recherche en Santé Respiratoire; Fondation Lefoulon-Delalande and the Fondation Legs Poix Chancellerie des Université de Paris; Therapeutic Innovation Doctoral School, Grant/Award Number: ED569; American Heart Association, Grant/Award Numbers: 20CDA35350059,

Abstract

Pulmonary arterial hypertension (PAH) is a severe disease caused by progressive distal pulmonary artery obstruction. One cause of PAH are loss-of-function mutations in the potassium channel subfamily K member 3 (KCNK3). KCNK3 encodes a two-pore domain potassium channel, which is crucial for pulmonary circulation homeostasis. However, our understanding of the pathophysiological mechanisms underlying KCNK3 dysfunction in PAH is still incomplete. Taking advantage of unique *Kcnk3*-deficient rats, we analyzed the transcriptomic changes in the lungs from homozygous *Kcnk3*-deficient rats and wild-type (WT) littermates and compared them to PAH patient transcriptomic data. Transcriptome analysis of lung tissue obtained from WT and *Kcnk3*-deficient rats identified 1915 down- or upregulated genes. In addition, despite limited similarities at the gene level, we found a strong common signature at the pathway level in PAH patients and *Kcnk3*-deficient rat lungs, especially for immune response. Using the dysregulated genes involved in the immune response, we identified Spleen Associated Tyrosine Kinase (SYK), a significantly downregulated gene in human PAH patients and *Kcnk3*-deficient rats, as a hub gene. Our data suggests that the altered immune system

23POST1022457; National Institutes of Health, Grant/Award Numbers: R01HL159865, R01HL162124

response observed in PAH patients may be partly explained by KCNK3 dysfunction through the alteration of SYK expression.

KEYWORDS
immune system, K2P3.1, PAH, RNAseq

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare, severe, and progressive disorder characterized by high blood pressure in the pulmonary arteries. PAH is the consequence of a progressive thickening of small pulmonary arteries (<500 μm in diameter), which increases right ventricular (RV) afterload and consequently leads to RV hypertrophy, RV failure, and, ultimately, death.[1] Approximately 15−20% of patients suffering from PAH have heritable forms of PAH (hPAH) due to mutations in several predisposing genes. Most cases of hPAH are due to a pathogenic variant of the bone morphogenic protein receptor type II (*BMPR2*) gene.[2] However, at least 20 other PAH-predisposing genes have recently been identified, including pathogenic variants in the potassium channel subfamily K member 3 (*KCNK3) gene*.[3,4] KCNK3 is an outward K^+ channel, also referred to as TWIK-related acid-sensitive potassium channel 1 (TASK-1) or two-pore-domain K^+ (K_{2P}) channel 3.1 (K_{2P}3.1).[5] KCNK3 is a member of the K2P channel family[6] and possesses several characteristics of background K^+ current, extracellular pH sensitivity, resistance to classic K^+ channel blockers, and insensitivity to intracellular calcium.[3,4] KCNK3 contributes to resting membrane potential in many different cell types, including pulmonary arterial smooth muscle cells (PASMC).[7,8]

Since 2013, 28 variants of *KCNK3* have been identified in different international PAH cohorts.[4] Most KCNK3 variants are heterozygous, and only three were homozygous.[4] Among these variants, only eight *KCNK3* variants have been functionally analyzed by patch-clamp experiments, and these studies have revealed a loss of function,[4] while the functionality of the other 20 KCNK3 variants has not yet been investigated. In addition to pathogenic *KCNK3* variations in PAH, we and others demonstrated cumulative evidence that decreased KCNK3 expression and function are hallmarks of different forms of PAH at pulmonary vascular and cardiac levels.[4,7,9–11]

As KCNK3 is not functional in mouse PASMC,[12] we have generated unique *Kcnk3*-deficient rats. Using this model, we previously highlighted that genetic inactivation of *Kcnk3* induces significant pulmonary vascular alterations, facilitating the development of age-dependent-PAH, exacerbated pulmonary arterial constriction, impaired pulmonary artery relaxation, increased number of muscularized pulmonary vessels, increased perivascular collagen deposition, as well as altered pulmonary arterial compliance.[13,14] In addition to pulmonary vascular cells, KCNK3 is known to be ubiquitously expressed in the adrenal gland,[15] the brain, and carotid bodies, where it contributes to the regulation of breathing,[16–20] and also in lymphocytes (T and B cells) were KCNK3 inhibition reduced lymphocyte proliferation and cytokine production.[21,22]

Despite several pieces of evidence pointing to the contribution of KCNK3 dysfunction in the physiopathology of PAH, the mechanisms underlying KCNK3 dysfunction in PAH are not entirely understood. In the present study, using an unbiased comparative transcriptomic approach of PAH patients and *Kcnk3*-deficient rat lungs, we aimed to unravel the molecular signatures of KCNK3 deficiency that predispose to PAH.

METHODS

Kcnk3-deficient rats

Kcnk3-deficient rats were generated using CRISPR/Cas9 with a specific sgRNA-rKCNK3 and Cas9 messenger RNA (mRNA)[13,14,16,23] targeting the first exon of the *Kcnk3* gene to induce a shift in the reading frame of exon 1 of the *Kcnk3* gene. We used a strain with 94 bp deleted in the first exon of *Kcnk3* (Δ94ex1), as previously described.[13,14,16] In one newborn rat, a deletion of 94 bp (Δ94ex1) was found, which resulted in an out-of-frame shift in the open reading frame, leading to a premature stop codon and generation of a completely different amino acid (aa) sequence. However, premature stop codons can cause mRNA degradation.[24] The deletion of 94 bp in the mRNA was not associated with the absence of mRNA, indicating the absence of mRNA degradation.[24] We studied homozygous rats ($Kcnk3^{\Delta 94ex1/\Delta 94ex1}$, named *Kcnk3*-deficient rats in this study) and wild-type (WT) littermates. Only male rats at 3 months old were analyzed in this study.

As we previously described,[13,14,16] a putative translation of the truncated mRNA could produce a truncated

90 aa protein instead of the 411 aa of the WT protein and share only the first 14 aa with the WT protein. Sequencing of the *Kcnk3* mRNA from $Kcnk3^{+/+}$ and $Kcnk3^{\Delta 94ex1/\Delta 94ex1}$ rats confirmed the deletion of the 94 bp and an aberrant protein sequence with eight potential premature stop codons.[13]

The founder animal with the Δ94ex1 deletion was crossed with a WT partner, and the deletion was transmitted to the offspring, as shown by genotypic DNA analysis, demonstrating that the rats were either $Kcnk3^{+/+}$, heterozygous $Kcnk3^{\Delta 94ex1/+,}$ or homozygous $Kcnk3^{\Delta 94ex1/\Delta 94ex1}$. In this study, we used only $Kcnk3^{+/+}$ and homozygous $Kcnk3^{\Delta 94ex1/\Delta 94ex1}$.

RNA sequencing and data analysis

RNA extraction and purification

Total RNA was extracted from lung tissues using TRIzol (Invitrogen) with DNase digestion. RNA concentration and purity were evaluated on a NanoDrop (Thermo Scientific) spectrophotometer measuring absorbance at 230, 260, and 280 nm. RNA integrity was controlled on a Bioanalyzer 2100 using the RNA 6000 Nano kit (Agilent TechnologiesA). RNA concentration was measured on a Xenius (Safas, Monaco) fluorimeter using the Quantifluor® RNA System kit (Promega).

Transcriptome analysis by RNA sequencing

Directional RNA-Seq Libraries were constructed from 1 μg of total RNA using the TruSeq Stranded mRNA Library Prep kit (Illumina), following the manufacturer's instructions. Final libraries were qualified on a Bioanalyzer 2100 using the High Sensitivity DNA Kit (Agilent Technologies), and library concentration was measured on a Xenius fluorimeter (Safas) using the PicoGreen™ kit (Invitrogen). Libraries were pooled in equimolar proportions and sequenced in one 100-bp single read P2 run on a NextSeq. 2000 instrument (Illumina) by the I2BC High-throughput sequencing facility.

RNA-seq data analysis

All analyses were performed using R (https://www.r-project.org/) and RStudio (http://www.rstudio.com/). Reads were independently mapped to the rat genome mRatBN7.2 using a subread aligner[25] from the Rsubread package.[26] Mapped reads were counted with gtf ensembl release 110 at the gene level using featureCounts[27] from Rsubread.[26] Genes with a raw count mean lower than ten were discarded for downstream analyses. Then, data were first normalized using TPM,[28] TMM normalization[29] was done using edgeR packages,[30] and data were linearized, and quantile normalized using voom function[31] from limma package.[32] Then, we applied a one-way analysis of variance for the treatment factor for each gene and made pairwise Tukey's post hoc tests between groups.[33]

Human microarray analysis

We obtained the human lung microarray data from GSE117261.[34] We compared lungs from failed donors for transplantation ($n = 25$) to lungs from PAH patients ($n = 58$). We performed the differential gene expression analysis using the R limma package (R Core Team (2018).[32]

Bioinformatic analysis and statistical analysis

Bioinformatic analysis was carried out following the comprehensive workflow described hereafter. 1. We utilized Gene Set Enrichment Analysis (GSEA) software in conjunction with human Gene Ontology biological process gene sets with 1000 permutations, a gene set size between 15 and 1000, and a classic enrichment statistic method to analyze the human and rat transcriptomic datasets. We considered gene set dysregulation statistically significant for an FDR below 0.25, as per the GSEA documentation.[35,36] 2. We performed hierarchical clustering in Cytoscape using the significant and overlapping gene sets between humans and rats. 3. We employed String software to cluster genes involved in the immune response with a p-value below 0.05 in humans.[37,38] 4. To identify the most relevant gene, we correlated the clustered gene between the Edges and Nodes of the hierarchical clustering.

Real-time- quantitative PCR

Total RNA from lungs and PASMCs was isolated with TRIzol (Thermo Fisher) and reverse-transcribed with a combination of random primers and poly-dT primers using the Omniscript reverse transcription kit (Qiagen). Real-time polymerase chain reaction was performed on cDNA with specific primers using iTaq Universal SYBR (Bio-Rad). PARK7 (Parkinson Disease Protein 7) was used as a housekeeping gene to normalize the transcript

expression. Primer sequences used are as follows PARK7 Forward: CGGGGTGCAGGCTTGTAAA and Reverse: TGACCACATCACGGCTACAC; SYK Forward CTTGGT CAGCGGGTGGATAATCT and Reverse: AGGCTTTGGG AAGGAGTATGATTT.

RESULTS

To determine the molecular consequences of KCNK3 deficiency in the lung and its link to PAH pathophysiology, we conducted RNA-seq to profile the transcriptome of lung tissues from 5 WT and 5 *Kcnk3*-deficient rats. Through the RNA-seq experiments, we detected 17606 unique genes, including 2940 unidentified genes (noncoding genes or unconfirmed new genes) (Supporting Information S1: Table 1). The principal component analysis showed clear, distinct patterns of the gene expression profiles for WT and *Kcnk3*-deficient rats (Figure 1a).

RNA-seq analysis revealed 1915 differentially expressed genes (DEG) between WT and *Kcnk3*-deficient rat lungs ($p < 0.05$ and fold change > 1.2) (Figure 1b). As illustrated in Figure 1c, gene ontology analysis found that dysregulated genes (DEG) in lungs from *Kcnk3*-deficient rats were involved in the regulation of cell movement, cell communication, and immune regulation.

To unravel the molecular signature of KCNK3 deficiency that predisposes to PAH, we examined the gene expression relationship between *Kcnk3*-deficient rats and PAH patient lungs (Figure 2a–c). We integrated our RNA-seq on the lungs of *Kcnk3*-deficient rats with an available online human microarray comparing the gene expression of lungs from control subjects and PAH patients[34,39] (Figure 2a,b). We then overlapped the DEG from both comparisons and found a small number of commonly DEG, 0.5% of human DEG and 1.1% of rats DEG (Figure 2c).

Given the restricted overlap in DEG and the established link between PAH and KCNK3 dysfunction, we hypothesized that the relationship operates at the pathway level rather than the gene level (Figure 2d,e). Therefore, we identified commonly dysregulated biological processes in humans and rats and discovered 268 processes altered in PAH patients and *Kcnk3*-deficient rats, including 18 upregulated and 250 downregulated processes (Figure 2f).

To obtain a coherent image of these biological processes, we performed hierarchical clustering using Cytoscape (Figure 3). This analysis showed that downregulated processes in humans and rats were predominantly associated with the immune response. Subsequently, we employed String software to identify hub genes by clustering genes related to the immune response with a significant p-value (Figure 4a) to determine the most interconnected genes in the hierarchical clustering (Figure 4b). The analysis revealed that the downregulation of SYK (Spleen Associated Tyrosine Kinase) gene in Human PAH and *Kcnk3*-deficient rats (Figure 4c) was central to one cluster and displayed the highest connectivity (Figure 4a,b). In addition, using online lung single cell (LungGENS) on healthy adult human, we found that Syk was primarily expressed by expressed by dendritic cells and macrophages (Supporting Information S2: Figure 1).

Together, these data show that *Kcnk3*-deficiency induces a transcriptome-wide deregulation, vastly affecting immune response and creating a fertile ground for PAH genesis.

DISCUSSION

This study compared gene expression between the lungs of PAH patients and *Kcnk3*-deficient rats. We demonstrated that despite limited similarities in DEGs, numerous biological processes, especially the ones involved in immune response, were highly similar. Using the DEGs involved in the immune response, we identified SYK as a hub gene.

In a previous study, we found that KCNK3 function is impaired in PAH patients regardless of whether they carry a mutation or not.[7] Here, we demonstrate that the transcriptome landscape modification in *Kcnk3*-deficient rats is related to the one found in humans with PAH. This relationship is found at the pathway level rather than gene expression. Pathway analysis is based on the aggregation of genes into one biological pathway and how the expression of this group of genes changes between conditions. This gives access to readily interpretable data that may carry more information than gene expression alone.

This pathway-level analysis revealed that pathways involved in the immune response were at the core of similarities found between *Kcnk3*-deficient rat and PAH patient lungs. This finding is in line with the well-described dysregulation of immune/inflammatory response in PAH[40] (Supporting Information S2: Figure 2). In another animal model, using *Kcnk3* knockout mice fed with a Western diet, similar dysregulation of the immune system was observed.[41] We previously showed that chemical inhibition of KCNK3 in rats led to increased serum concentration of inflammatory markers, such as monocyte chemoattractant protein-1,

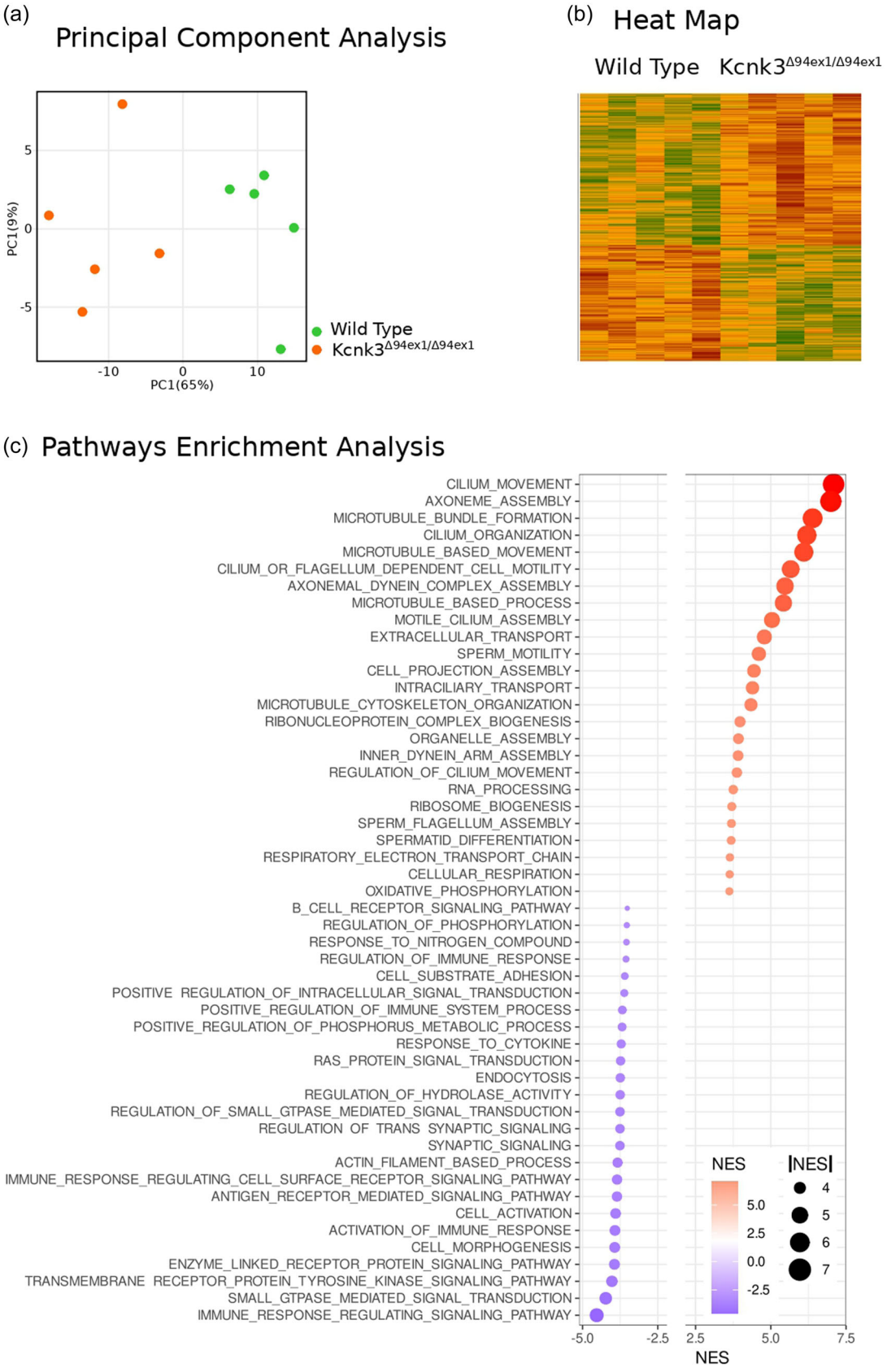

FIGURE 1 The consequence of KCNK3 deficiency at the transcriptomic level. (a) Principal component analysis (PCA) was performed on the relative mRNA expression level between lung tissues from WT and *Kcnk3*-deficient rats obtained by spectral counting (WT [Red] or *Kcnk3*-deficient rats [blue]). $n = 5$ rats. (b) Heatmap representation for RNA sequencing results in lung tissues from WT and *Kcnk3*-deficient rats. (c) Gene ontology analysis of upregulated (left) and downregulated (right) expressed genes between lung tissues from WT and *Kcnk3*-deficient rats. KCNK3, potassium channel subfamily K member 3; mRNA, messenger RNA; WT, wild-type.

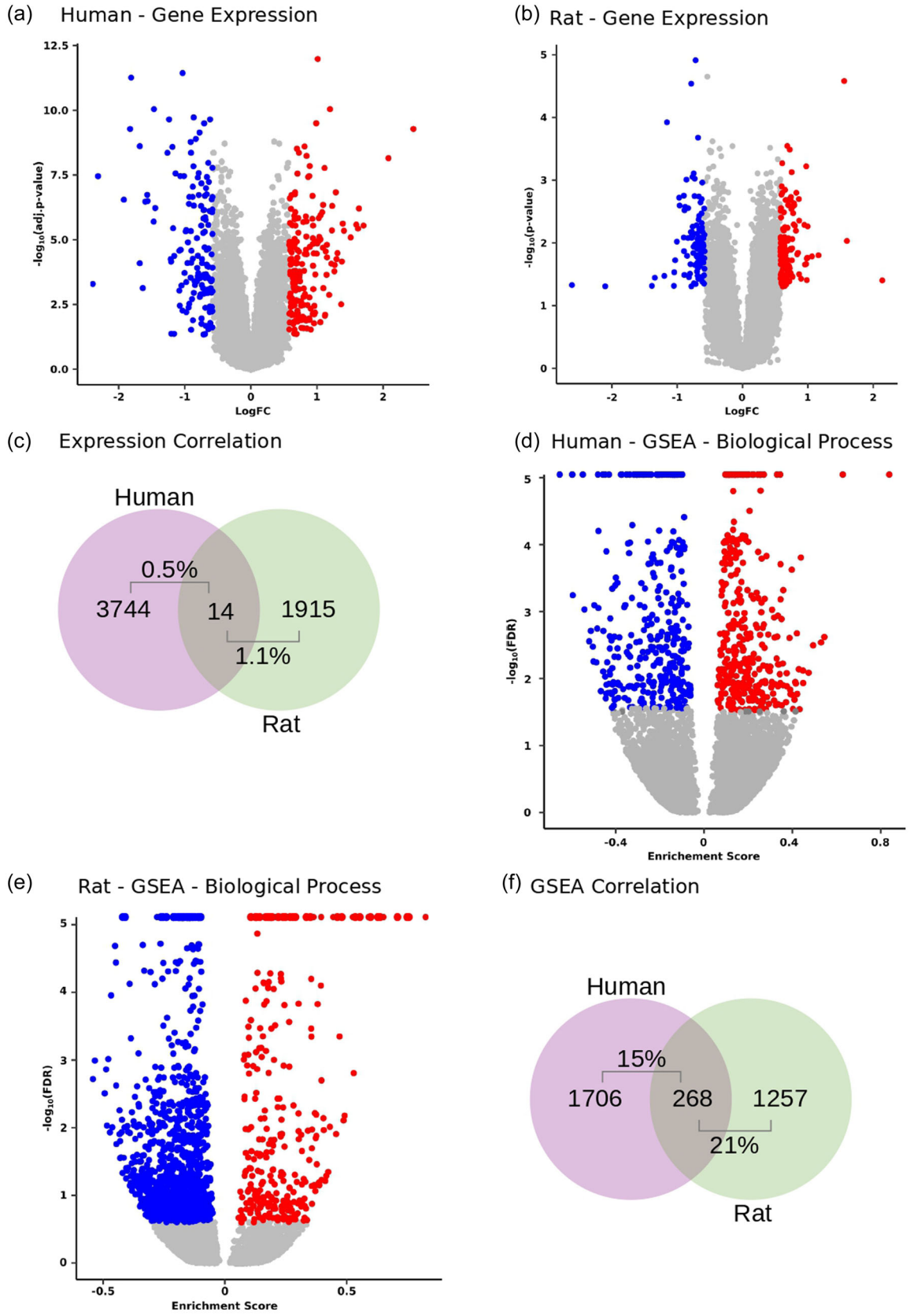

FIGURE 2 Expression profiling of lung genes in PAH and *Kcnk3*-deficient rats. (a) Volcano plot of differentially expressed genes in PAH lungs versus control lungs. (b) Volcano plot of differentially expressed genes in *Kcnk3*-deficient rat lungs versus control rat lungs. (c) Venn diagram of commonly dysregulated genes between humans and *Kcnk3*-deficient rats. (d) Gene set enrichment analysis of biological processes in PAH lungs. (e) Gene set enrichment analysis of biological processes in *Kcnk3*-deficient rat lungs. (f) Venn diagram of commonly enriched gene sets between humans and *Kcnk3*-deficient rats. KCNK3, potassium channel subfamily K member 3; PAH, pulmonary arterial hypertension.

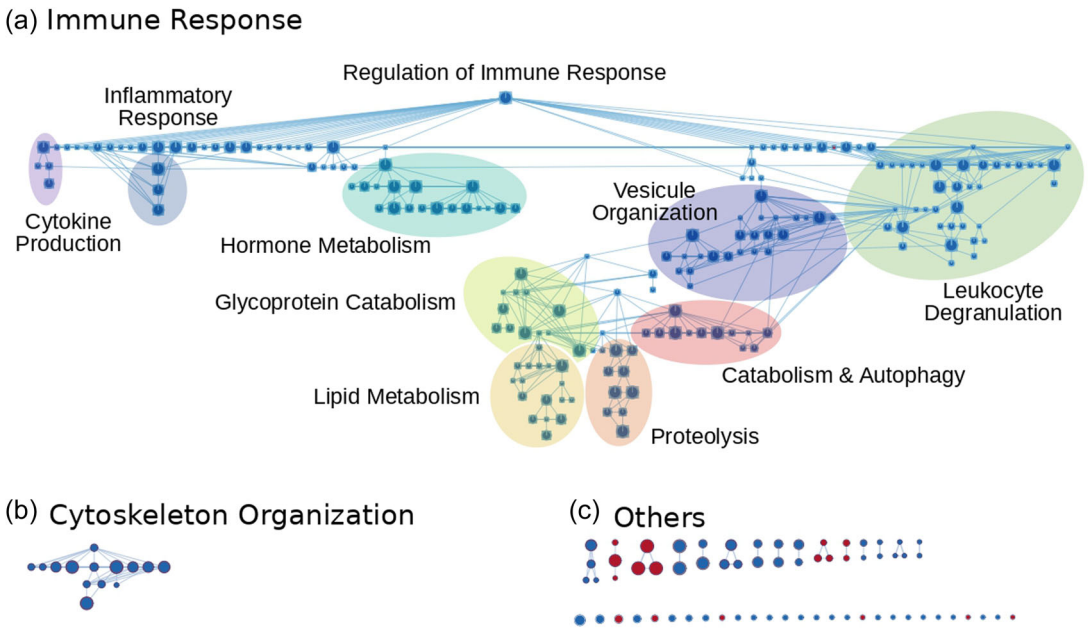

FIGURE 3 Hierarchical clustering of commonly dysregulated gene sets. (a) Immune response, (b) cytoskeleton organization, (c) other small clusters.

tissue inhibitor of metalloproteinase-1, and Gro-1 (melanoma growth stimulating activity, alpha 1).[7] Other studies showed that *Kcnk3* knockout mice had a decreased number of circulating lymphocytes[41] or impaired T cell effector functions.[22] Hence, this study adds strong support for the role of KCNK3 in the regulation of the immune response, which has yet to be uncovered.

We found the SYK gene as a hub common gene related to inflammation and dysregulated in PAH patients and *Kcnk3*-deficient rat lungs, as it was the most connected to the dysregulated inflammatory pathways. SYK is a cytoplasmic kinase, mainly expressed by dendritic cells and macrophages (Supporting Information S2: Figure 1), that binds to the immunoreceptor tyrosine-based activation motif of Fc receptors, C-type lectin receptors (CLECs), and B cell receptors.[42] SYK is involved in various biological processes, including innate immunity recognition, platelet activation, and vascular development,[43] all of which are known to play a role in pulmonary hypertension. In PAH, the role of SYK remains unknown. One study on primary cultured vascular SMC demonstrated whether it is protective or deleterious. The effect of platelet-derived growth factor subunit B (PDGF-BB) on SMC proliferation was, in part, attributable to SYK.[44] We recently identified the KCNK3 interactome using proximity labeling and mass spectrometry analyses,[45] and we have found that KCNK3 interacts with more than 1000 different proteins, notably different kinases or receptor tyrosine kinases, including Src Kinase, C-Jun, and VEGF-VEGFR2, PI3K/AKT, insulin receptor signaling cascade, PDGF.[45,46] In addition, we also found that KCNK3 knockdown in hPECs reduced VEGFR signaling, which could affect SYK expression and function since the VEGFR signaling pathway and SYK are intimately linked.[47,48]

Our data suggests that the altered immune system response observed in PAH patients could be partly explained by KCNK3 dysfunction through decreased SYK expression and open a new avenue of research in the role of KCNK3 in PAH physiopathology.

LIMITATIONS

Considering the sex differences that exist in PAH patients, it would be exciting to investigate the sex differences in the context of KCNK3 deficiency. However, no report thus far suggests a gender bias in KCNK3 expression, and we did not find a difference in KCNK3 expression between male and female patients in our Human PAH samples. Moreover, we previously demonstrated that female *Kcnk3*-deficient rats are predisposed to develop PAH similarly to males.[13] Further experiments are needed to investigate the consequence of *Kcnk3*-deficiency in female rats.

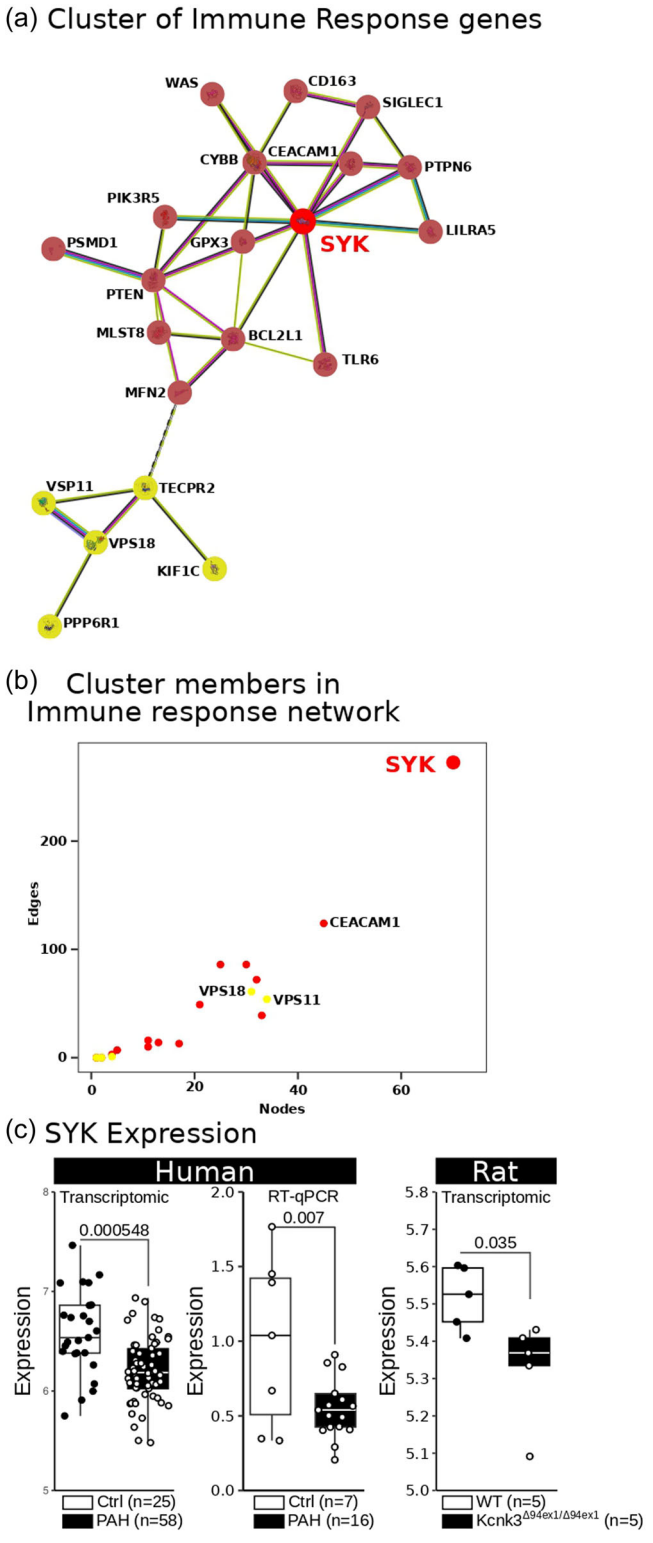

FIGURE 4 Clustering of genes related to the immune response in human and rat lungs using String software. (a) Map of clusters (red and yellow) showing genes significantly dysregulated in both species. (b) Plot displaying the distribution of genes between edges (number of connections between biological processes) and nodes (number of biological processes a gene is involved in). (c) SYK mRNA expression in Human and *Kcnk3*-deficient rat lungs. KCNK3, potassium channel subfamily K member 3; mRNA, messenger RNA; SYK, Spleen Associated Tyrosine Kinase.

AUTHOR CONTRIBUTIONS

Grégoire Ruffenach, Hélène Le Ribeuz, Florent Dumont and Fabrice Antigny designed the study. Grégoire Ruffenach, Hélène Le Ribeuz, Kristell El Jekmek, Florent Dumont, A. SMW and Fabrice Antigny collected the data. T. Grégoire Ruffenach, Hélène Le Ribeuz, Kristell El Jekmek, Florent Dumont, A. SMW and Fabrice Antigny analyzed the data. GF and Fabrice Antigny drafted the manuscript. All the authors participated in the development of the manuscript, provided final approval for submission. Fabrice Antigny is the guarantor of the content of the manuscript.

ACKNOWLEDGMENTS

The authors thank Claudine Delomenie and Florent Dumont for RNA sequencing experiments and bioinformatic analyses (ACTAGen and BIOINFO facilities, IPSIT Paris Saclay University). We also acknowledge the sequencing and bioinformatics expertize of the I2BC High-throughput sequencing facility (Gif-sur-Yvette, France), supported by France Génomique (funded by the French National Program "Investissement d'Avenir" ANR-10-INBS-09). The authors thank Yvonne Dutheil from INSERM UMR_S 999 and Hospital Marie Lannelongue for all her help. This study was supported by grants from the French National Institute for Health and Medical Research (INSERM), the Université Paris-Saclay, the Marie Lannelongue Hospital, the Federation Française de Cardiologie, and the French National Agency for Research (ANR) (grant no. ANR-18-CE14-0023 [KAPAH]). F. Antigny receives funding from the Fondation du Souffle et Fonds de Dotation Recherche en Santé Respiratoire from the Fondation Lefoulon-Delalande and the Fondation Legs Poix Chancellerie des Université de Paris. A.S-M W are supported by the Therapeutic Innovation Doctoral School (ED569). G Ruffenach is supported by the American Heart Association (20CDA35350059). M. Eghbali is supported by the National Institutes of Health (R01HL159865 and R01HL162124). Lejla Medzikovic is supported by the American Heart Association (23POST1022457).

CONFLICT OF INTEREST STATEMENT

Marc Humbert has relationships with drug companies, including Acceleron, AOP Orphan, Janssen, Merck, hiesi, Ferrer, Janssen, Merck, MorphogenIX, Shou Ti, Tiakis, and United Therapeutics. David Montani has relationships with drug companies, including Actelion, Bayer, GSK, Novartis, and Pfizer. In addition to being investigators in trials involving these companies, other relationships include consultancy services and memberships to scientific advisory boards. The remaining authors declare no conflict of interest.

ETHICS STATEMENT

The animal facility was licensed by the French Ministry of Agriculture (agreement N° C92-019-01). This study was approved by the Committee on Ethics of Animal Experiments (CEEA26; CAP Sud). Animal experiments were approved by the French Ministry of Higher Education, Research, and Innovation (N°7757). Animal experiments were performed in accordance with the guidelines from Directive 2010/63/EU on September 22, 2010 of the European Parliament on the protection of animals used for scientific purposes and complied with the French institution's animal care and handling guidelines.

ORCID

Grégoire Ruffenach http://orcid.org/0000-0001-6419-4332
Anaïs Saint-Martin Willer http://orcid.org/0000-0002-2078-3271
Fabrice Antigny http://orcid.org/0000-0002-9515-6571

REFERENCES

1. Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, Olschewski AJ, Pullamsetti SS, Schermuly RT, Stenmark KR, Rabinovitch M. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. Eur Respir J. 2019;53(1):1801887.
2. Machado RD, Southgate L, Eichstaedt CA. Pulmonary arterial hypertension: a current perspective on established and emerging molecular genetic defects. Hum Mutat. 2015;36(12):1113–27. https://doi.org/10.3390/biom10091261
3. Le Ribeuz H, Capuano V, Girerd B, Humbert M, Montani D, Antigny F. Implication of potassium channels in the pathophysiology of pulmonary arterial hypertension. Biomolecules. 2020;10(9):1261.
4. Saint-Martin Willer A, Santos-Gomes J, Adão R, Brás-Silva C, Eyries M, Pérez-Vizcaino F, Capuano V, Montani D, Antigny F. Physiological and pathophysiological roles of the KCNK3 potassium channel in the pulmonary circulation and the heart. J Physiol. 2023;601(17):3717–37.
5. Ketchum KA, Joiner WJ, Sellers AJ, Kaczmarek LK, Goldstein SAN. A new family of outwardly rectifying potassium channel proteins with two pore domains in tandem. Nature. 1995;376(6542):690–5.
6. Duprat F. TASK, a human background K+ channel to sense external pH variations near physiological pH. EMBO J. 1997;16(17):5464–71.
7. Antigny F, Hautefort A, Meloche J, Belacel-Ouari M, Manoury B, Rucker-Martin C, Péchoux C, Potus F, Nadeau V, Tremblay E, Ruffenach G, Bourgeois A, Dorfmüller P, Breuils-Bonnet S, Fadel E, Ranchoux B, Jourdon P, Girerd B, Montani D, Provencher S, Bonnet S, Simonneau G, Humbert M, Perros F. Potassium channel subfamily K member 3 (KCNK3) contributes to the development of pulmonary arterial hypertension. Circulation. 2016;133(14):1371–85.
8. Olschewski A, Li Y, Tang B, Hanze J, Eul B, Bohle RM, Wilhelm J, Morty RE, Brau ME, Weir EK, Kwapiszewska G, Klepetko W, Seeger W, Olschewski H. Impact of TASK-1 in human pulmonary artery smooth muscle cells. Circ Res. 2006;98(8):1072–80.
9. Lambert M, Boet A, Rucker-Martin C. Loss of KCNK3 is a hallmark of RV hypertrophy/dysfunction associated with pulmonary hypertension. Cardiovasc Res. 2018;114:880–93.
10. Callejo M, Morales-Cano D, Mondejar-Parreño G, Barreira B, Esquivel-Ruiz S, Olivencia MA, Moreno L, Cogolludo A, Perez-Vizcaino F. Restoration of vitamin D levels improves endothelial function and increases TASK-Like K+ currents in pulmonary arterial hypertension associated with vitamin D deficiency. Biomolecules. 2021;11(6):795.
11. Hautefort A, Mendes-Ferreira P, Sabourin J, Manaud G, Bertero T, Rucker-Martin C, Riou M, Adão R, Manoury B, Lambert M, Boet A, Lecerf F, Domergue V, Brás-Silva C, Gomez AM, Montani D, Girerd B, Humbert M, Antigny F, Perros F. Bmpr2 mutant rats develop pulmonary and cardiac characteristics of pulmonary arterial hypertension. Circulation. 2019;139(7):932–48.
12. Manoury B, Lamalle C, Oliveira R, Reid J, Gurney AM. Contractile and electrophysiological properties of pulmonary artery smooth muscle are not altered in TASK-1 knockout mice. J Physiol. 2011;589(Pt 13):3231–46.
13. Lambert M, Capuano V, Boet A, Tesson L, Bertero T, Nakhleh MK, Remy S, Anegon I, Pechoux C, Hautefort A, Rucker-Martin C, Manoury B, Domergue V, Mercier O, Girerd B, Montani D, Perros F, Humbert M, Antigny F. Characterization of Kcnk3-Mutated rat, a novel model of pulmonary hypertension. Circ Res. 2019;125(7):678–95.
14. Lambert M, Mendes-Ferreira P, Ghigna MR, LeRibeuz H, Adão R, Boet A, Capuano V, Rucker-Martin C, Brás-Silva C, Quarck R, Domergue V, Vachiéry JL, Humbert M, Perros F, Montani D, Antigny F. Kcnk3 dysfunction exaggerates the development of pulmonary hypertension induced by left ventricular pressure overload. Cardiovasc Res. 2021;117(12):2474–88.
15. Heitzmann D, Derand R, Jungbauer S, Bandulik S, Sterner C, Schweda F, Wakil AE, Lalli E, Guy N, Mengual R, Reichold M, Tegtmeier I, Bendahhou S, Gomez-Sanchez CE, Isabel Aller M, Wisden W, Weber A, Lesage F, Warth R, Barhanin J. Invalidation of TASK1 potassium channels disrupts adrenal gland zonation and mineralocorticoid homeostasis. EMBO J. 2008;27(1):179–87.
16. Yegen CH, Lambert M, Beurnier A, Montani D, Humbert M, Planès C, Boncoeur E, Voituron N, Antigny F. KCNK3 channel is important for the ventilatory response to hypoxia in rats. Respir Physiol Neurobiol. 2023;318:104164.
17. Trapp S, Isabel Aller M, Wisden W, Gourine AV. A role for TASK-1 (KCNK3) channels in the chemosensory control of breathing. J Neurosci. 2008;28(35):8844–50.
18. Buckler KJ. TASK channels in arterial chemoreceptors and their role in oxygen and acid sensing. Pflügers Archiv - European Journal of Physiology. 2015;467(5):1013–25.
19. Buckler KJ. TASK-like potassium channels and oxygen sensing in the carotid body. Respir Physiol Neurobiol. 2007;157(1):55–64.

20. Bayliss DA, Barhanin J, Gestreau C, Guyenet PG. The role of ph-sensitive TASK channels in central respiratory chemoreception. Pflügers Archiv Euro J Physiol. 2015;467(5):917–29.
21. Meuth SG, Bittner S, Meuth P, Simon OJ, Budde T, Wiendl H. TWIK-related acid-sensitive K+ channel 1 (TASK1) and TASK3 critically influence T lymphocyte effector functions. J Biol Chem. 2008;283(21):14559–70.
22. Bittner S, Bauer MA, Ehling P, Bobak N, Breuer J, Herrmann AM, Golfels M, Wiendl H, Budde T, Meuth SG. The TASK1 channel inhibitor A293 shows efficacy in a mouse model of multiple sclerosis. Exp Neurol. 2012;238(2):149–55.
23. Remy S, Chenouard V, Tesson L. Generation of gene-edited rats by delivery of CRISPR/Cas9 protein and donor DNA into intact zygotes using electroporation. Sci Rep [Internet]. 2017;7:16554. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5707420/
24. Chang YF, Imam JS, Wilkinson MF. The nonsense-mediated decay RNA surveillance pathway. Annu Rev Biochem. 2007;76:51–74.
25. Liao Y, Smyth GK, Shi W. The subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acids Res. 2013;41(10):e108.
26. Liao Y, Smyth GK, Shi W. The R package rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. Nucleic Acids Res. 2019;47(8):e47.
27. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 2014;30(7):923–30.
28. Li B, Ruotti V, Stewart RM, Thomson JA, Dewey CN. RNA-Seq gene expression estimation with read mapping uncertainty. Bioinformatics. 2010;26(4):493–500.
29. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 2010;11(3):R25.
30. Chen Y, Lun ATL, Smyth GK, Chen Y, Lun ATL, Smyth GK. From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using rsubread and the edger quasi-likelihood pipeline. F1000Research. 2016;5:1438.
31. Law CW, Chen Y, Shi W, Smyth GK. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol. 2014;15(2):R29.
32. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.
33. Dumont F, Ponsardin E, Bernadat G. MOAL: Multi-Omic analysis at lab. A simplified methodology workflow to make reproducible omic bioanalysis [Internet]. bioRxiv. 2023. https://www.biorxiv.org/content/10.1101/2023.10.17.562686v1
34. Stearman RS, Bui QM, Speyer G, Handen A, Cornelius AR, Graham BB, Kim S, Mickler EA, Tuder RM, Chan SY, Geraci MW. Systems analysis of the human pulmonary arterial hypertension lung transcriptome. Am J Respir Cell Mol Biol. 2019;60(6):637–49.
35. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Nat Acad Sci. 2005;102(43):15545–50.
36. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstråle M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC. PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nature Genet. 2003;34(3):267–73.
37. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, Gable AL, Fang T, Doncheva NT, Pyysalo S, Bork P, Jensen LJ, von Mering C. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023;51(D1):D638–46.
38. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, Doerks T, Stark M, Muller J, Bork P, Jensen LJ, Mering C. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res. 2011;39(Database issue):D561–8.
39. Romanoski CE, Qi X, Sangam S, Vanderpool RR, Stearman RS, Conklin A, Gonzalez-Garay M, Rischard F, Ayon RJ, Wang J, Simonson T, Babicheva A, Shi Y, Tang H, Makino A, Kanthi Y, Geraci MW, Garcia JGN, Yuan JXJ, Desai AA. Transcriptomic profiles in pulmonary arterial hypertension associate with disease severity and identify novel candidate genes. Pulm Circ. 2020;10(4):2045894020968531.
40. Liu SF, Nambiar Veetil N, Li Q, Kucherenko MM, Knosalla C, Kuebler WM. Pulmonary hypertension: linking inflammation and pulmonary arterial stiffening. Front Immunol. 2022;13:959209.
41. West JD, Austin ED, Rizzi EM, Yan L, Tanjore H, Crabtree AL, Moore CS, Muthian G, Carrier EJ, Jacobson DA, Hamid R, Kendall PL, Majka S, Rathinasabapathy A. KCNK3 mutation causes altered immune function in pulmonary arterial hypertension patients and mouse models. Int J Mol Sci. 2021;22(9):5014.
42. Wigerblad G, Warner SA, Ramos-Benitez MJ, Kardava L, Tian X, Miao R, Reger R, Chakraborty M, Wong S, Kanthi Y, Suffredini AF, Dell'Orso S, Brooks S, King C, Shlobin O, Nathan SD, Cohen J, Moir S, Childs RW, Kaplan MJ, Chertow DS, Strich JR. Spleen tyrosine kinase inhibition restores myeloid homeostasis in COVID-19. Sci Adv. 2023;9(1):eade8272.
43. Biswas P, Dey D, Rahman A, Islam MA, Susmi TF, Kaium MA, Hasan MN, Rahman MH, Mahmud S, Saleh MA, Paul P, Rahman MR, Al Saber MA, Song H, Rahman MA, Kim B. Analysis of SYK gene as a prognostic biomarker and suggested potential bioactive phytochemicals as an alternative therapeutic option for colorectal cancer: an In-Silico Pharmaco-Informatics investigation. J Pers Med. 2021;11(9):888.
44. Luo Q, Gao ZX, Cao LL, Yu L, Wang T, Liu HM. Effect of spleen tyrosine kinase on the proliferation of pulmonary vascular smooth muscle cells in rats. Zhongguo Dang Dai Er Ke Za Zhi. 2010;12(11):886–90.
45. Ribeuz HL, Willer ASM, Chevalier B, Sancho M, Masson B, Eyries M, Jung V, Guerrera IC, Dutheil M, Jekmek KE, Laubry L, Carpentier G, Perez-Vizcaino F, Tu L, Guignabert C, Chaumais MC, Péchoux C, Humbert M, Hinzpeter A, Mercier O, Capuano V, Montani D, Antigny F. Role of KCNK3 dysfunction in dasatinib-associated pulmonary arterial hypertension and endothelial cell dysfunction. Am J Respir Cell Mol Biol. 2024;71(1):95–109.

46. Le Ribeuz H, Dumont F, Ruellou G, Lambert M, Balliau T, Quatredeniers M, Girerd B, Cohen-Kaminsky S, Mercier O, Yen-Nicolaÿ S, Humbert M, Montani D, Capuano V, Antigny F. Proteomic analysis of KCNK3 loss of expression identified dysregulated pathways in pulmonary vascular cells. Int J Mol Sci. 2020;21(19):7400.
47. Van Daele M, Kilpatrick LE, Woolard J, Hill SJ. Characterisation of tyrosine kinase inhibitor-receptor interactions at VEGFR2 using sunitinib-red and nanoBRET. Biochem Pharmacol. 2023;214:115672.
48. Kazerounian S, Duquette M, Reyes MA, Lawler JT, Song K, Perruzzi C, Primo L, Khosravi-Far R, Bussolino F, Rabinovitz I, Lawler J. Priming of the vascular endothelial growth factor signaling pathway by thrombospondin-1, CD36, and spleen tyrosine kinase. Blood. 2011;117(17):4658–66.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ruffenach G, Le Ribeuz H, Dutheil M, El Jekmek K, Dumont F, Willer AS-M, Humbert M, Capuano V, Medzikovic L, Eghbali M, Montani D, Antigny F. Transcriptome analyses reveal common immune system dysregulation in PAH patients and *Kcnk3*-deficient rats. Pulm Circ. 2024;14:e12434. https://doi.org/10.1002/pul2.12434