Identification of early biomarkers of transcriptomics in alveolar macrophage for the prognosis of intubated ARDS patients

Songchang Shi1†, Shuo Wei2†, Xiaobin Pan1†, Lihui Zhang1, Shujuan Zhang1, Xincai Wang1, Songjing Shi4* and Wei Lin3*

Abstract

Background: Currently, the rate of morbidity and mortality in acute respiratory distress syndrome (ARDS) remains high. One of the potential reasons for the poor and ineffective therapies is the lack of early and credible indicator of risk prediction that would help specific treatment of severely affected ARDS patients. Nevertheless, assessment of the clinical outcomes with transcriptomics of ARDS by alveolar macrophage has not been performed.

Methods: The expression data GSE116560 was obtained from the Gene Expression Omnibus databases (GEO) in NCBI. This dataset consists of 68 BAL samples from 35 subjects that were collected within 48 h of ARDS. Differentially expressed genes (DEGs) of different outcomes were analyzed using R software. The top 10 DEGs that were up- or down-regulated were analyzed using receiver operating characteristic (ROC) analysis. Kaplan–Meier survival analysis within two categories according to cut-off and the value of prediction of the clinical outcomes via DEGs was verified. GO enrichment, KEGG pathway analysis, and protein–protein interaction were also used for functional annotation of key genes.

Results: 24,526 genes were obtained, including 235 up-regulated and 292 down-regulated DEGs. The gene ADORA3 was chosen as the most obvious value to predict the outcome according to the ROC and survival analysis. For functional annotation, ADORA3 was significantly augmented in sphingolipid signaling pathway, cGMP-PKG signaling pathway, and neuroactive ligand-receptor interaction. Four genes (ADORA3, GNB1, NTS, and RHO), with 4 nodes and 6 edges, had the highest score in these clusters in the protein–protein interaction network.

Conclusions: Our results show that the prognostic prediction of early biomarkers of transcriptomics as identified in alveolar macrophage in ARDS can be extended for mechanically ventilated critically ill patients. In the long term, generalizing the concept of biomarkers of transcriptomics in alveolar macrophage could add to improving precision-based strategies in the ICU patients and may also lead to identifying improved strategy for critically ill patients.

†Songchang Shi, Shuo Wei, and Xiaobin Pan have contributed equally to this work

*Correspondence: songjingshi@163.com; caolalin0929@163.com

1 Department of Endocrinology, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, Fuzhou 350001, People’s Republic of China
2 Department of Critical Care Medicine, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, Fuzhou 350001, People’s Republic of China

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
**Introduction**

Acute respiratory distress syndrome (ARDS), accompanied by increased pulmonary vascular permeability, and loss of aerated lung tissue, is an acute lung injury [1]. A series of interventions have been proposed for ARDS, such as lower tidal volumes [2], higher positive end-expiratory pressure (PEEP) [3], prone positioning [4], and extracorporeal membrane oxygenation [5]. It was found by a recent international, multicenter observational cohort study that morbidity and mortality of ARDS were still high [6]. The prevalence period of ARDS among ICUs in 50 countries was 10.4% of ICU admissions [6]. Overall, up to 40% of patients with ARDS died in the hospital [6].

One of the potential reasons for the lack of effective therapies is the absence of early, credible indicator for risk prediction, which would help in the precise treatment of acute ARDS [7, 8]. It was shown that 1/3 of the genes in blood leukocytes were differentially expressed between sub-phenotypes of ARDS, supporting the biological heterogeneity of patients [9]. These biological sub-phenotypes are suggested to provide prediction for a precision-based therapeutic strategy [10].

Recent studies have suggested that transcriptomic analyses using whole-blood leukocyte RNA might not accurately reflect all the lung processes of ARDS [11]. Other findings suggest that alveolar macrophages (AMs) may contribute towards the inflammation and injury in ARDS [12]. Nevertheless, assessment of the clinical outcomes with ARDS by AM has not been performed [12].

In this study, we examined the transcriptome of AMs isolated from patients on the first day after the onset of ARDS. Our study attempted to performed the assessment of the clinical outcomes with transcriptomics of ARDS by alveolar macrophage (AM).

**Methods**

**Study population and design**

The data was obtained from the Gene Expression Omnibus databases (GEO) in NCBI, a public functional genomics data repository. The expression data (https://www.ncbi.nlm.nih.gov/geo/; GSE116560) was based on the Illumina GPL6883 platform (Illumina Human Ref-8 v3.0 expression BeadChip), which was submitted by Charib SA et al. This data performed unbiased genome-wide transcriptional profiling of AMs purified from bronchoalveolar lavage fluid collected from patients with ARDS. This dataset [12] consists of 68 samples from 35 subjects that occurred within 48 h of ARDS, whose BALF (BAL fluid) were taken at Day 1, Day 4 and Day 8. The average age was 45, comprising 22 men and 13 women. The factors of the ARDS were trauma (19), sepsis (17), pneumonia (9), and other (4). Participants who extubated were not selected for bronchoscopy on Day 4 or Day 8. Twenty patients were successfully extubated within 28 days. Fifteen patients were unsuccessfully extubated at 28 days, including five patients who died. At present, 28 days have been observed as an important time point for prognosis in many studies on ARDS [13–15]. The patients who were released from mechanical ventilation within 28 days were defined as the “good” group, those who did or were still dependent on mechanical ventilation at Day 28 were defined as the “poor” group. The data was prepared, processed and analyzed by the software R (version 3.6.3). The flowchart of this study is shown in Fig. 1. The procedures followed were in accordance with the ethical standards of the Responsible Committee on Human Experimentation and with the Helsinki Declaration.

**Identification of differentially expressed genes**

The data was normalized and corrected by the package limma of R software [16]. Differentially expressed genes (DEGs) of different outcomes were screened with criteria.
**Results**

**Identification of DEGs**

24,526 genes were screened from the GSE116560 dataset, including 235 up-regulated and 292 down-regulated DEGs. It was shown that some of the transcriptional programs of AM were different between the patients released from mechanical ventilation within 28 days and those who died or were still dependent on mechanical ventilation at Day 28. The volcano map of all DEGs is shown in Fig. 2A. The heat map of top 100 DEGs shows top 20 up-regulated genes, and top 20 down-regulated genes, Fig. 2B–D.

**Identification of the top 10 DEGs with outcome**

The top 10 up- and down-regulated DEGs were identified based on ROC analysis. The group of genes identified showed the best outcome with the setting AUC > 0.7, except for ICA1 (Fig. 3). The AUC for the gene FRAT1 was 0.773, with a significant change of gene expression as up-regulated DEGs (Fig. 3A). The AUC for the gene UNC45A was 0.866, with the most significant change of gene expression as down-regulated DEGs (Fig. 3B). The best observed concentration of cut-off to predict was chosen after ROC analysis.

**Identification the key genes via survival analysis**

Kaplan–Meier survival analysis using two categories based on the cut-off was performed and the value of prediction of the clinical outcomes via DEGs was verified (Table 1). The gene ADORA3 showed a significant change with this outcome (Fig. 4A and Additional file 1). This was chosen as the most appropriate value to predict the outcome according to the survival analysis (Fig. 4B), \( p = 0.059 \) (< 0.10).

**Functional annotation of gene ADORA3**

The key gene ADORA3 was further evaluated by GO categories and KEGG pathways. Results of GO [24, 25] analysis found ADORA3 significantly enriched in pathways, such as adenosine P1 receptors, nucleotide-like (purinergic) receptors, G alpha (i) signaling events, GPCR ligand binding, GPCR downstream signaling, signaling by GPCR, and signal transduction (Fig. 5A). The KEGG pathway results revealed that ADORA3 was significantly augmented in sphingolipid signaling pathway, cGMP-PKG signaling pathway, and neuroactive ligand-receptor interaction (Fig. 5B, C).

**Protein–protein interaction network**

The PPI network of 237 up-regulated DEGs from ARDS patients identified using STRING analysis contained 123 nodes and 146 edges. A total of 6 clusters were generated in MCODE. 4 genes were selected as the most important clusters by the scores evaluated in MCODE. MCODE 1, consisting of four genes (ADORA3, GNB1, NTS, and RHO), with 4 nodes and 6 edges, had the highest score in these clusters (Fig. 5D).

It was shown that transcriptional programs of AM were different between the patients released from mechanical ventilation within 28 days and those who died or were still dependent on mechanical ventilation at Day 28. Among them, ADORA3 might be a key gene.
Discussion

This study investigated the prognostic and predictive enrichment of ARDS sub-phenotypes of intubated patients. This is the first study to find the different prognosis between patients with significantly high or low expression of gene ADORA3 in ARDS. In the predictive model of our survival analysis, it forecasted that different expression levels of ARDOA3 at above or below the cut-off, the incidence of extubating were different at 5, 10, 15, 20, and 25 days. Our study revealed that ADORA3 and its related pathways might be associated with 28-day outcomes. As we know, ADORA3 might play an important part in the resolution and initiation of ARDS.

Fig. 2  Identification of DEGs
ADROA3 may affect the physiological functions of cells and organs by participating in signal transduction in several signal pathways. Purinergic receptors family includes the GPCR P2Y purinergic receptors and adenosine P1 receptors [26]. Purinergic receptors are involved in cellular functions such as vascular reactivity, apoptosis and cytokine secretion [27]. P1 receptors are purinergic receptors such as G-protein coupled receptors with their endogenous ligand adenosine. There are four adenosine receptors in humans, with distinct functions. They might play important roles in the heart, brain, and might be involved in inflammation and immune responses [28]. The classical mechanism is inhibition of the cAMP dependent pathway via inhibition of adenylate cyclase [29]. Decrease in production of cAMP from ATP leads to decreased activity of cAMP-dependent protein kinases [29].

Putten et al. found that ADORA3 and its pathway induced proinflammatory cytokines [30]. Another research concluded the protective effect of preventing
immune mediated damage and excessive immune response via ADORA3 activation [31]. Lung fibroblasts are promoted into myofibroblasts by adenosine receptors. This suggests a potential involvement of ADORA3 in the processes of fibrotic lung disease [32]. Until now, studies on ADORA3 and ARDS pathogenesis is insufficient, and the mechanisms remain to be investigated further.

Some researchers have found the role of ADORA3 in heart. The signaling of ADORA3 protects cardiomyocytes against the damage of ischemia, as well as protects it from energy depletion and contractile dysfunction [33–37]. Moreover, activation of ADORA3 might induce myocardial cells apoptosis [38]. Del et al. found that mRNA expression of ADORA3 in left ventricle of failing minipig heart was higher than in hearts of control healthy minipig [39]. In addition, ADORA3 is overexpressed in inflammation and up-regulated in peripheral blood mononuclear cells of autoimmune diseases. Activation of ADORA3 resulting in downregulation of nuclear factor kappa B (NF-kB) and tumor necrosis factor-α (TNFα) [40], leads to the inhibition of inflammatory cytokines [41].

The package of limma was used from Bioconductor to correct for multiple testing issue. Through the analysis of differential genes by ROC analysis, survival analysis, functional annotation, and PPI network. We narrowed the scope of target genes. The top10 up-regulated and top 10 down-regulated genes had been analyzed by survival analysis for all.

In the survival analysis of ADORA3, it was found that the two groups of survival analysis curves were far apart within the first 3 weeks, and gradually approached closer to each other in the last week. It might be considered that ADORA3 may be useful in the early stages of ARDS to predict mortality, but not in the later stages of the disease. Furthermore, we will stratify the disease course and expand the sample size in the further study.

This datasets we used were obtained from a public database. With this datasets, Morrel et al. [12] identified genes between groups at each time point using linear models, while temporal expression analysis was

### Table 1  Survival analysis of the top 10 up-regulated and down-regulated genes

| Genes | OR   | Lower 95% | Upper 95% | \( p \) value |
|--------|------|-----------|-----------|---------------|
| Top 10 of the up-regulated DEGs | | | | |
| SSH2   | 0.516 | 0.166     | 1.603     | 0.252         |
| FKBPS  | 1.02  | 0.313     | 3.882     | 0.879         |
| TSC22D3| 0.368 | 0.048     | 2.827     | 0.337         |
| DDIT4L (cut-off = 9.305) | 1.862 | 0.487     | 7.116     | 0.363         |
| DDIT4L (cut-off = 8.951) | 0.692 | 0.269     | 1.780     | 0.445         |
| ICA1   | 0.757 | 0.217     | 2.636     | 0.662         |
| FRAT1  | 0.512 | 0.194     | 1.347     | 0.175         |
| ADORA3 | 2.285 | 0.926     | 8.614     | 0.068*        |
| TFCP2L1| 0.918 | 0.302     | 2.794     | 0.881         |
| FAR1P1 | 0.607 | 0.235     | 1.566     | 0.301         |
| PP2447 | 0.757 | 0.268     | 2.141     | 0.600         |
| KIAA0256 | 0.614 | 0.240     | 1.571     | 0.309         |
| AMFR (cut-off = 10.391) | 1.120 | 0.440     | 2.851     | 0.812         |
| AMFR (cut-off = 10.614) | 0.760 | 0.293     | 1.968     | 0.571         |
| CTSZ   | 0.907 | 0.258     | 3.195     | 0.879         |
| PPP3R1 | 0.564 | 0.217     | 1.465     | 0.239         |
| MYOM2 (cut-off = 9.843) | 2.715 | 0.354     | 20.840    | 0.337         |
| MYOM2 (cut-off = 10.211) | 0.877 | 0.287     | 2.678     | 0.818         |
| MYOM2 (cut-off = 10.348) | 0.883 | 0.337     | 2.314     | 0.800         |
| MYOM2 (cut-off = 10.751) | 0.728 | 0.286     | 1.852     | 0.505         |
| UNC45A | 1.152 | 0.332     | 4.004     | 0.824         |
| CD86   | 0.414 | 0.119     | 1.444     | 0.166         |
| C21orf57 | 0.719 | 0.253     | 2.044     | 0.535         |
| IL18BP | 0.786 | 0.302     | 2.047     | 0.622         |
| DCAL1  | 0.632 | 0.141     | 2.842     | 0.550         |

DEG differentially expressed genes

\*\( p < 0.10 \)
performed using the STEM. Zhao et al. [39] analyzed that GSE116560 was only one of the datasets, which was based on different platform. In our study, we narrowed the scope by screening differential genes and ROC, and finally performing survival analysis on related genes with a distinctive combination of time and outcome.

Although this study is promising and opens up a new perspective on the impact of ADORA3 and its signaling on the outcome in ARDS, the potential limitations of our research should also be contemplated. First, the dataset was obtained from a public database. All results were obtained using bioinformatics. Future studies are currently being designed for examining the exact roles of ADORA3 and its signaling in ARDS. Second, because of the limited sample size, studies on a larger population are required to better define the role of ADORA3 in ARDS.

Fig. 4 Identification the key genes via survival analysis
In conclusion, our results show that the prognostic prediction of early biomarkers of transcriptomics as identified in alveolar macrophage in ARDS can be applied to mechanically ventilated critically ill patients. In the long term, generalizing the concept of biomarkers of transcriptomics in alveolar macrophage could add to improving precision-based strategies in the ICU population and lead to identifying treatable therapy for all critically ill.

**Table 1** Functional annotation of gene ADORA3

| Pathway name                        | Entities | Reactions |
|-------------------------------------|----------|-----------|
|                                     | found    | ratio     | p-value  | FDR* | found | ratio |
| Adenosine P1 receptors              | 1/4      | 3.45e-04  | 3.45e-04 | 0.003 | 1/2   | 1.49e-04 |
| Nucleotide-like (purinergic) receptors | 1/16    | 0.001    | 0.001    | 0.006 | 1/15 | 0.001  |
| G alpha (i) signalling events       | 1/317    | 0.027    | 0.027    | 0.04  | 3/74 | 0.006 |
| Class A/1 (Rhodopsin-like receptors) | 1/333    | 0.029    | 0.029    | 0.04  | 1/158 | 0.012 |
| GPCR ligand binding                 | 1/469    | 0.04     | 0.04     | 0.04  | 1/185 | 0.014 |
| GPCR downstream signalling          | 1/631    | 0.054    | 0.054    | 0.054 | 3/168 | 0.013 |
| Signaling by GPCR                   | 1/706    | 0.061    | 0.061    | 0.061 | 4/353 | 0.026 |
| Signal Transduction                 | 1/2,565  | 0.221    | 0.221    | 0.221 | 4/2,402 | 0.179 |

* False Discovery Rate

**Fig. 5** Functional annotation of gene ADORA3
Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12890-022-02130-8.

Additional file 1. Supplementary 1 Different expression of the key genes with the outcome.

Acknowledgements

We would like to thank the participants for providing the information used in this study and for kindly making the arrangements for data collection.

Author contributions

SS performed the formal analysis, methodology, and writing-original draft; SW, XP, LZ, SZ, and XW performed the data curation, and resources; WL and SS performed the conceptualization, formal analysis, writing-original draft, and project administration; WL is the guarantor of this manuscript and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

Funding

This study was supported by the Natural Science Foundation of Fujian Province (Grant No. 2020J011088), the Innovation Project of Fujian Provincial Health Commission (2021CXA003).

Availability of data and materials

The datasets analyzed during the current study are publicly available in the [Gene Expression Omnibus database](https://www.ncbi.nlm.nih.gov/geo/; GSE116560). The Gene Expression Omnibus database, [https://www.ncbi.nlm.nih.gov/geo/; GSE116560].

Declarations

Ethics approval and consent to participate

The study has been approved by the Fujian Provincial Hospital Ethics Committee. The study follows the principles of the Declaration of Helsinki. All study participants provided informed consent for this study.

Disclosure statement

This manuscript is an honest, accurate and transparent account of the study, case or topic being reported; that no important aspects have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

1 Department of Critical Care Medicine, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital South Branch, Fujian Provincial Hospital, Fuzhou 350001, People’s Republic of China.
2 Department of Infectious Disease, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, Fuzhou 350001, People’s Republic of China.
3 Department of Endocrinology, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, Fuzhou 350001, People’s Republic of China.
4 Department of Critical Care Medicine, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, Fuzhou 350001, People’s Republic of China.

Received: 26 May 2022 Accepted: 30 August 2022 Published online: 02 September 2022

References

1. Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012;307(23):2526–33.
2. Acute Respiratory Distress Syndrome Network, Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, Wheeler A. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med. 2000;342(18):1301–8.
3. Briel M, Meade M, Mercat A, Brower RG, Talmor D, Walter SD, Slutsky AS, Pullenayegum E, Zhou Q, Cook D, et al. Higher vs lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome: systematic review and meta-analysis. JAMA. 2010;303(9):865–73.
4. Guerin C, Reignier J, Richard JC. Prone positioning in the acute respiratory distress syndrome. N Engl J Med. 2013;369(10):980–1.
5. Peek GE, Mugu ford M, Tiruvospati R, Wilson A, Allen E, Thalannany MM, Hibbert CL, Trueciale A, Clemens F, Cooper N, et al. Efficacy and economic assessment of conventional ventilatory support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR): a multicentre randomised controlled trial. Lancet. 2009;374(9698):1351–63.
6. Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, Gattinoni L, van Haren F, Larsson A, McAuley DF, et al. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. JAMA. 2016;315(8):788–800.
7. Luo L, Shaver CM, Zhao Z, Koyama T, Calfee CS, Bastarache JA, Ware LB. Clinical predictors of hospital mortality differ between direct and indirect ARDS. Chest. 2017;151(4):755–63.
8. Villar J, Blanco J, del Campo R, Andaluz-Ojeda D, Diaz-Dominguez FJ, Muriel A, Corcoles V, Suarez-Sippmann F, Tarancón C, González-Higuera S, et al. Assessment of PaO2/FIO2 for stratification of patients with moderate and severe acute respiratory distress syndrome. BMJ Open. 2015;5(3):e006812.
9. Bos LDJ, Sicluna BP, Ong DSY, Cremer O, van der Poll T, Schultz MJ. Understanding heterogeneity in biologic phenotypes of acute respiratory distress syndrome by leukocyte expression profiles. Am J Resp Crit Care Med. 2019;200(1):42–50.
10. Wang X, Zhou J, Wang T, George SL. On enrichment strategies for bioinformatics differential expression analyses for RNA-sequencing and microarray observational study. Lancet Respir Med. 2020;8(12):1209–18.
11. Morrell ED, Radella F 2nd, Manicone AM, Mikacenic C, Stapleton RD, Gharib SA, Wurfel MM. Peripheral and alveolar cell transcriptional programs are distinct in acute respiratory distress syndrome. Am J Resp Crit Care Med. 2018;197(4):526–32.
12. Morrell ED, Bhattrajuk PK, Mikacenic CR, Radella F 2nd, Manicone AM, Stapleton RD, Wurfel MM, Gharib SA. Alveolar macrophage transcriptional programs are associated with outcomes in acute respiratory distress syndrome. Am J Resp Crit Care Med. 2019;200(6):732–41.
13. Bos LDJ, Spodding M, Sinha P, Bravani SV, Lyons PG, Bevley AF, Botta M, Tsonas AM, Serpa Neto A, Schultz MJ, et al. Longitudinal respiratory subphenotypes in patients with COVID-19 related acute respiratory distress syndrome: results from three observational cohorts. Lancet Respir Med. 2021;9(12):1377–86.
14. Writing Committee and Steering Committee for the RELAx Collaborative Group, Algera AG, Pisani L, Serpa Neto A, den Boer SS, Bosch FFH, Bruin K, Kloorster PM, Van der Meer NJM, et al. Effect of a lower vs higher positive end-expiratory pressure strategy on ventilator-free days in ICU patients without ARDS: a randomized clinical trial. JAMA. 2020;324(24):2509–20.
15. Sinha P, Calfee CS, Chianan S, Brealey D, Cutler S, King C, Killick C, Richards O, Cheema Y, Bailey C, et al. Prevalence of phenotypes of acute respiratory distress syndrome in critically ill patients with COVID-19: a prospective observational study. Lancet Respir Med. 2020;8(12):1209–18.
16. Ritchie ME, Prakash B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. Imma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.
17. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, Muller M. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinform. 2011;12:77.
18. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000;25(1):25–9.
19. Jassal B, Matthews L, Viteri G, Gong C, Lorente P, Fabregat A, Sidiropoulos K, Cook J, Gillespie M, Haw R, et al. The reactome pathway knowledgebase. Nucleic Acids Res. 2020;48(D1):D498–503.
20. Kaneshia M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27–30.
21. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, et al. STRING v10: protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Res. 2015;43(Database issue):D447–452.
22. Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape StringApp: network analysis and visualization of proteomics data. J Proteome Res. 2019;18(2):623–32.
23. Bandettini WP, Kellman P, Mancini C, Booker OJ, Vasi S, Leung SW, Wilson JR, Shanbhag SM, Chen MY, Araie AE. MultiContrast Delayed Enhancement (MCDE) improves detection of subendocardial myocardial infarction by late gadolinium enhancement cardiovascular magnetic resonance: a clinical validation study. J Cardiovasc Magn Reson. 2012;14:83.
24. Fabregat A, Jupe S, Matthews L, Sidiropoulos K, Gillespie M, Garapati P, Haw R, Jassal B, Koning N, May B, et al. The reactome pathway knowledgebase. Nucleic Acids Res. 2018;46(D1):D649–55.
25. Fabregat A, Koning N, Viteri G, Sidiropoulos K, Marin-Garcia P, Ping P, Wu G, Stein L, D’Eustachio P, Hermjakob H. Reactome graph database: efficient access to complex pathway data. PLoS Comput Biol. 2018;14(1):e1005968.
26. Burnstock G. Purinergic signalling—an overview. Novartis Found Symp. 2006;276:26–48 (discussion 48–57, 275–281).
27. Abbacchio MP, Burnstock G, Vhraltsky A, Zimmermann H. Purinergic signalling in the nervous system: an overview. Trends Neurosci. 2009;32(1):19–29.
28. Friedholm BB, Lizerman AP, Jacobson KA, Linden J, Muller CE. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors—an update. Pharmacol Rev. 2011;63(1):1–34.
29. Dessauer CW, Chen-Goodspeed M, Chen J. Mechanism of Galphaim-mediated inhibition of type V adenyl cyclase. J Biol Chem. 2002;277(32):28823–9.
30. van der Putten C, Verh J, Sukiurova L, Zuidervijk-Sick EA, Simonetti E, Koenen H, Burm SM, van Noort JM, Lizerman AP, van Hjum S, et al. TLR-induced IL-12 and CCL2 production by myeloid cells is dependent on adenosine A3 receptor-mediated signaling. J Immunol. 2019;202(8):2421–30.
31. D’Antongiovanni V, Fornai M, Pellegrini C, Benvenuti L, Blandizzi C, Antonioni L. The adenosine system at the crossroads of intestinal inflammation and neoplasia. Int J Mol Sci. 2020;21(1):5089.
32. Della Latta V, Cabati M, Rocchiccioli S, Del Ry S, Morales MA. The role of the adenosinergic system in lung fibrosis. Pharmacol Res. 2013;76:182–9.
33. Liu GS, Richards SC, Olsson RA, Mullane K, Walsh RS, Downey JM. Evidence that the adenosine A3 receptor may mediate the protection afforded by preconditioning in the isolated rabbit heart. Cardiovasc Res. 1994;28(7):1057–61.
34. Maldonado C, Qiu Y, Tang XL, Cohen MV, Auchampach J, Bollr R. Role of adenosine receptors in late preconditioning against myocardial stunning in conscious rabbits. Am J Physiol. 1997;273(3 Pt 2):H1324–1332.
35. Thouruni VH, Ronson RS, Jordan JE, Guyton RA, Vinten-Johansen J. Adenosine A3 pretreatment before cardioplegic arrest attenuates postischemic cardiac dysfunction. Ann Thorac Surg. 1999;67(6):1752–7.
36. Tracey WR, Magee W, Masamune H, Kennedy SP, Knight DR, Buchholz RA, Hill RJ. Selective adenosine A3 receptor stimulation reduces ischemic myocardial injury in the rabbit heart. Cardiovasc Res. 1997;33(2):410–5.
37. Xu Z, Jang Y, Mueller RA, Norfleet EA. IB-MECA and cardioprotection. Cardiovasc Drug Rev. 2006;24(3–4):227–38.
38. Hannon JP, Pfrankhuche HJ, Fozard JR. A role for mast cells in adenosine A3 receptor-mediated hypotension in the rat. Br J Pharmacol. 1995;115(6):945–52.
39. Del Ry S, Cabati M, Martino A, Simonucci A, Morales MA, Picano E, Adenosine receptor mRNA expression in normal and failing minipig hearts. J Cardiovasc Pharmacol. 2011;58(2):149–56.
40. Fishman P, Bar-Yehuda S, Madi L, Rath-Wolfson L, Ochaion A, Cohen S, Baharav E. The P13K-NF-kappaB signal transduction pathway is involved in mediating the anti-inflammatory effect of IB-MECA in adjuvant-induced arthritis. Arthritis Res Ther. 2006;8(1):R33.
41. Hasko G, Szabo C, Nemeth ZH, Kvetan V, Pastores SM, Vizi ES. Adenosine receptor agonists differentially regulate IL-10, TNF-alpha, and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. J Immunol. 1996;157(10):4634–40.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.