Different Characteristics of Mitochondrial Dynamics-related miRNAs on the Hemodynamics of Pulmonary Artery Hypertension and Chronic Thromboembolic Pulmonary Hypertension

Short running title: **Mitochondrial dynamics-related miRNA and PH**

Noriko Iwatani (MD), Kayoko Kubota (MD, PhD), Yoshiyuki Ikeda (MD, PhD)*, Akihiro Tokushige (MD, PhD), Sunao Miyanaga (MD, PhD), Kenjuro Higo (MD, PhD), Mitsuru Ohishi (MD, PhD, FJCC)

Department of Cardiovascular Medicine and Hypertension, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

*Corresponding author:

Yoshiyuki Ikeda, MD, PhD,

Department of Cardiovascular Medicine and Hypertension,

Graduate School of Medical and Dental Sciences, Kagoshima University

8-35-1 Sakuragaoka, Kagoshima City, Kagoshima 890-8520, Japan

Tel: +81992755318

Fax: +81992658447

E-mail: alltezza@m2.kufm.kagoshima-u.ac.jp

Key words: Pulmonary artery hypertension; Chronic thromboembolic pulmonary hypertension; Mitochondrial dynamics; miRNA

Abstract

Background: Mitochondria are dynamic organelles that undergo fission or fusion. These mitochondrial dynamics are reported to be associated with pulmonary hypertension (PH). PH is divided into 5 groups, including pulmonary artery hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH), based on its pathogenesis. However, it is still unknown whether and how miRNAs related to mitochondrial dynamics (MD) affect PAH and CTEPH.

Methods: We investigated patients who underwent right heart catheterization between October 2016 and January 2019. Out of 34 PH patients, 12 were diagnosed with PAH, and 22 were diagnosed with CTEPH. In addition, there were 30 patients diagnosed with left heart disease. We enrolled the 34 PH patients as the PH group and 30 left heart disease patients as the control group.

Results: Among MD-related miRNAs, the circulating levels of miR-140-3p were higher, and those of miR-485-5p were lower in the PH group than in the control group (p<0.01), suggesting that miRNAs inducing mitochondrial fission are related to PH. The miR-140-3p levels in the PAH and CTEPH groups were higher than those in the control group (p<0.01). The levels of miR-140-3p and miR-485-5p in the PAH group correlated with pulmonary vascular resistance (r=0.582, p=0.046) and cardiac index (r=-0.36, p=0.04), respectively. The miR-485-5p levels in the CTEPH group correlated with right atrium pressure (r=-0.456, p=0.049).

Conclusion: MD-related miRNAs levels change to induce fission and are closely related to the hemodynamics of PAH and CTEPH.

Introduction

Pulmonary hypertension (PH) is defined by a mean pulmonary arterial pressure (mPAP) ≥25 mmHg at rest as assessed by right heart catheterization (RHC) [1]. PH is a severe disease characterized by remodeling of the vasculature and increased resistance, stiffness, and fibrosis of the pulmonary artery [2]. These factors lead PH patients to right-sided heart failure and death. This disease is divided into 5 groups depending on the location of lesions [3]. Group 1: Pulmonary artery hypertension (PAH); Group 2: PH due to left heart disease; Group 3: PH due to lung diseases and/or hypoxia; Group 4: Chronic thromboembolic pulmonary hypertension (CTEPH); Group 5: PH with unclear and/or multifactorial mechanisms. Among the 5 PH groups, PAH and CTEPH are pre-capillary PH and the lesion focus exists on their pulmonary artery [1,4]. In the past two decades, the diagnostic and treatment methods for these diseases have changed markedly [5,6]. However, it remains a disease with poor prognosis, and the cause of its pathophysiology is unclear.

The recent accumulation of evidence demonstrates that PAH is modulated by microRNAs (miRNAs) which are small non-coding single-stranded RNA molecules around 22 nucleotides long that bind to mRNA and downregulate relevant gene expression by degrading mRNA. Recently it has been revealed that some miRNAs modulating PAH are related to mitochondrial dynamics [7,8]. Mitochondria play a role in generating ATP as energy for cells, regulating apoptosis and necrosis, and producing secondary reactive oxygen species upon ATP production, which is responsible for the development of cardiovascular diseases. Mitochondria are dynamic organelles that change their morphology through fusion and fission to meet various cell functional demands. Mitochondrial fusion combines individual mitochondrial membranes by stimulation, and conversely, mitochondrial fission is marked by the

fragmentation of mitochondria and mitochondrial networks in response to stress [9,10]. It has recently been demonstrated that mitochondrial dynamics are associated with several cardiovascular diseases, including PAH, and play a pivotal role in regulating cell proliferation in the pathophysiology of PAH [11]. However, most of these reports were based on animal experiments and only focusing on PAH. Therefore, it is little known 1) whether miRNAs related to mitochondrial dynamics are clinically involved in PH, and 2) how the miRNA level changes due to the difference in each PH group, especially PAH and CTEPH. In this study, we examined whether and how miRNAs related to mitochondrial dynamics were involved in PAH and CTEPH.

Methods

Patients and study design

From October 2016 to January 2019, we registered patients who underwent RHC to evaluate right and left cardiac function, such as cardiac output and pulmonary blood pressure, in our hospital, which was a referral-type hospital with approximately 600 beds. Based on these results, we diagnosed and classified these patients according to the PH guidelines (JCS 2017/JPCPHS 2017) [1]. When the pulmonary artery wedge pressure (PAWP) was <15 mmHg and mPAP ≥25 mmHg, we diagnosed the patient with pre-capillary PH. Out of the 41 pre-capillary PH patients, we excluded 7 patients with severe lung disease as there was a distinct change in not only the pulmonary artery but also the pulmonary alveolus. Among the remaining 34 PH patients, 12 patients were diagnosed with PAH, and 22 patients were diagnosed with CTEPH. PAH patients contained 3 idiopathic PAH patients, 3 connective tissue diseases, 3 congenital heart disease, 1 human immunodeficiency virus infection, 1 portopulmonary hypertension, and 1 pulmonary veno-occlusive disease. We enrolled these 34 patients

diagnosed with PAH and CTEPH as the PH group. In addition, there were 30 patients who underwent RHC for evaluating hemodynamics because of left heart disease (5 patients with congenital heart disease patients, 13 with valvular disease patients, 6 with ischemic heart disease, and 6 with heart failure). We enrolled 30 patients as the control group, and all of them were not pre-capillary PH patients. All patients who were about to undergo RHC were informed about this study, and only patients who consented to the study were enrolled. This study complied with the standards of the Declaration of Helsinki and current ethical guidelines and was approved by our hospital ethics committee and the approval number of the ethics committee is 160090 (28-98). (Fig. 1).

Hemodynamic measurements and blood sampling

RHC was performed at rest in the supine position. We introduced a sheath through the internal jugular vein or femoral vein followed by a 6Fr or 7Fr Swan-Gantz catheter. Then, 15 mL of blood was sampled from the sheath and centrifuged at 1,000 g for 15 min to obtain the serum sample. PAP, PAWP, right atrium pressure (RAP), right ventricle (RV) pressure, cardiac index (CI), and pulmonary vascular resistance (PVR) were measured.

Measurement of circulating miRNA

We measured circulating miRNA by quantitative real-time polymerase chain reaction (qRT-PCR) as described previously [12]. In brief, total RNA was isolated from serum samples using the miRNeasy Serum/Plasma Kit (QIAGEN, Hilden, Germany). Reverse transcription into cDNA and RT-PCR were performed using the TaqMan Advanced miRNA Assays Kit (Thermo Fisher Scientific, Waltham, USA), according to the manufacturer’s instructions. To date, it has been reported that miR-30, miR-485, and miR-499 induce mitochondrial fusion, and miR-140 causes mitochondrial fission [13-

18]. The levels of miR-30a-3p, miR-30a-5p, miR-140-3p, miR-140-5p, miR-485-3p, miR-485-5p, and miR-499a-5p were measured and normalized to the cel-miR-39-3p [19]. Primers for human miRNA were purchased from Invitrogen(Carlsbad, USA), and miRNA sequences and their miRBase accession numbers are shown in Table 1.

Statistical analysis

As there was substantial skewing of miRNA values, logarithmic transformation was applied to subordinate the skewed values. Comparison of basic characteristics between the two groups, control vs. PH, was performed using Student's t-test for parametric data or the Wilcoxon test for nonparametric data. A chi-square test was performed to compare sex between the two groups. Comparison of the miRNA levels in both the control and PH groups was performed using Student's t-test. Analysis of variance was performed to compare the levels of miRNAs in control, PAH, and CTEPH groups. We used linear regression analysis to evaluate the correlation between miRNA levels and hemodynamic data. If the hemodynamic data were nonparametric, we applied logarithmic transformation. Statistical analysis was performed using the JMP statistical analysis package (JMP Pro version 14, SAS, Cary, NC, USA). We assumed statistical significance at a *p*-value of <0.05.

Results

Patient characteristics

Patient characteristics and hemodynamics data are shown in Table 2. The median age of the control group was higher than that of the PH group (p=0.034), 67.4±12.0 years vs. 60.2±14.0 years, respectively. There were 14 females in the control group (46.7%) and 29 females in the PH group (85.3%). The rate of females in the PH group was significantly higher than that in the control group (p=0.001). We tested 6-minute

walk test (6MWD) in the PH group. The median value of 6MWD was 371.0 m (293.0-433.0 m) in the PH group, and there was no difference between the PAH group and the CTEPH group. Serum B-type natriuretic peptide level was higher in the control group (p=0.002), and there was no difference between the PAH group and the CTEPH group. There was no difference in the RAP and CI measures. mPAP and PVR were significantly higher in the PH group than in the control group (p<0.001). The PAWP of the control group was higher than that of the PH group (p<0.001). Nine patients from the PAH and CTEPH groups, each, had been treated with specific pulmonary vasodilators. Fifteen patients from the control group had been treated with diuretics, and 10 patients from the PAH group and 8 patients from the CTEPH group had been treated with diuretics. We measured mixed venous oxygen saturation (SVO_2) in PH patients through RHC. In the PH group, the median value of SVO_2 was 68.9% (62.8-73.9%). There was no significant difference between the PAH and CTEPH groups. There were 10 patients from PAH group and 9 patients from CTEPH group who were treated with home oxygen therapy.

The circulation levels of miR-140-3P and miR-485-5P were involved in the pathophysiology of PH

We compared miRNA levels in the PH group to those in the control group. The circulating miRNA levels of miR-140-3p were higher, while those of miR-485-5p were lower, in the PH group compared to those in the control group. The circulation levels of miRNA-30, miRNA-140-5p, miRNA-485-3p, and miRNA-499 did not differ between the two groups (Fig. 2).

Next, we compared the circulating miRNA-140 and miRNA-485 levels between the PAH, CTEPH, and control groups. The circulating levels of miRNA-140-3p were higher, while those of miRNA-485-5p were lower, in both PAH and CTEPH groups, compared

to those in the control group. Furthermore, miRNA-140-3p and miRNA-485-5p levels did not differ between the PAH and CTEPH groups. Also, miRNA-140-5p and miRNA-485-3p levels did not differ between the PAH, CTEPH, and control groups (Fig. 3).

Moreover, we investigated the relationship between the circulating miRNA-140 or miRNA-485 levels and the clinical data, and the hemodynamic parameters. The serum B-type natriuretic peptide levels and the 6MWD were not correlated with the level of miR-140 and miR-485 in any groups. In the PAH+CTEPH group, the miRNA-485-5p level was related to RAP. In the PAH group, the miRNA-140 level was related to PVR and the miRNA-485-5p level was related to CI. In the CTEPH group, the miRNA-485-5p level was related to RAP. We also investigated the relationship between miRNAs and SVO_2 and found that there was no correlation (Table 3). The levels of miRNAs did not differ between patients based on whether they had received medical treatment with specific pulmonary vasodilators.

Discussion

In this study, we demonstrated that the miR-140-3p levels in the PH group were significantly higher than those in the control group, and the miR-485-5p levels in the PH group were significantly lower than those in the control group. Additionally, miR-140-3p and miR-485-5p levels were related to hemodynamic parameters. Other mitochondrial dynamics-related miRNAs, such as miR-30 and miR-499 did not differ between the PH and control groups.

Previous clinical studies demonstrated that either circulating levels of miR-140 and miR-485 were related to coronary artery diseases, such as acute coronary syndrome [20-22]. However, precise mechanisms by which either circulating miR-140 or miR-485 is regulated in coronary artery diseases are still unclear, and there are few clinical

reports to show the relationship among miR-140, miR-485, and PH. A previous study using animal models and in vitro experiments reported that miRNA-140 levels were increased in PH [23]. Another study revealed that miR-140-5p was downregulated in patients with PAH and experimental in vitro models of PAH [24]. Since there are few reports clinically examined in PH patients including not only PAH but also CTEPH, it is still controversial whether miRNAs related to mitochondrial dynamics were involved in PAH and CTEPH. Our paper is a novel clinical study revealing that mitochondrial dynamics-related miRNAs are involved in not only PAH but also CTEPH and have different characteristics in the relationship between hemodynamics and the difference in each PH group, PAH and CTEPH.

Mitochondrial dynamics are controlled by mitochondrial fission proteins, Drp1 and Fis1, and fusion proteins, mitofusin 1 and 2 (Mfn1 and Mfn2), and OPA1, through opposing actions. A previous experimental study revealed that reactive oxygen species and doxorubicin induced mitochondrial fission and apoptosis in cardiomyocytes [16]. In this experimental study, Mfn1 was downregulated, whereas miR-140 was upregulated upon apoptotic stimulation. Furthermore, knockdown of miR-140 increased the expression of Mfn1, inhibited mitochondrial fission, and reduced myocardial infarct sizes in an animal model. Another experimental study demonstrated that overexpression of miR-485-5p blocked mitochondrial fission and myocardial hypertrophy [17]. Since circulating levels of miR-140 increased and miR-485 decreased in PH patients in our clinical study, such alteration of miRNA is thought to be related to the change of mitochondrial dynamics to fission. Marsboom et al. showed that excessive fission with activation of the fission factor, Drp1, occurred in PAH, which led to an increase in intracellular calcium, activity of the mitosis promoter cyclin B1/CDK1, and normoxic activation of hypoxia-induced factor (HIF-1α) [8].

Downregulation of miR-485-5p is also reported to be involved in promoting the proliferation of several cells, including vascular cells [25-27]. These results, altogether, suggest that the increase of miR-140 and the decrease of miR-485 may contribute to force mitochondrial dynamics to fission and promote pulmonary vascular cell proliferation.

In PAH lung, arterial media and intima gradually augment their thickness, leading to an increase in PVR [3]. Indeed, our study showed that the alteration of circulating miR-140-3p levels correlated to PVR only in the PAH group but not in the CTEPH group, even though the miR-140-3p levels were higher in both PAH and CTEPH groups. Intimal thickening and remodeling of small pulmonary vessels are also observed in the CTEPH lung [9]. This change was explained by the shear stress of the non-occluded area. However, the main cause of pathology in CTEPH is thromboembolic obstruction [28]. Therefore, alteration of miR-140-3p and miR-485-5p levels, specifically the increase in miR-140-3p levels, may contribute to the etiology of PAH through pathological changes.

On the other hand, the miR-485-5p levels correlated with CI in the PAH group and RA in the CTEPH group. A previous study reported that miR-485-5p was related to cardiomyocyte hypertrophy [17]. Overexpression of miR-485-5p blocked mitochondrial fission and hypertrophy induced by phenylephrine by inhibiting MAPL expression and increasing the levels of fusion factor, Mfn2, in cultured primary cardiomyocytes. MAPL is a small ubiquitin like modifier E3 ligase and an important contributor in the mitochondrial fission process. In our study, miR-485-5p levels in the CTEPH group correlated to RAP, which represents pressure overload of the right heart, and may facilitate right atrial and ventricular cardiomyocyte remodeling. Therefore, a decrease in miR-485-5p levels may indicate cellular remodeling in PH patients.

In our study, the decrease in miR-485-5p levels was also correlated with the decrease of CI in the PAH group. Regarding the cardiac function in the pathophysiology of PH, there is ventricular interdependence between the RV and left ventricle (LV) [29]. Pressure overload applies mechanical stress on the RV, and the RV is dilated. RV overload and dilation, in turn, decrease LV compliance and stroke volume [30]. Considering that RV overload leads to an increase in RAP, which correlates with the miR-485-5p level, the correlation between the miR-485-5p level and CI may be derived from ventricular interdependence through RV overload. However, the mechanisms underlying how these correlations are dependent on the disease type (PAH or CTEPH) are unclear. Since CI and RA are indicators of clinical prognosis in PH patients [31,32] alteration of circulating miR-485-5p levels may also represent clinical severity.

SVO_2, which reflects the oxygen supply to the whole body, is used in risk assessment of patients with PAH [31,32]. However, there was no correlation between miRNAs and SVO_2 in this study. We examined miRNAs using only hemodynamics and SVO_2 data gathered by RHC. Further studies with other modality data are needed to evaluate the association between SVO_2 and miRNAs.

Limitations

Due to the clinical experimentation using patients with PH, we could not investigate the pathological features of pulmonary vessels or the status of mitochondrial morphology (fission and fusion) on vascular lesions. The origins of miRNAs were also unclear. Since PH is a rare disease, the number of patients enrolled in this study was small. In this experiment, we measured mitochondrial dynamics related miRNAs which were previously reported to be associated to cardiovascular diseases, such as coronary artery disease and cardiac hypertrophy. Since there are many other miRNAs

related to mitochondrial dynamics in other than cardiovascular system, it would be beneficial to examine whether these miRNAs are also associated with PH. Furthermore, since this was a cross-sectional study, we could not evaluate the changes in miRNA levels over time, i.e. whether the miRNA levels changed along the course of the specific PH therapy.

Conclusion

Mitochondrial dynamics-related circulating miRNAs, miR-140-3p and miR-485-5p, show different characteristics and may play crucial roles in regulating hemodynamic changes in clinical PAH and CTEPH.

Acknowledgments

We thank Ms Michiko Shimokawa, Ms Ayako Kuroki, and Ms Aya Yanagi for their excellent technical assistance and Dr Osamu Yasuda for his instrumental supply.

Sources of Funding

This research was partially supported by the Japan Society for the Promotion of Science KAKENHI (Grant No. 19K07891).

Disclosure

None

Table 1 miRNA sequences.

miRNA	**Sequence**	**miRBase Accession Number**
miR-30a-3p	CUUUCAGUCGGAUGUUUGCAGC	MIMAT0000088
miR-30a-5p	UGUAAACAUCCUCGACUGGAAG	MIMAT0000087
miR-140-3p	UACCACAGGGUAGAACCACGG	MIMAT0004597
miR-140-5p	CAGUGGUUUUACCCUAUGGUAG	MIMAT0000431
miR-485-3p	GUCAUACACGGCUCUCCUCUCU	MIMAT0002176
miR-485-5p	AGAGGCUGGCCGUGAUGAAUUC	MIMAT0002175
miR-499a-5p	UUAAGACUUGCAGUGAUGUUU	MIMAT0002870
Cel-miR-39-3p	UCACCGGGUGUAAAUCAGCUUG	MIMAT0000010

Table 2 Patients' characteristics.

	control n=30	PH (PAH+CTEPH) n=34	PAH n=12	CTEPH n=22	*p*-value (control vs PH)
Age (years)	67.4±12.0	60.2±14.0	51.0±17.2	65.2±8.9	0.034
Female (%)	14 (46.7)	29 (85.3)	10 (83.3)	19 (86.4)	0.001
NYHA Ⅰ/Ⅱ/Ⅲ/Ⅳ	2/20/5/3	0/19/14/1	0/8/3/1	0/11/11/0	
6MWD (m)	-	371.0 (293.0-433.0)	236.5 (67.5-362.0)	381.0 (318.0-450.0)	-
BNP (pg/mL)	108.4 (44.4-338.9)	47.4 (12.2-165.6)	25.3 (8.8-64.3)	53.8 (18.5-209.2)	0.002
mPAP (mmHg)	16.0 (14.0-24.0)	33.5 (25.75-43.0)	27.0 (25.0-38.25)	37.0 (29.8-43.5)	<0.001
PAWP (mmHg)	11.2±5.3	7.3±3.1	7.6±2.5	7.1±3.5	<0.001
RA (mmHg)	4.5 (3.0-7.0)	4 (3.0-6.3)	4.5 (4.0-6.0)	3.0 (2.8-7.3)	0.311
CI (L/min/㎡)	2.7±0.6	2.6±0.9	3.3±1.1	2.2±0.4	0.340

PVR (Wood・units)	1.6 (1.2-2.0)	6.3 (5.9-12.3)	4.5 (2.4-6.0)	7.8 (5.9-12.4)	<0.001
SVO_2(%)	-	68.9 (62.8-73.9)	73.8 (56.9-77.6)	67.5 (62.8-72.0)	-
Diuretics	15	18	10	8	-
HOT	0	19	10	9	-

PAH, pulmonary artery hypertension; PH, pulmonary hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; NYHA, New York Heart Association functional classification; 6MWD, 6-minute walking distance; BNP, B-type natriuretic peptide; mPAP, mean pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; RA, right atrial pressure; CI, cardiac index; PVR, pulmonary vascular resistance; SVO_2, mixed venous oxygen saturation; HOT, home oxygen therapy.

Table 3 Correlation of miRNAs levels and hemodynamics data.

PH (PAH + CTEPH)							
	log (RA)	CI	log (mPAP)	PVR	log (SVO_2)	log (BNP)	log (6MWD)
log (140-3p)	r = 0.169	r = −0.035	r = 0.282	r = 0.351	r = −0.052	r = −0.171	r = 0.003
	p = 0.369	p = 0.850	p = 0.123	p = 0.052	p = 0.784	p = 0.356	p = 0.989
log (485-5p)	r = −0.421	r = 0.146	r = 0.135	r = 0.173	r = 0.011	r = 0.295	r = 0.346
	p = 0.020	p = 0.439	p = 0.465	p = 0.352	p = 0.950	p = 0.106	p = 0.158

PAH							
	log (RA)	CI	log (mPAP)	PVR	log (SVO_2)	log (BNP)	log (6MWD)
log (140-3p)	r = −0.087	r = −0.356	r = 0.454	r = 0.582	r = −0.227	r = 0.034	r = 0.331
	p = 0.787	p = 0.282	p = 0.138	p = 0.046	p = 0.500	p = 0.914	p = 0.293
log (485-5p)	r = 0.171	r = 0.606	r = 0.379	r = 0.301	r = 0.230	r = 0.231	r = 0.507
	p =	p =	p =	p =	p =	p =	p =

	0.596	0.048	0.224	0.340	0.495	0.496	0.492

CTEPH							
	log (RA)	CI	log (mPAP)	PVR	log (SVO_2)	log (BNP)	log (6MWD)
log (140-3p)	r = 0.223	r = −0.090	r = 0.241	r = 0.190	r = −0.068	r = −0.190	r = −0.080
	p = 0.373	p = 0.713	p = 0.312	p = 0.434	p = 0.781	p = 0.435	p = 0.784
log (485-5p)	r = −0.531	r = 0.145	r = −0.022	r = −0.059	r = 0.218	r = 0.245	r = 0.346
	p = 0.023	p = 0.552	p = 0.927	p = 0.807	p = 0.369	p = 0.310	p = 0.224

PAH, pulmonary artery hypertension; PH, pulmonary hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; RA, right atrial pressure; CI, cardiac index; mPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; SVO_2, mixed venous oxygen saturation; BNP, B-type natriuretic peptide; 6MWD, 6-minute walk distance.

References

[1] Fukuda K, Date H, Doi S, Fukumoto Y, Fukushima N, Hatano M, et al. Guidelines for the treatment of pulmonary hypertension (JCS 2017/JPCPHS 2017). Circ J 2019;83:842-945.

[2] Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, et al. Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol 2013;62:D34-41.

[3] Stacher E, Graham BB, Hunt JM, Gandjeva A, Groshong SD, McLaughlin VV, et al. Modern age pathology of pulmonary arterial hypertension. Am J Respir Crit Care Med 2012;186:261-72.

[4] Simonneau G, Torbicki A, Dorfmüller P, Kim N. The pathophysiology of chronic thromboembolic pulmonary hypertension. Eur Respir Rev 2017;26:160112.

[5] Minatsuki S, Kodera S, Kiyosue A, Saito A, Maki H, Hatano M, et al. Balloon pulmonary angioplasty improves quality of life in Japanese patients with chronic thromboembolic pulmonary hypertension. J Cardiol 2020;76:205-10.

[6] Fukumitsu M, Suzuki K. Mesenchymal stem/stromal cell therapy for pulmonary arterial hypertension: Comprehensive review of preclinical studies. J Cardiol 2019;74:304-12.

[7] Dasgupta A, Wu D, Tian L, Xiong PY, Dunham-Snary KJ, Chen KH, et al. Mitochondria in the pulmonary vasculature in health and disease: oxygen-sensing, metabolism, and dynamics. Compr Physiol 2020;10:713-65.

[8] Marsboom G, Toth PT, Ryan JJ, Hong Z, Wu X, Fang YH, et al. Dynamin-related protein 1-mediated mitochondrial mitotic fission permits hyperproliferation of vascular smooth muscle cells and offers a novel therapeutic target in pulmonary hypertension. Circ Res 2012;110:1484-97.

[9] Ikeda Y, Shirakabe A, Brady C, Zablocki D, Ohishi M, Sadoshima J. Molecular mechanisms mediating mitochondrial dynamics and mitophagy and their functional roles in the cardiovascular system. J Mol Cell Cardiol 2015;78:116-22.

[10] Ikeda Y, Sciarretta S, Nagarajan N, Rubattu S, Volpe M, Frati G. New insights into the role of mitochondrial dynamics and autophagy during oxidative stress and aging in the heart. Oxid Med Cell Longev 2014;2014:210934.

[11] Rothman A, Restrepo H, Sarukhanov V, Evans WN, Wiencek JRG, Williams R, et al. Assessment of microRNA and gene dysregulation in pulmonary hypertension by endoarterial biopsy. Pulm Circ 2017;7:455-64.

[12] Namino F, Yamakuchi M, Iriki Y, Okui H, Ichiki H, Maenosono R, et al. Dynamics of soluble thrombomodulin and circulating miRNAs in patients with atrial fibrillation undergoing radiofrequency catheter ablation. Clin Appl Thromb Hemost 2019;25:1076029619851570.

[13] Gu D, Zou X, Ju G, Zhang G, Bao E, Zhu Y. Mesenchymal stromal cells derived extracellular vesicles ameliorate acute renal Ischemia reperfusion Injury by inhibition of mitochondrial fission through miR-30. Stem Cells Int 2016;2016:2093940.

[14] Li J, Donath S, Li Y, Qin D, Prabhakar BS, Li P. MiR-30 regulates mitochondrial fission through targeting p53 and the dynamin-related protein-1 pathway. PLoS Genet 2010;6:e1000795.

[15] Yang S, Li H, Chen L. MicroRNA-140 attenuates myocardial ischemia-reperfusion injury through suppressing mitochondria-mediated apoptosis by targeting YES1. J Cell Biochem 2019;120:3813-21.

[16] Li J, Li Y, Jiao J, Wang J, Li Y, Qin D, et al. Mitofusin 1 is negatively regulated by microRNA 140 in cardiomyocyte apoptosis. Mol Cell Biol 2014;34:1788-99.

[17] Zhao Y, Ponnusamy M, Liu C, Tian J, Dong Y, Gao J, et al. MiR-485-5p

modulates mitochondrial fission through targeting mitochondrial anchored protein ligase in cardiac hypertrophy. Biochim Biophys Acta Mol Basis Dis 2017;1863:2871-81.

[18] Wang JX, Jiao JQ, Li Q, Long B, Wang K, Liu JP, et al. MiR-499 regulates mitochondrial dynamics by targeting calcineurin and dynamin-related protein-1. Nat Med 2011;17:71-8.

[19] Kondratova K, Kurapeevb D, Popovb M, Sidorovac M, Minasianb S, Galagudzab M, et al. Heparinase treatment of heparin-contaminated plasma from coronary artery bypass grafting patients enables reliable quantification of microRNAs. Biomol Detect Quantif 2016;8:9-14.

[20] Li X, Yang Y, Wang L, Qiao S, Lu X, Wu Y, et al. Plasma miR-122 and miR-3149 potentially novel biomarkers for acute coronary syndrome. PLoS One 2015;1;10:e0125430.

[21] Karakas M, Schulte C, Appelbaum S, Ojeda F, Lackner KJ, Münzel T, et al. Circulating microRNAs strongly predict cardiovascular death in patients with coronary artery disease-results from the large AtheroGene study. Eur Heart J 2017;38:516-23.

[22] D'Alessandra Y, Carena MC, Spazzafumo L, Martinelli F, Bassetti B, Devanna P, et al. Diagnostic potential of plasmatic MicroRNA signatures in stable and unstable angina. PLoS One 2013;15;8:e80345.

[23] Joshi SR, Dhagia V, Gairhe S, Edwards JG, McMurtry IF, Gupte SA. MicroRNA-140 is elevated and mitofusin-1 is downregulated in the right ventricle of the Sugen5416/hypoxia/normoxia model of pulmonary arterial hypertension. Am J Physiol Heart Circ Physiol 2016;311:H689-98.

[24] Zhang Y, Xu J. MiR-140-5p regulates hypoxia-mediated human pulmonary artery smooth muscle cell proliferation, apoptosis and differentiation by targeting Dnmt1 and

promoting SOD2 expression. Biochem Biophys Res Commun 2016;473:342-8.
[25] Musri MM, Coll-Bonfill N, Maron BA, Peinado VI, Wang RS, Altirriba J, et al. MicroRNA dysregulation in pulmonary arteries from chronic obstructive pulmonary disease. Relationships with vascular remodeling. Am J Respir Cell Mol Biol 2018;59:490-9.
[26] Wang FR, Xu SH, Wang BM, Wang F. MiR-485-5p inhibits metastasis and proliferation of osteosarcoma by targeting CX3CL1. Eur Rev Med Pharmacol Sci 2018;22:7197-204.
[27] Han DL, Wang LL, Zhang GF, Yang WF, Chai J, Lin HM, et al. MiRNA-485-5p, inhibits esophageal cancer cells proliferation and invasion by down-regulating O-linked N-acetylglucosamine transferase. Eur Rev Med Pharmacol Sci 2019;23:2809-16.
[28] Lang IL, Dorfmuller P, Noordegraaf AF. The pathobiology of chronic thromboembolic pulmonary hypertension. Ann Am Thorac Soc 2016;13 (Suppl. 3):S215-21.
[29] Naeije R, Badagliacca R. The overloaded right heart and ventricular interdependence. Cardiovasc Res 2017;113:1474-85.
[30] Andersen S, Nielsen-Kudsk JE, Vonk Noordegraaf A, de Man FS. Right ventricular fibrosis. Circulation 2019;139:269-85.
[31] Benza RL, Gomberg-Maitland M, Miller DP, Frost A, Frantz RP, Foreman AJ, et al. The REVEAL registry risk score calculator in patients newly diagnosed with pulmonary arterial hypertension. Chest 2012;141:354-62.
[32] Nickel N, Golpon H, Greer M, Knudsen L, Olsson K, Westerkamp V, et al. The prognostic impact of follow-up assessments in patients with idiopathic pulmonary arterial hypertension. Eur Respir J 2012;39:589-96.

1

2

Figure Legends

Figure 1 Division of the RHC patients. Patients who underwent RHC because of left heart disease were the control group (n=30). There were 41 pre-capillary PH patients, whose mean pulmonary arterial pressure was 25 mmHg or over, and whose pulmonary artery wedge pressure was less than 15 mmHg, and we excluded 7 patients with pulmonary disease from these 41 patients. The remaining 34 PH patients were classified as the PH group.

PAH, pulmonary artery hypertension; PH, pulmonary hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; RHC, right heart catheterization.

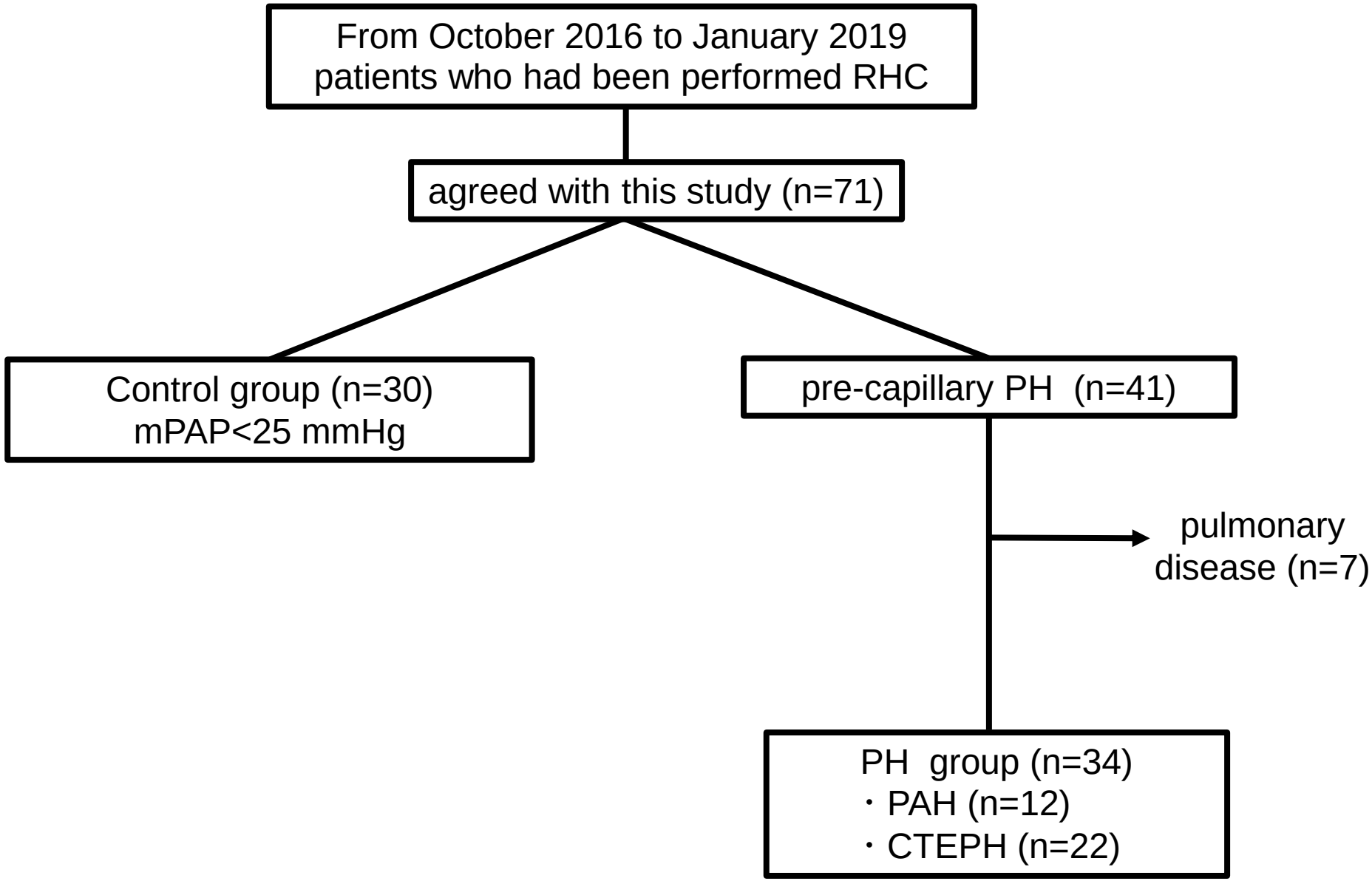
Figure 2 Comparison of serum miRNAs levels; control group vs PH group.

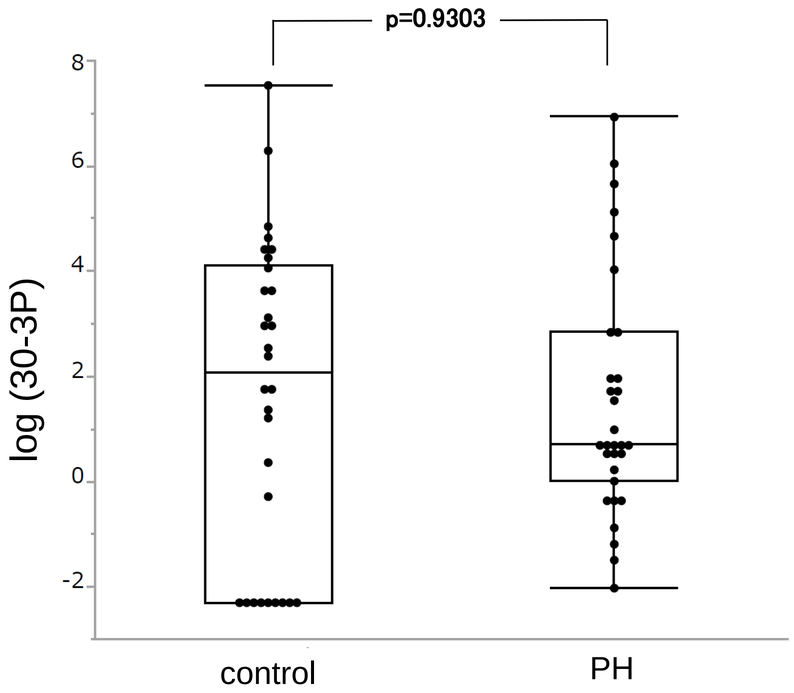
The miRNA levels of miR-140-3p were higher and those of miR-485-5P were lower in the PH group than in the control group.

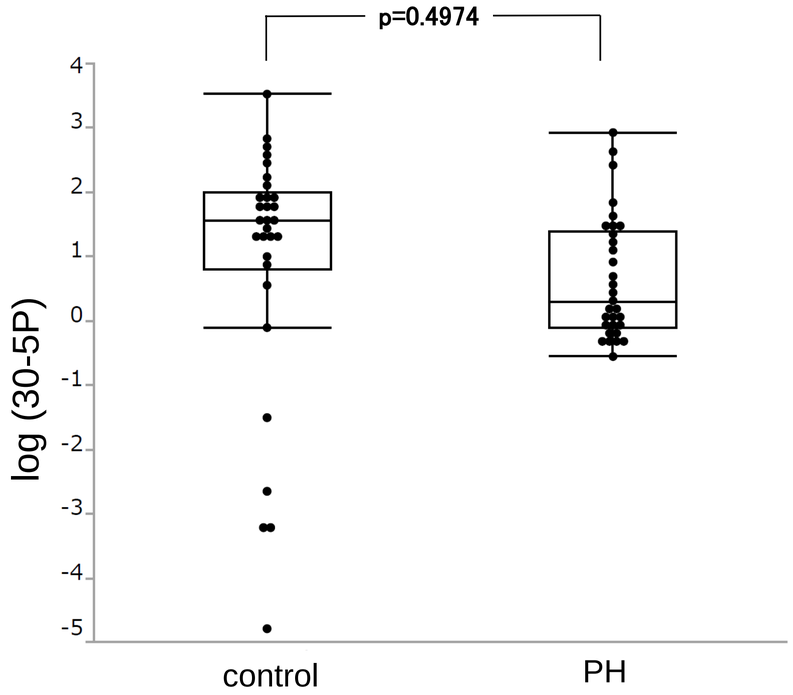
PH, pulmonary hypertension.

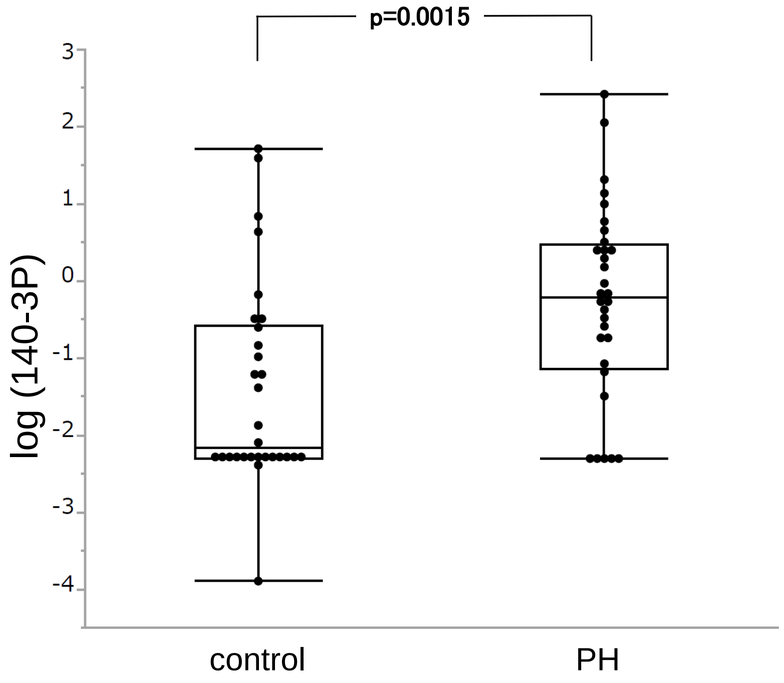
Figure 3 Comparison of miR140-3p and miR485-5p levels; control vs PAH vs CTEPH. The miRNA-140-3p level was higher and that of miRNA-485-5p was lower in the PAH and CTEPH groups than in the control group. There was no change in the miRNA-140-3p level between the PAH group and CTEPH group.

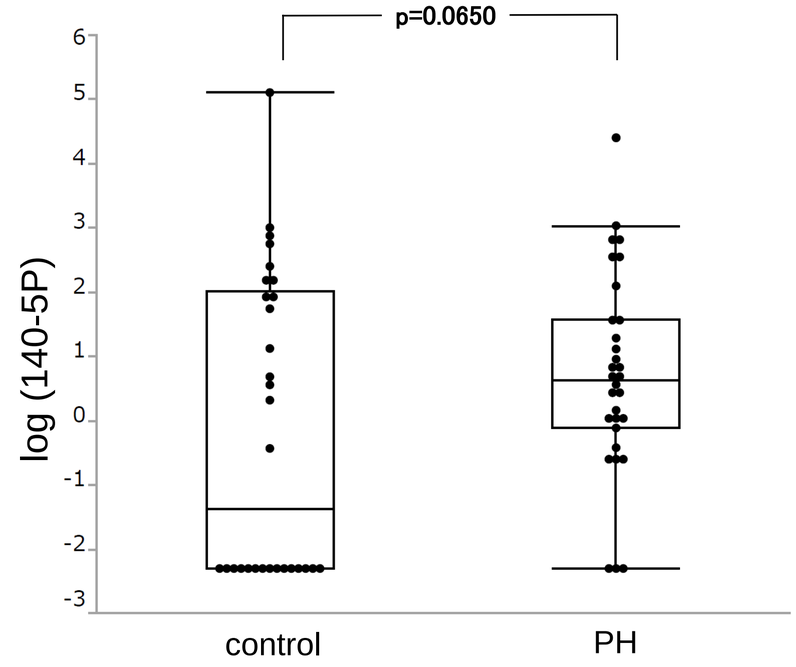
PAH, pulmonary artery hypertension; CTEPH, chronic thromboembolic pulmonary hypertension.

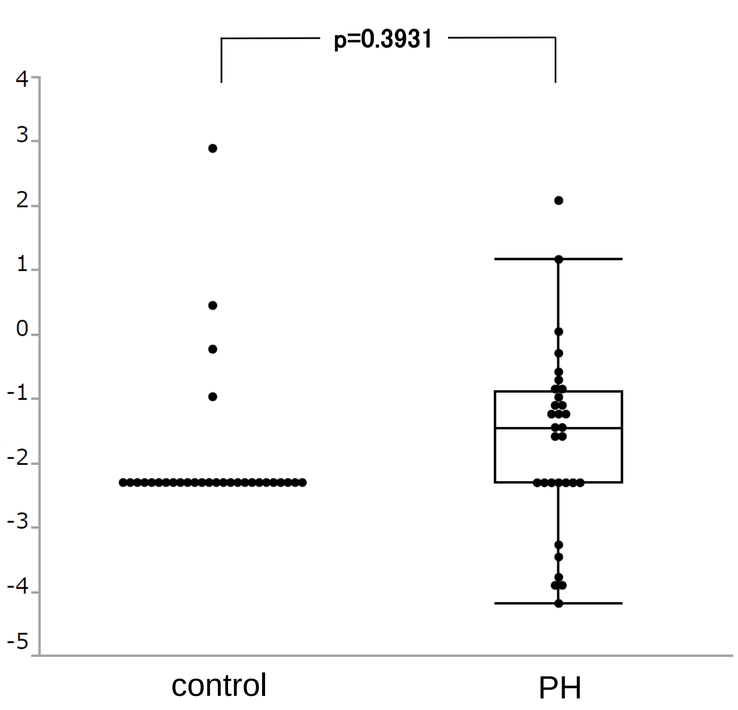

From October 2016 to January 2019
patients who had been performed RHC
agreed with this study (n=71)
Control group (n=30)
mPAP<25 mmHg
pre-capillary PH (n=41)
pulmonary
disease (n=7)
PH group (n=34)
・PAH (n=12)
・CTEPH (n=22)

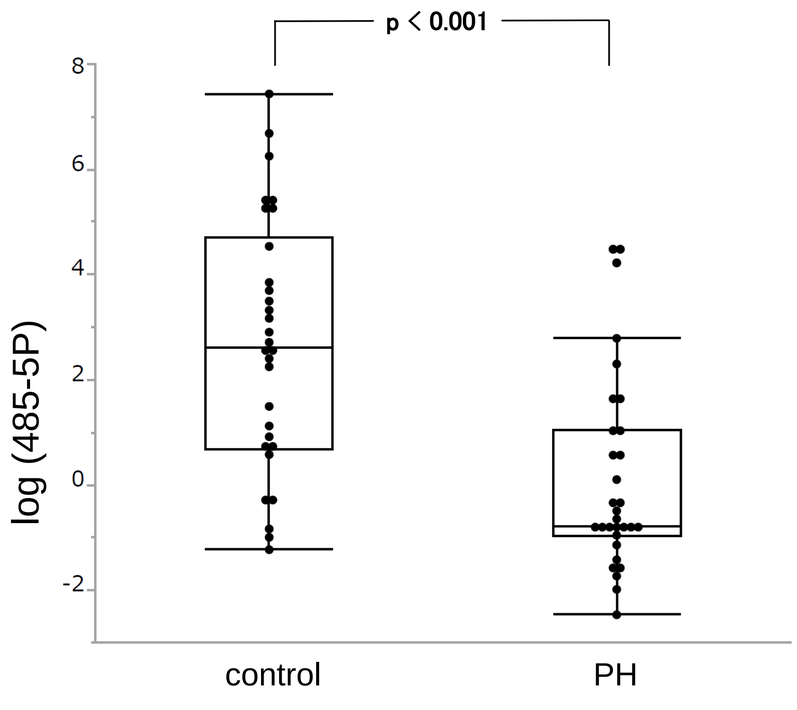

p=0.9303
log (30-3P)
8
6
4
2
0
-2
control
PH

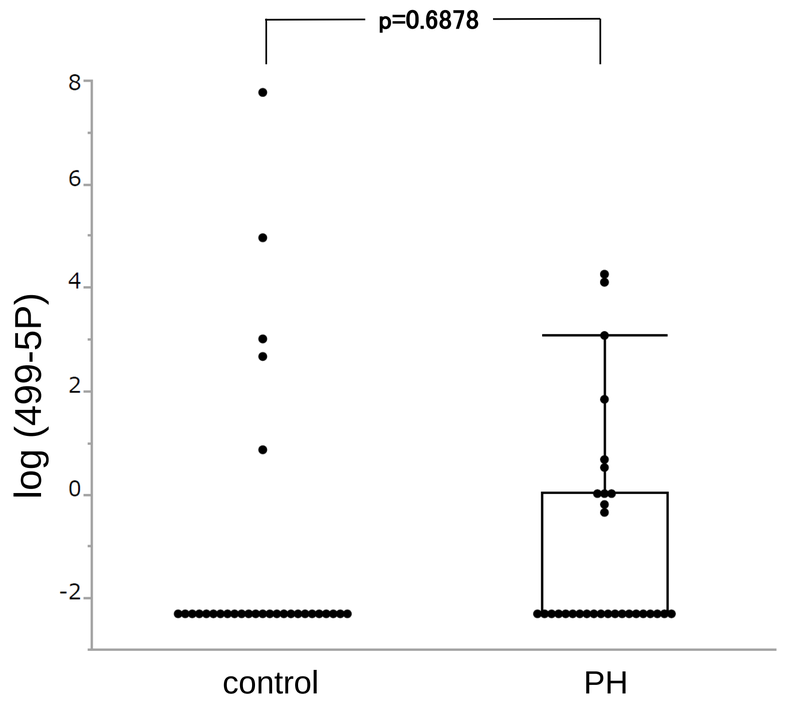

p=0.4974
log (30-5P)
4
3
2
1
0
-1
-2
-3
-4
-5
control
PH

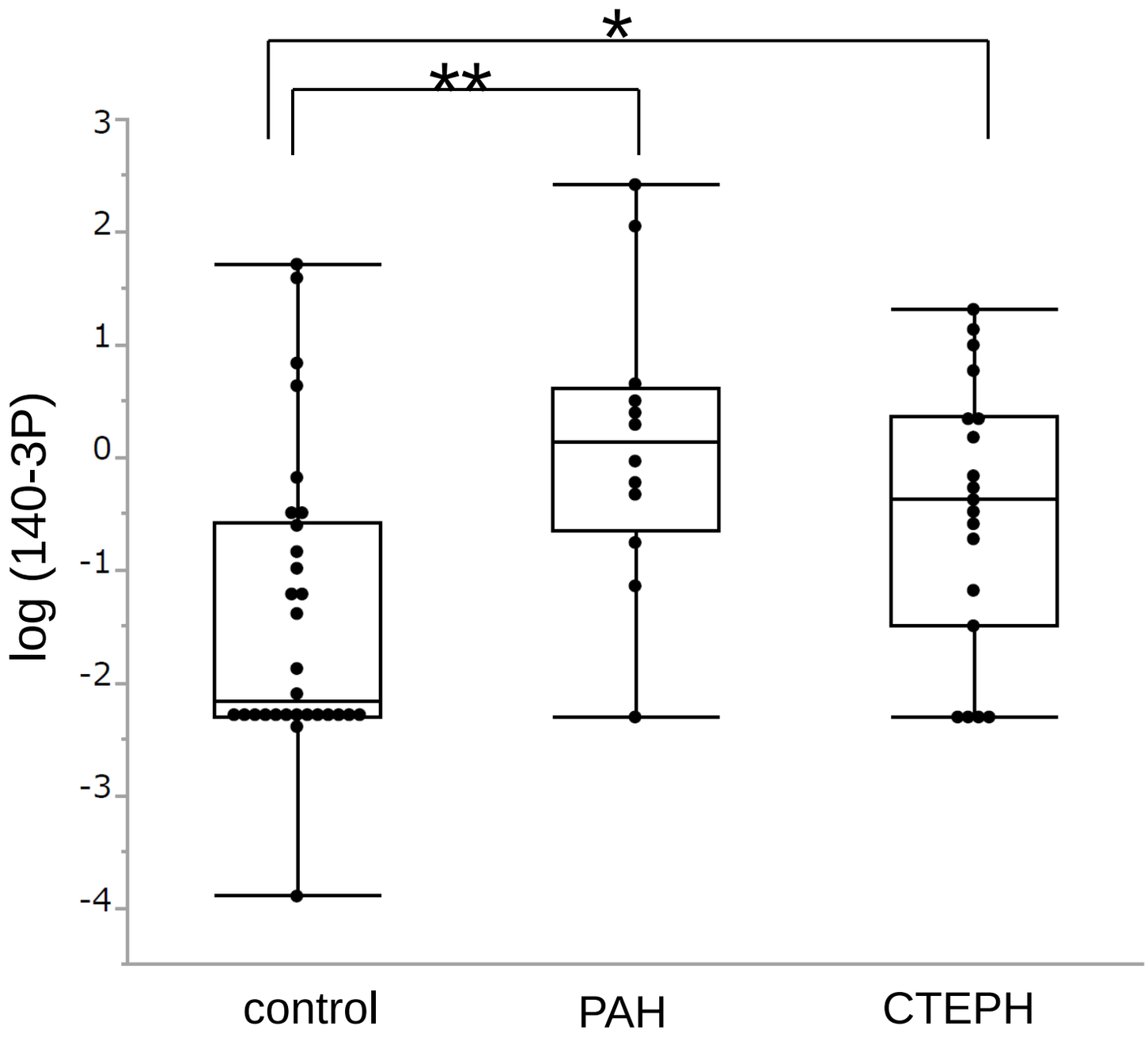

p=0.0015
log (140-3P)
3
2
1
0
-1
-2
-3
-4
control
PH


p=0.0650
log (140-5P)
6
5
4
3
2
1
0
-1
-2
-3
control
PH

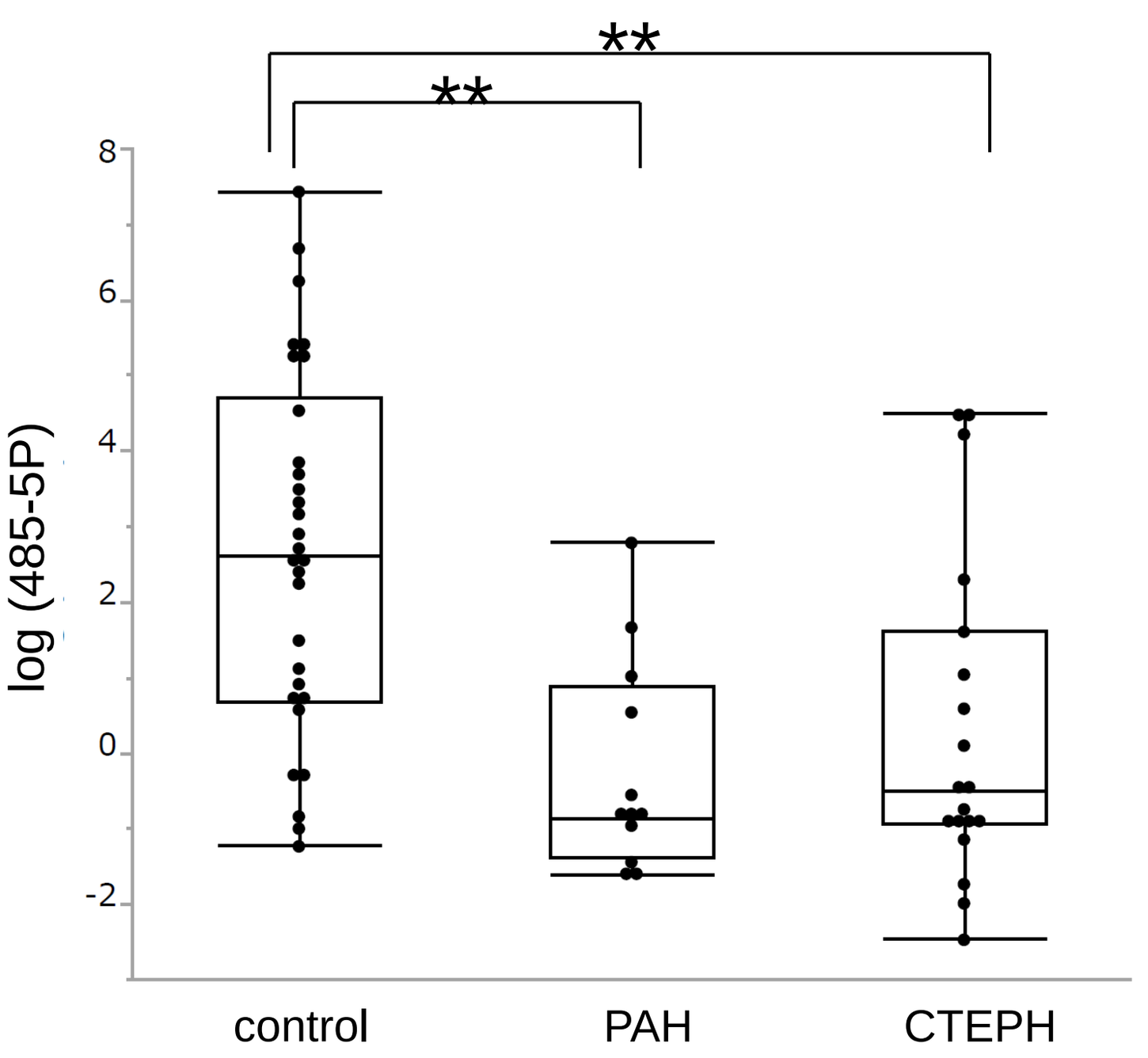

p=0.3931
log (485-3P)
4
3
2
1
0
-1
-2
-3
-4
-5
control
PH


p < 0.001
log (485-5P)
8
6
4
2
0
-2
control
PH


p=0.6878
log (499-5P)
8
6
4
2
0
-2
control
PH



*p < 0.01
**p< 0.001



**p< 0.001