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PERM1: A NOVEL REGULATOR OF MITOCHONDRIAL ENERGETICS IN CARDIOMYOCYTES

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BACKGROUND

Heart failure is a leading cause of mortality in developed countries. Heart failure is a chronic, progressive condition, in which the heart is unable to pump blood to meet the perfusion and metabolic needs of the body. The failing heart is known as the "energy-starved heart", where mitochondria are impaired[1]. It has been postulated that mitochondria are the therapeutic target of heart failure[2]. Our recent study demonstrated that the histone methyltransferase Smyd1 regulates mitochondrial energetics in the heart[3]. Through screening of genes that are regulated by Smyd1, we identified Perm1 (PGC-1- and ERR-induced regulator, muscle 1) as a downstream target in the metabolic network. However, the role of Perm1 in the heart is unknown.

SIGNIFICANCE

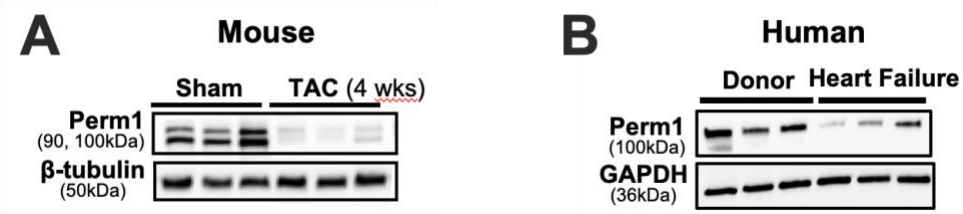


Figure 1. Perm1 is downregulated in the failing heart. (A) Western blotting analysis showing that the protein level of Perm1 was decreased in the mouse heart that was subjected to transverse aortic constriction (TAC) (32.0 ± 6.7% of sham hearts, and (B) in cardiac tissue that was collected from patients before the implantation of a left ventricular assist device (55.2 ± 13.1% of donors, as compared with donor hearts). *: $p<0.05$. Oka et al. PLoS ONE. 2020.

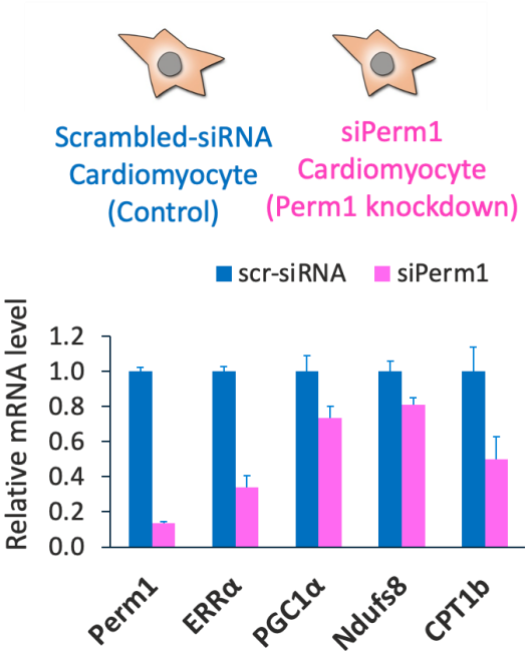


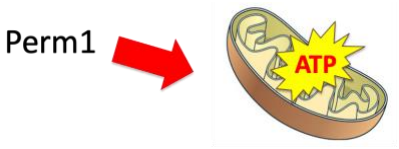
Figure 2. Downregulation of metabolic genes by siRNA-mediated Perm1 knockdown (siPerm1) in cardiomyocytes. Quantitative PCR (qPCR) showed that Perm1 knockdown leads to downregulation of ERRα and PGC-1α, both which are the key regulators of mitochondrial bioenergetics. Ndufs8 (OXPHOS gene) and CPT1b (FAO gene) were also downregulated in siPerm1 cardiomyocytes. *: $p<0.05$. Modified from Oka et al. PLoS ONE. 2020.

HYPOTHESIS

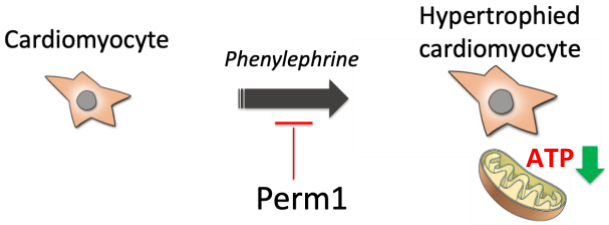
Overexpression of Perm1 enhances mitochondrial energetics.

OBJECTIVES

Aim 1: To investigate the effects of overexpression of Perm1 on the expression of genes involved in mitochondrial bioenergetics.



Aim 2: To test the hypothesis if overexpression of Perm1 rescues downregulation of metabolic genes during cellular hypertrophic stress.



METHODS

Neonatal rat cardiomyocytes were transfected to overexpress Perm1 using adenovirus infection. Cellular hypertrophy was induced by incubating with phenylephrine for 48 hours. RNA was then extracted from neonatal rat cardiomyocytes. Reverse transcription was performed to make cDNA from RNA. Quantitative PCR (qPCR) using Taqman primers allowed for quantification of gene expression.

RESULTS

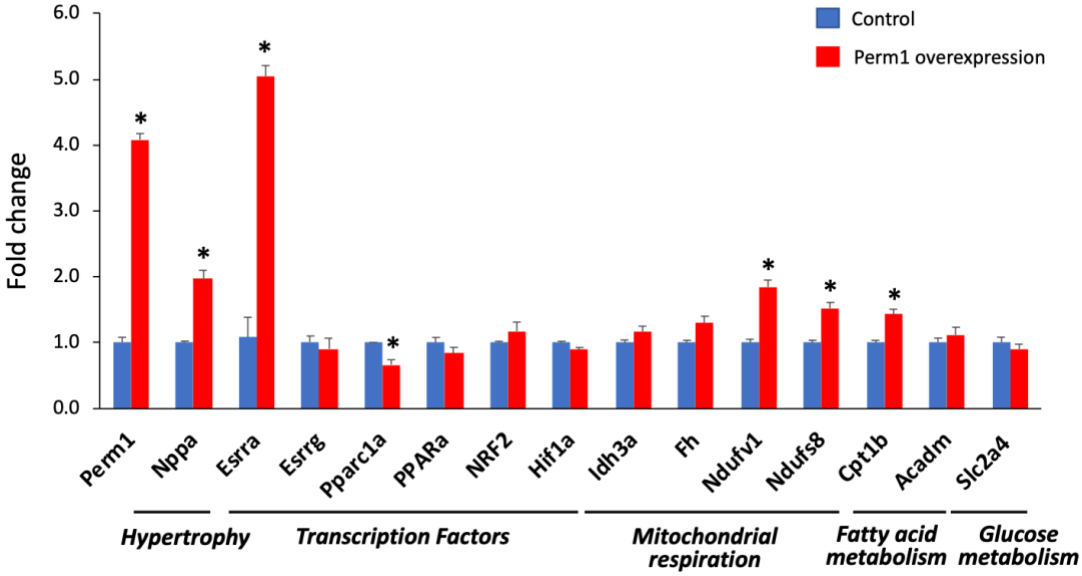


Figure 3. Perm1 induces some metabolic genes in cardiomyocytes. Adenovirus-mediated overexpression of Perm1 (Ad-PM1) in led to the significant increase of mRNA levels of ERRα and genes involved in mitochondrial energetics (Ndufv1; Ndufs8; Cpt1b), as compared with control (Ad-null). *: <0.05. Oka et al. PLoS ONE. 2020.

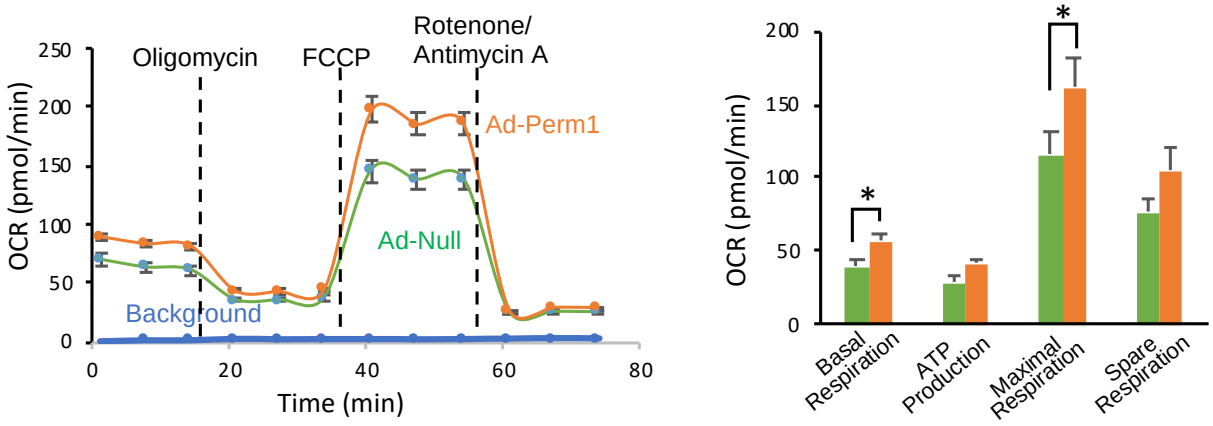


Figure 4. Perm1 overexpression in cardiomyocytes enhances mitochondrial respiration capacity. Cell Mito Stress Test was performed using a Seahorse XF flux analyzer. Glucose was used as a major substrate of mitochondria. *: <0.05. Oka et al. PLoS ONE. 2020.

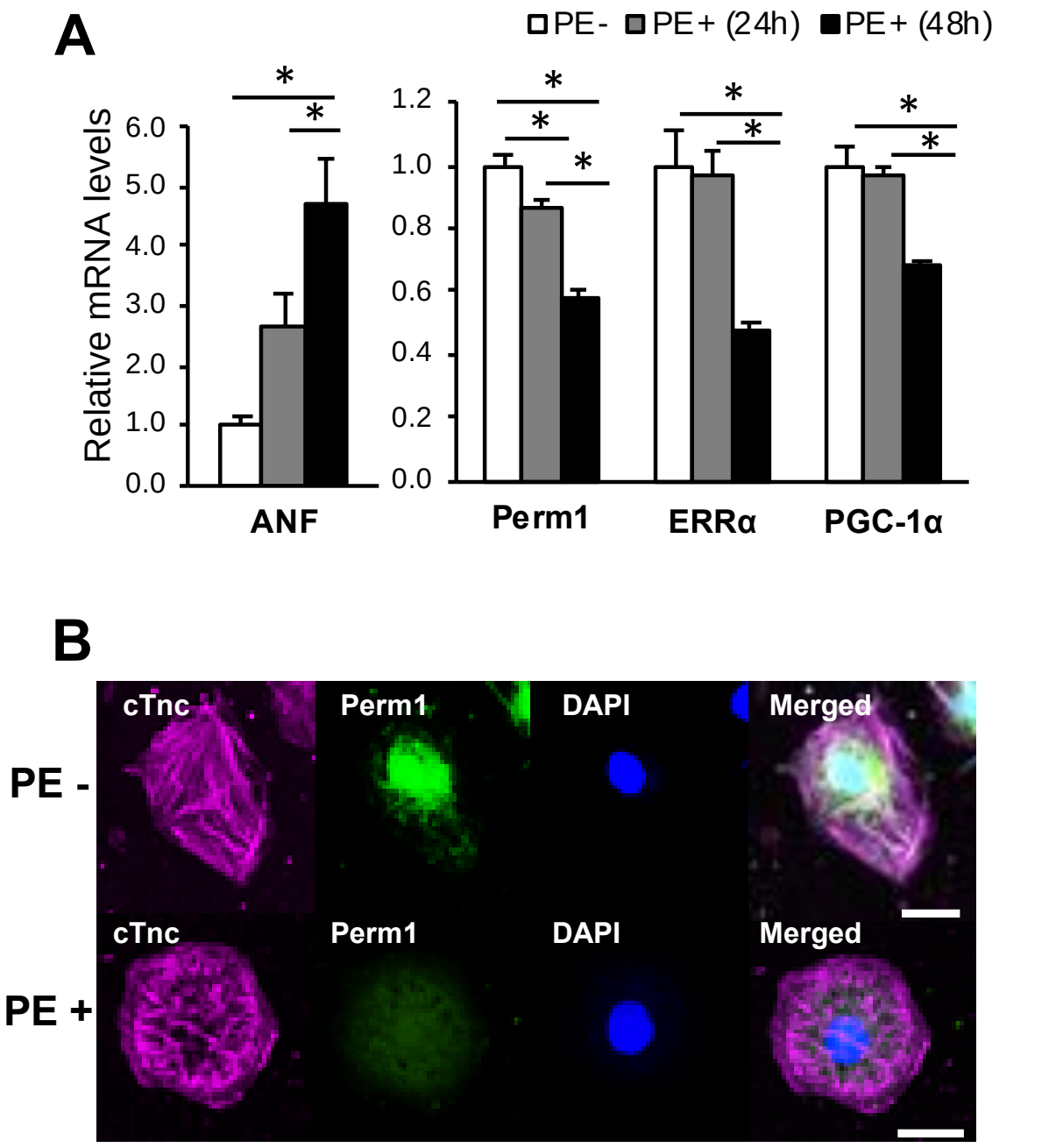


Figure 5. Perm1 is downregulated by phenylephrine (PE)-induced cellular hypertrophic stress. (A) qPCR shows that Perm1 was downregulated in cardiomyocytes after 24h incubation with PE (50 μM), followed by downregulation of ERRα and PGC-1α (at 48h incubation), when the mRNA level of ANP, a marker of hypertrophy, was significantly increased. *: $p<0.05$ (B) Immunostaining of cardiomyocytes shows that Perm1 was predominantly localized in the nucleus in cardiomyocytes. After incubation with PE (24h), Perm1 was less localized in the nucleus, showing the diffused expression pattern (scale bar: 20 μM). Oka et al. PLoS ONE. 2020.

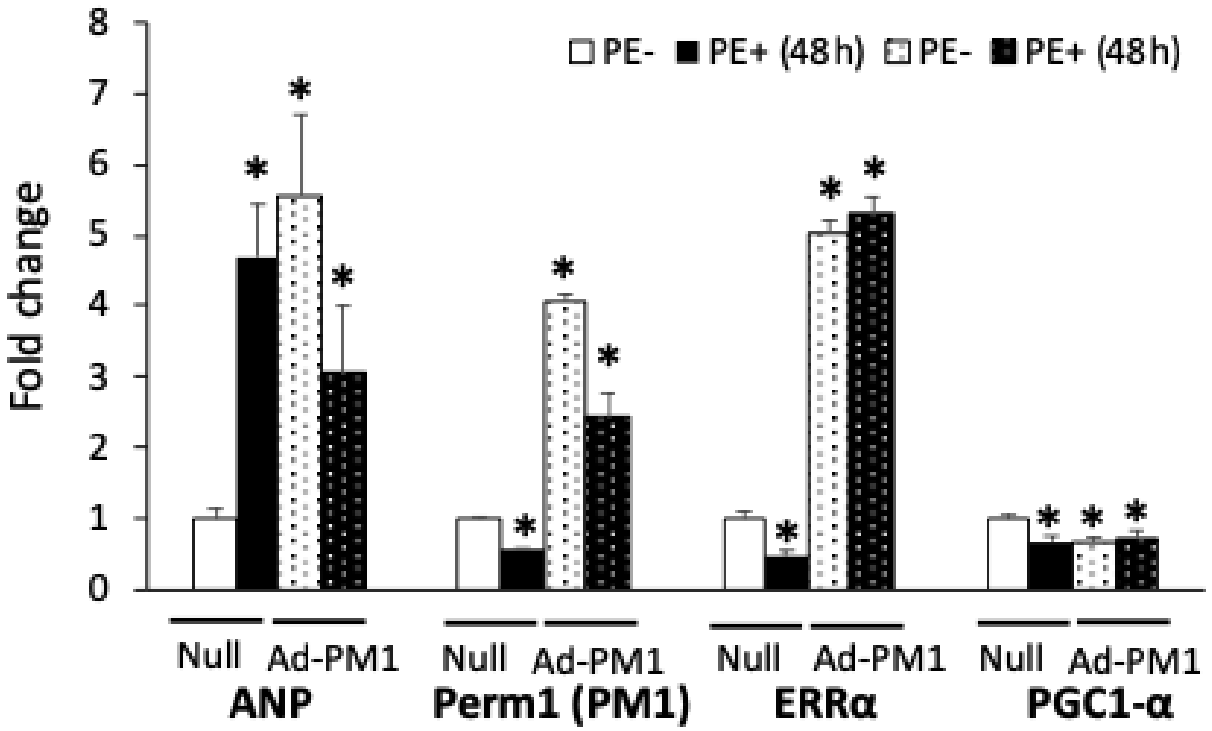


Figure 6. Perm1 rescues downregulation of ERRα in cardiomyocytes. qPCR shows that Perm1 overexpression prior to PE-induced hypertrophic stress prevents downregulation of ERRα, whereas the downregulation of PGC-1α was not rescued by overexpression of Perm1. *: $p<0.05$ vs. control (null).s

SUMMARY
Overexpression of Perm1 in cardiomyocytes led to the upregulation of a subset of metabolic genes, in association with enhanced mitochondrial energetics. Perm1 overexpression rescued the downregulation of ERRα, caused by phenylephrine-induced cellular hypertrophy.

CONCLUSIONS
Perm1 positively regulates mitochondrial energetics in cardiomyocytes.

ACKNOWLEDGMENTS
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