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REVIEW ARTICLE

Influence of estrogen on right ventricular mitochondrial function in pulmonary hypertension

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Abstract

There is a female predominance in pulmonary arterial hypertension (PAH) with a ~4:1 female-to-male ratio. However, female PAH patients exhibit better right ventricular (RV) function and thus better survival than the males. The majority of the current PAH therapies target pulmonary vascular remodeling and/or vasoconstriction in the pulmonary vasculature. However, no therapies directly target the RV, partially because the underlying mechanisms of RV failure in PAH are not fully understood. Since the RV serves as the main determinant of mortality, clarifying the mechanism of RV failure and the associated sex differences in PAH may promote the development of novel therapeutic strategies. Numerous molecular abnormalities have been detected in RV in PAH, particularly due to the suppression of mitochondrial function, including the inhibition of glucose oxidation and a shift to uncoupled aerobic glycolysis (Warburg metabolism) and excessive mitochondrial fission. The mitochondrial suppression is associated with hypocontractility and increased apoptosis in RV cardiomyocytes, and the hyperproliferative, pro-fibrotic, and apoptosis-resistant phenotypes in RV fibroblasts in PAH. Mitochondria also serve as the site for sex steroid synthesis, and in turn, the sex steroids, particularly estradiol (E2), influence the mitochondria both indirectly and directly. It is not well understood how E2 affects mitochondrial function in RV in PAH. Hence, this review focuses on the key mitochondrial genes and proteins that are influenced by PAH, E2, and sex.

Keywords: Estradiol; Pulmonary arterial hypertension; Right ventricle; Mitochondria; Sex differences

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1. Introduction

Pulmonary hypertension (PH) is a progressive and life-threatening disease that is characterized by a sustained increase in pulmonary artery pressure and subsequent right ventricular (RV) dysfunction and failure.[1] Hemodynamically, this condition can be defined as a mean pulmonary artery pressure greater than 20 mmHg at rest.[2] The World Health Organization (WHO) has categorized PH into five different groups (Table 1).[2] In this review, we will focus on group 1, pulmonary arterial hypertension (PAH). PAH can be further subcategorized into seven different subtypes: idiopathic PAH, heritable PAH, PAH due to or associated with drugs and toxins, connective tissue disease, congenital heart disease, etc., PAH due to long-term response to calcium channel blockers, PAH

Table 1. Pulmonary hypertension (PH) groups as classified by the World Health Organization (WHO)

Group	Definition
Group 1	Pulmonary arterial hypertension
Group 2	Pulmonary hypertension due to left heart disease
Group 3	Pulmonary hypertension due to lung diseases and/or hypoxia
Group 4	Pulmonary hypertension due to pulmonary artery obstructions
Group 5	Pulmonary hypertension with unclear and/or multifactorial mechanisms

Note: Adapted from reference Humbert *et al*[2]

with overt features of venous/capillaries involvement, and persistent PH of the newborn syndrome.[2]

PAH is a rare disease with an estimated prevalence of around 5 – 52 cases/million.[3] Although rare, PAH is a fatal disease with high mortality. More than 30 years ago, one registry reported 1-year survival rate of 68% and 5-year survival rate of 34% in patients with heritable PAH.[4] As more treatments are developed and the management of PAH is improved, the estimated 1-year and 5-year survival rates have improved to ~86 – 90% and 61 – 65%, respectively, based on recent registries on PAH[5-7] and an estimated 1-year survival rate of 94% and 10-year survival rate of 60% in another recent registry on PAH associated with connective tissue disease.[8] At present, the mortality rate of PAH patients remains high and better therapeutics are needed.

In PAH, the pathology begins with an obstructive vasculopathy in the pulmonary circulation. As a result, many studies and therapeutic development have focused on the pulmonary vasculature. However, the prognosis in PAH is mainly determined by the response of the RV to the RV pressure overload, with RV failure accounting as the major cause of death in PAH patients.[9,10] At present, most PAH therapies only target the pulmonary vasculature component, with no therapies directly and effectively targeting the RV. Since RV is the major determinant of mortality, it has thus been of great interest in recent years. Note that when assessing the response of RV to increased RV afterload, RV function alone such as RV contractility is not sufficient, rather RV-pulmonary artery coupling is a better metric to assess the cardiopulmonary function and provide prognosis.[11-13] Another important fact in PAH is the sex paradox. PAH predominantly affects females in which there is a ~4:1 female-to-male ratio.[14-17] Although female sex represents a clinical risk factor, male PAH patients have worse survival rates particularly due to poorer RV status and hemodynamic profiles.[11,18-20] This is evidenced by higher RV contractility and RV-pulmonary artery coupling, lower RV mass, and better diastolic adaptation in female PAH patients than the males.[11] In addition, females also reportedly respond better to approved PAH therapies than males.[20] Hence, there is a critical need to understand the sexual dimorphism in PAH and the underlying mechanisms.

The sex paradox is also known as the estrogen paradox. Endogenous estrogens, particularly, estradiol (E2) and its metabolites, are thought to play an important role in pulmonary vascular remodeling and thus, cause more women to develop PAH.[21-23] One example of sex difference is related to the mutation of the gene encoding bone morphogenetic protein receptor 2 (*BMPR2*). *BMPR2* mutation can result in loss of the BMPR2 function and may lead to PAH. It has been reported that females have a higher penetrance of the *BMPR2* mutation (40%) versus males (14%).[23,24] A mutation in the *BMPR2* gene is found in 80% of heritable PAH patients and approximately 20% of idiopathic PAH patients.[25-27] In addition, E2 may predispose women to PAH as E2 signaling leads to a reduction in BMPR2 function in normal pulmonary artery smooth muscle cells (PASMCs) without *BMPR2* mutation and drives a pro-proliferative phenotype in the PASMCs, potentially resulting in the development of PAH.[28] Although the higher incidence rate of PAH in females is probably related to E2, E2 on the other hand, is thought to have beneficial and protective effects on RV, leading to better RV function in female PAH patients than the males.[21] One evidence is that exogenous E2 administered through subcutaneous pellets demonstrates a protective role within the RV and, thus, enhances survival in the Sugen/hypoxia (SuHx) animal model of PH.[29] However, recent clinical trials of anastrozole to lower estrogen levels in PAH patients did not demonstrate any effect on RV function, suggesting that endogenous estrogen was neither protective nor pathogenic pathogenic ([30]; unpublished data from Kawut *et al.*) The effects of endogenous and exogenous E2 on cardiopulmonary function require more studies.

The effects of estrogen on RV function in PAH may be partially attributed to its interaction with mitochondria. During the progression of PAH, mitochondria in the RV undergo metabolic changes, notably mitochondrial fragmentation (fission) and a shift toward uncoupled aerobic glycolysis, known as the Warburg metabolism.[31-34] Mitochondrial metabolic function has been proposed to be linked to many observed molecular abnormalities in the RV and the resulting RV dysfunction including reduced contractility and increased fibrosis.[35] Therefore, studying mitochondrial function in RV in PAH has been of great interest to reveal the underlying mechanisms for developing novel, promising therapeutics. E2 has been known to interact with mitochondrial function

both directly through targeting the mitochondria and indirectly through the nucleus.[36] However, the role of E2 in sex-dependent mitochondrial and RV function in PAH is not well understood. In this review, we will summarize the molecular pathways through which E2 affects mitochondrial function and the expression of genes and proteins in these pathways in the RV in PAH. The role of the E2 metabolites is beyond the scope of this review and will not be discussed.

2. Sex differences in mitochondrial function in RV in PAH

Under normal circumstances, the mitochondria generate energy in the form of adenosine triphosphate (ATP) through oxidative phosphorylation. In many PAH cases, mitochondrial abnormalities result in reduced ATP production, which is synthesized predominantly through glycolysis. Clinically, mitochondrial metabolic changes can be detected by an increase in glucose uptake as measured through 2-deoxy-2-[^{18}F]-fluoro-D-glucose (FDG) uptake.[33,37-39] In addition, an increase in fatty acid utilization as indicated with iodine-123-beta-methyl iodophenyl pentadecanoic acid (BMIPP)[40,41] or [^{18}F]-fluoro-6-thia-heptadecanoic acid (FTHA),[37] has been observed in the RV in PAH patients. This indicates a shift toward glycolysis and fatty acid oxidation, which are key hallmarks of mitochondrial dysfunction. However, possible differences in glucose uptake or fatty acid oxidation between male and female PAH patients and associated RV changes have not been investigated, probably due to the limited number of PAH patients. As glucose uptake or fatty acid utilization is inversely correlated to RV function[33,37,38,40] and female patients have better RV function than males, it might be expected that mitochondrial metabolism is better preserved in the RV of female PAH patients and sex is involved in the mitochondrial abnormalities in PAH-associated RV failure, although this needs to be confirmed clinically.

RV mitochondrial abnormalities have also been demonstrated in animal models of PH. Monocrotaline (MCT) rats display mitochondrial depolarization, increased mitochondrial fission, and a shift to glycolysis in RV.[42-44] In other animal models of PH including SuHx rats, Fawn-Hooded rats, and rats with pulmonary artery banding (PAB), there is also a shift toward glycolysis in the RV.[32,45-47] Interestingly, while a shift toward fatty acid oxidation was observed in the RV myocytes in SuHx and PAB rats,[45,47] there is a decrease in fatty acid oxidation in the RV myocytes in Fawn-Hooded rats.[46] Glutaminolysis is less studied and has been seen to increase in RV in MCT rats.[48] These studies have shown that mitochondrial abnormalities are correlated with reduced RV contractility and increased RV stiffness resulting in reduced RV systolic and diastolic function and RV-pulmonary artery coupling in animal models of both PH and PAH. Hence, we propose that normalizing mitochondrial metabolism could improve RV function and RV-pulmonary artery coupling through increasing RV contractile function and reducing cell proliferation and collagen production in RV fibroblasts. This has been demonstrated in PAB, MCT, Fawn-Hooded, and SuHx rat models of PH.[29,32,43,45-48] These abnormalities have been observed in both RV myocytes and fibroblasts. It is unknown whether RV myocytes or fibroblasts (or other cell types) are more sensitive to changes in mitochondrial function or hormones. However, the loss of RV contractile function is known to be at least partly due to a decrease in mitochondrial respiration in the RV myocytes and probably an increase in RV stiffness, given that normalizing mitochondrial respiration or metabolism can improve RV contractile function and RV compliance by reducing cell proliferation and collagen production in RV fibroblasts in animal models of PH.[29,42,45-47]

Note the animal model of PH discussed above focused on the males and did not include the females to examine the sex differences. Glucose transporter 1 (GLUT1) is a key regulator and marker for glucose utilization. GLUT1 was shown to increase in the RV of male MCT, Fawn-Hooded, and PAB rats and associated with increased glycolysis when compared to their respective controls.[32,46,47] A study on SuHx rat RV found an increase in GLUT1 expression only in the females but not in the males.[29] The reason for the difference in RV GLUT1 expression between SuHx rat model and other rat models of PH is not clear. Other mitochondrial metabolisms such as fatty acid metabolism also play an important role in RV function in PAH. In addition, the RV of female SuHx rats is in a compensated stage rather than a more severe or failure stage in male SuHx and MCT rats. Studies on mitochondrial metabolism at different stages (i.e., compensated and decompensated) of RV in both sexes and the associated RV function and RV-pulmonary artery coupling in PAH may shed light on the role of different mitochondrial changes in different animal models of PH and be useful for the development of therapeutics.

3. Estrogen receptors

Cholesterol serves as the precursor molecule for E2 synthesis. The downstream reactions are then catalyzed predominantly by the cytochrome P450 enzymes and aromatase.[49] Once synthesized, E2 will mediate its signaling through coupling to one of its three receptors: estrogen receptor alpha (ERα, *ESR1*), estrogen receptor beta (ERβ, *ESR2*), and G protein-coupled estrogen receptor (GPER or

GRP30) (Figure 1).[49,50] ERα and ERβ share a great deal of similarities although they have significant differences. ERα is composed of an amino-terminal transcription control domain (AF-1), which is its main region of interaction with regulatory binding proteins; however, ERβ does not have a strong AF-1 domain within its amino terminus.[51] Instead, ERβ contains a repressor domain that modulates ERα activity.[51] Meanwhile, GPER is unrelated to the ERs but does indeed, mimic ER signaling.[52] ERα and ERβ reside in the nucleus, mitochondria, and cytoplasm,[53,54] while GPER is expressed in the plasma membrane as well as the endoplasmic reticulum.[53] Sex differences have been detected in ERα protein expression, with ERα expression significantly higher in female cardiomyocytes than in males. Meanwhile, ERβ protein expression in cardiomyocytes is similar in both males and females.[55,56]

E2 will diffuse into the cell where it will locate the nuclear ER and trigger receptor dimerization. These dimers then interact directly with specific DNA sequences, known as estrogen response elements (ERE), which transactivate gene expression. Alternatively, E2 can interact indirectly through the tethering of other DNA transcription factors, leading to the recruitment of activator proteins.[57,58] GPER will be activated through the classical G protein-coupled receptor mechanism. The genomic effects mediated by the ERs (ERα, ERβ) occur over hours to days while the non-genomic effects mediated by GPER occur rapidly within seconds to minutes.[59,60]

4. Influence of estrogen on mitochondrial function

E2 affects the mitochondria both indirectly through targeting the nucleus or directly by regulating the expression of mitochondrial genes. Both ERα and ERβ have been detected within the mitochondria, with ERβ accounting as the main receptor (Figure 2).[61,62] Mitochondrial ERs are encoded by the same genes that encode nuclear ERα and ERβ as knock-out of ERα and ERβ demonstrates a complete absence of mitochondrial ER in mice.[63] The localization of these receptors within the mitochondria varies depending on the cell type. The classical estrogen signaling mechanism is when E2 passes through the plasma membrane and binds directly with intracellular ERα and ERβ in the cytoplasm (Figure 2). This binding triggers receptor phosphorylation and dimerization, in which this newly formed complex translocates into the nucleus where it binds to the chromatin at ERE sequences, enhancer regions, and 3'-untranslated regions of target genes (Figure 2).[57,64] ERα and ERβ can also be phosphorylated and activated in a ligand-independent manner.[49,57] There are over 70,000 EREs within the mouse and human genomes.[65] These nuclear effects can then influence mitochondrial DNA (mtDNA) gene transcription and function. For instance, E2 can regulate nuclear respiratory factor 1 (*NRF1*), which promotes the transcription of mitochondrial transcription factor A (*TFAM*) that targets the mtDNA genes.[66] E2 can also promote the upregulation of peroxisome proliferator-

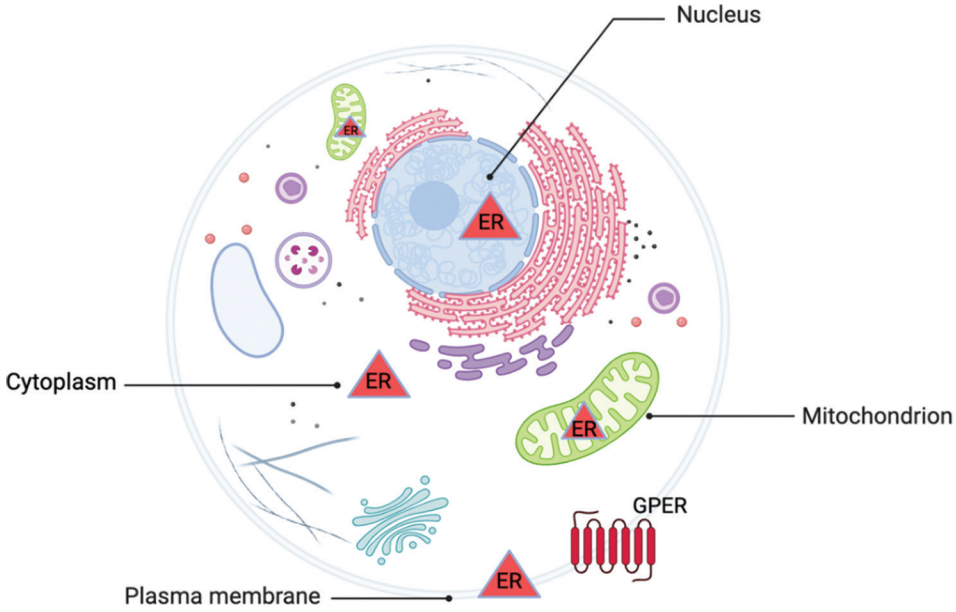

Figure 1. Location of the estrogen receptors (ERs). ERα and ERβ have been found to reside in the nucleus, cytoplasm, mitochondria, and plasma membrane, which belong to the type I nuclear receptor family. Meanwhile, extranuclear receptor G protein-coupled estrogen receptor (GPER) is expressed in the plasma membrane. Source: Created by BioRender.com.

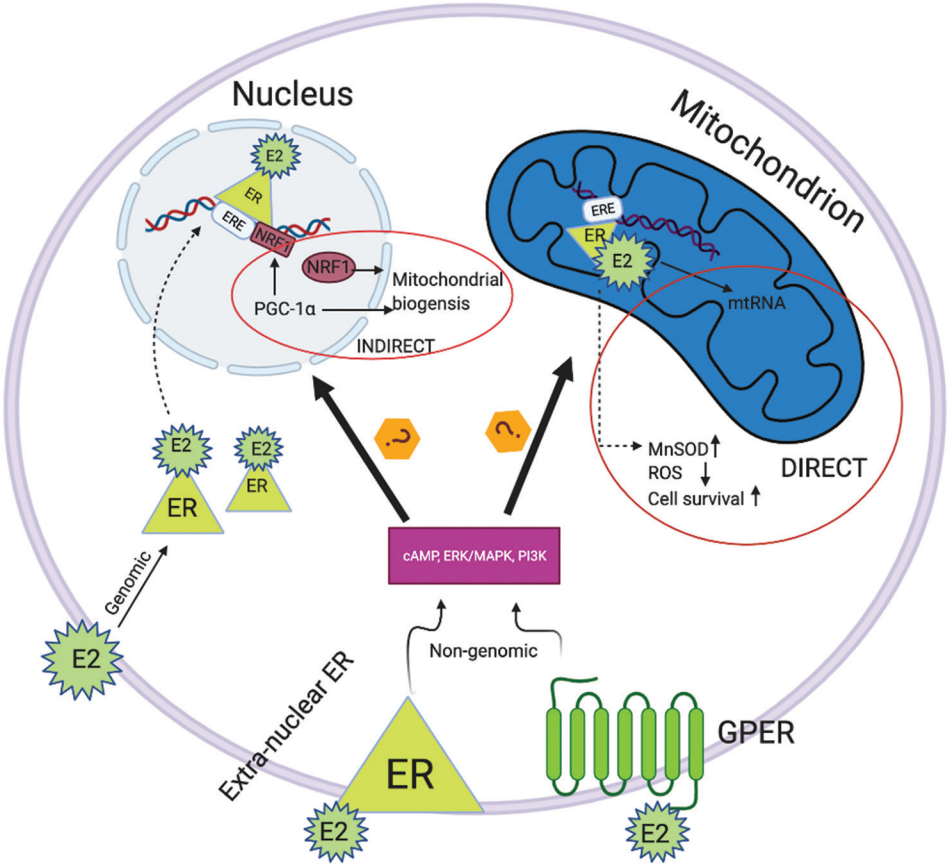

Figure 2. Effects of estradiol (E2) on mitochondrial function. Genomic activity: E2 diffuses into the cell and locates the estrogen receptor (ER) in the cytoplasm, subsequently undergoes dimerization. In the genomic activity, the ERs are then translocated to the nucleus where they bind to the estrogen response element (ERE) and regulate the expression of transcription factors including nuclear respiratory factor 1 (NRF1). Concomitantly, peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) expression is enhanced and together this stimulates mitochondrial biogenesis, which is responsible for the indirect effects on mitochondrial function. Direct effects of E2: E2 binds to ER within mitochondria and induces an increase in mitochondrial RNA (mtRNA) activity and manganese superoxide dismutase (MnSOD) and reduces reactive oxygen species (ROS) generation. Non-genomic activity: E2 binds to extranuclear ER and G protein-coupled estrogen receptor (GPER), activating cyclic adenosine monophosphate (cAMP), extracellular signal-regulated kinase 1/2 (ERK), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K). Adapted from Velarde.[36]

activated receptor gamma coactivator-1 alpha (PGC-1α), which plays a crucial role in mitochondrial biogenesis.[67,68]

Within the human mitochondria, a variety of ERE sites have been detected in the following genes: cytochrome oxidase (CO) II, 7S rRNA, 112S rRNA, D-loop region, tRNA-met, and unidentified reading frame (URF) 1 and 5.[60] Similarly, in the rat genome, they have been detected in CO I, CO II, tRNA-gIn, CO b, URF 4, URF 5, 12S rRNA, 16S rRNA, and D-loop region.[60] Once E2 is imported into the mitochondria, it is shown to selectively bind to ERE in the D-loop region in both human and mouse mtDNA.[60,66] The binding of ER to ERE within mitochondria is proposed to upregulate the expression of mitochondrial genes that play a role in the electron transport chain (ETC).[36] ERβ can also bind directly to mitochondrial proteins including ATP synthase.[69] Although ER can directly influence mitochondrial activity, the nucleus controls E2's major effects on mitochondrial function.

E2 can bind to GPER at the plasma membrane. This results in activation in p38 mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinase 1/2 (ERK1/2), and c-Jun NH_2-terminal protein kinase (JNK).[68] MAPK and related signaling pathways have been linked to myocardial hypertrophy induction through activation of ERβ.[70] These non-genomic effects mediated by GPER do not rely on gene expression to execute their activity, unlike the nuclear receptors.

5. Influence of mitochondria on estrogen synthesis and regulation

Estrogens are synthesized from cholesterol, which takes place predominately in the ovaries, placenta, and

to a lesser extent in the skin, liver, adipose tissue, and brain.[71] Cholesterol undergoes a series of reactions, which are mediated largely by members of the cytochrome P450 (CYP) family and the key estrogen synthase, aromatase, allowing E2 to be formed.[71] The mitochondria are also the site for E2 biosynthesis and contain many of the required enzymes.[36] Even when E2 is administered exogenously, it is preferentially translocated to the mitochondria.[72] The high fluidity of the mitochondria membranes and the lipophilic nature of E2 allow for it to diffuse easily into the mitochondria. Thus, the mitochondria essentially act as an "E2 sink".[72] On the other hand, E2 helps to maintain low levels of ROS, thereby protecting mitochondria from oxidative damage.[36] If the mitochondria become damaged, this will inhibit E2 biosynthesis leading to a decline in E2 levels and an increase in ROS production, which, further, accelerates mitochondrial dysfunction.[36]

6. Estradiol (E2) levels and the regulated mitochondria-associated genes in PAH

6.1. Circulating E2 levels

The normal circulating E2 level is 43 – 113 pmol/L for healthy males aged between 26 – 77 years.[73] Male PAH patients display an increase in E2 levels when compared with control subjects in several clinical studies: 154 versus 106 pmol/L,[74] 78 versus 59 pmol/L,[75] and 150 versus 102 pmol/L,[76] respectively. While, in pre-menopausal women, the normal circulating E2 level is 275 – 1650 pmol/L, and in post-menopausal women, this falls to < 40 pmol/L. Meanwhile, in pregnant women, E2 levels can increase to as much as 26 nmol/L. Pre-menopausal women with PAH illustrated higher levels of E2 and shorter 6-min walking distances when compared with healthy controls.[77] Interestingly, tricuspid annular plane systolic excursion (TAPSE) fluctuated depending on the menstrual cycle. In addition, E2 was shown to influence the transcription of extracellular vesicle miRNA-21, miRNA-29c, and miRNA-376a, which promote vascular proliferation and are implicated in the pathobiology of PAH.[77]

Post-menopausal women with PAH have demonstrated higher levels of E2 compared with healthy controls.[78] These PAH patients also had shorter 6-min walking distances, worse RV afterload, RV dilatation, and overall RV function.[78] Similarly in males, worse RV function was found to be linked to higher levels of E2 and reduced dehydroepiandrosterone sulfate (DHEAS) levels.[76]

One of the major E2 production enzymes, aromatase (*CYP91A1*), was found to contain a single-nucleotide polymorphism (SNP) in the *CYP19A1* gene (rs7175922).[79] This was associated with increased aromatase activity and as a result, elevated levels of circulating E2 in both male (64%) and female (36%) portopulmonary hypertension-PAH patients.

6.2. PGC-1α

PGC-1α, located in the cell nucleus and cytoplasm, is a versatile transcription coactivator.[80] It has been shown to be predominantly expressed in tissues in which the mitochondria are most abundant, such as the RV.[81] Here, it interacts with a variety of transcription factors that play a role in many responses including those involved in mitochondrial biogenesis.[81,82] The exact mechanism by which PGC-1α achieves this is still being studied; however, it has been shown to involve *NRF1/2* and signaling through the estrogen-related receptor α (ERRα).[82] The relationship between PGC-1α and the NRF system is considered to be major as PGC-1α directly and dramatically modulates *NRF1/2* gene expression and its downstream gene, *TFAM*, the key gene involved in mtDNA replication and repair.[83] Meanwhile, mutated *NRF1* can equally inhibit PGC-1α-stimulated cell proliferation. Particularly, within the RV, PGC-1α has been found to be significantly reduced in male MCT and SuHx rats and ovariectomized female SuHx rats and displays a decreasing trend in RV of limited PAH patients.[67,84,85] Reduced PGC-1α level is associated with impaired mitochondrial and RV function in male SuHx rats and ovariectomized female SuHx rats[67,84,85] and the treatment with E2 before the induction of PAH preserved PGC-1α level and mitochondrial and RV function in the female SuHx rats.[67]

PGC-1α is also involved in other systems that influence mitochondrial activity including the fission and fusion events. The dynamin-like GTPases mitofusin 1 and 2 (MFN1/2) are responsible for mediating the fusion (joining) of the mitochondria.[34] PGC-1α is able to induce *MFN2* transcription with the help of binding to ERRα.[86] Furthermore, the ability of PGC-1α to induce mitochondrial biogenesis has been linked to MFN2 expression, with the loss of MFN2 reducing PGC-1α activity.[86,87] Female PAH patients and female SuHx and MCT rats with MFN2 and PGC-1α deficiencies have been correlated to mitochondrial dysfunction including a notable shift to excessive fission in PASMCs.[87] Meanwhile, overexpression of MFN2 has been found to reduce the rates of cell proliferation, enhance apoptosis, and reverse mitochondrial fragmentation in PASMCs both *in vitro* and *in vivo*.[87] In addition, MFN2 has also been found to be downregulated in male SuHx rats, which is associated with mitochondrial dysfunction and RV cardiomyocyte hypertrophy.[88] Therefore, PGC-1α is a potential therapeutic target for RV in PAH.

6.3. NRF1/2

To date, two members of the NRF family have been identified: NRF1 and several isoforms of NRF2, also known as GA-binding protein (GABPA). The NRF family has been shown to be activated by PGC-1α and once activated, targets several genes related to mitochondria and respiration.[89,90] Once activated, for instance by PGC-1α, NRF1 and NRF2 will then bind to three key mitochondrial transcription factors, TFAM, mitochondrial transcription factor B1 (TFB1M), and mitochondrial transcription factor B2 (TFB2M) to promote upregulation of their expression.[90] These three transcription factors can then be translocated to the mitochondria to induce mtDNA replication, transcription, and hence, biogenesis. Mutation in the *NRF1* binding site has been shown to completely abolish the activation of TFAM, with mutations in the *NRF2* binding site shown to only dampen TFAM activity.[91]

The role of the NRF family is just beginning to be understood in PAH. One study illustrated that *NRF1* mRNA expression showed no changes in the RV of male MCT rats.[85] Another study found an increased NRF1 in the RV of male rats following 14 days in hypoxia.[92] Since chronic hypoxia alone only results in an adaptive or compensated RV, the reason for this increased NRF1 in hypoxic rats could be due to positive feedback in the RV to prevent RV failure as the NRFs mediate protective effects. Given the potential role of the NRF family in mitochondrial function and its sex dependence (Figure 2), it is worth studying the NRF family as potential therapeutic targets.

6.4. TFAM

TFAM binds to the D-loop of mtDNA whereby it initiates activation of mitochondrial replication and transcription. It is the key enhancer that promotes the unwinding of the mitochondrial RNA to allow for mitochondrial RNA polymerase (POLRMT) to bind to mtDNA promoters to be transcribed.[90] Ultimately, TFAM is deemed essential for increasing mitochondrial mass. Although, the previous studies have indicated that despite its ability to stimulate mtDNA transcription, it is not able to do so for mtDNA copy number.[93] Moreover, they reported that excessive TFAM concentration can also lead to an inhibition effect on the transfection rate.[93]

Investigations into TFAM in PAH are limited and even more so in the context of the RV. The mRNA expression of *TFAM* was found to decrease in RV of male SuHx and male Wistar MCT rats but not male PAB rats.[84,85] In compensated male MCT rats, mRNA expression of *TFAM* had a decreasing trend in RV.[85] This suggests that the reduction in TFAM is independent of RV pressure overload. Further studies on how TFAM is regulated by E2 and its role in regulating RV mitochondrial function in PAH are required.

6.5. ERK1/2 (MAPK)

When E2 binds to the plasma membrane GPER (also known as GPR30), it activates Gα, Gβ, and Gγ subunits,[94,95] which then stimulates cAMP and cyclic guanosine monophosphate (cGMP) production.[95] The production of cAMP and cGMP activates several signaling pathways including the indirect activation of calcium.[94-96] The intracellular Ca^{2+} release is thought to be one of the main mechanisms by which GPER influences the mitochondria.[68] In addition, the major kinase enzyme family activated through the GPER, MAPK, and one of its subfamilies ERK1/2 play a key role in regulating proliferation in response to oxidants, hormones, and stress factors,[97] hence influencing mitochondrial function in PAH.[98]

Although the data on ERK1/2 in RV in PAH is limited, there are few studies on the lungs in PAH. ERK1/2 levels are significantly elevated in the lungs of human PAH patients (40% male and 60% female) and in hypoxic male rats.[97] There is also an increase in ERK1/2 phosphorylation in the pulmonary arteries of male MCT rats.[28,99] An increase in ERK1/1 was found to stimulate dynamin-related protein 1 (DRP1), resulting in excess mitochondrial fission and proliferation in PASMCs of male MCT rats.[99] An ERK

Table 2. List of mitochondria and estrogen-related genes and their changes in the right ventricle (RV) in pulmonary arterial hypertension (PAH)

Genes	Protein/mRNA expression in RV in PAH	References
PGC-1α	• Downregulated in male SuHx Sprague-Dawley rats[84] • Downregulated in male MCT Wistar rats[85] • A decreasing trend in human PAH patients[84]	84,85
NRF2	Unknown	
ERK	Unknown	
NRF1	• Downregulated in male MCT Wistar rats	85
TFAM	• Downregulated in male SuHx Sprague-Dawley rats[84] • Downregulated in male MCT Wistar rats[85]	84,85
GPER	• No change in male and female SuHx Sprague-Dawley rats	29
ERα	• No change in male and female SuHx Sprague-Dawley rats	29
ERβ	• No change in male and female SuHx Sprague-Dawley rats	29

Abbreviations: SuHx: Sugen/hypoxia; MCT: Monocrotaline; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator; TFAM: Mitochondrial transcription factor A; GPER: G protein-coupled estrogen receptor 1; ER: Estrogen receptor; NRF: Nuclear respiratory factor; ERK: Extracellular signal-regulated kinase.

inhibitor (U0126) prevented and reversed PH in male hypoxic rats, reducing both pulmonary pressure and RV hypertrophy.[97] All these studies suggested that ERK1/2 may be a therapeutic target in PAH.

6.6. Summary

The expression of these above-mentioned genes in RV in PAH is summarized in Table 2 and the associated molecular pathway is presented in Figure 2**.** Due to limited data on these E2- and mitochondria-related genes and proteins and data mainly obtained from males (Table 2), the role of each gene or protein in altering RV function in different sexes at different RV stages (compensated and decompensated) in PAH is still unclear. More studies are required to examine the mechanistic pathways in Figure 2 and their importance to RV function in PAH.

7. Conclusions

PAH is a sex dimorphism condition, with females being at a higher risk than males. The mortality of PAH relies on the status of the RV and has been associated with alterations in mitochondrial functions. E2 can regulate mitochondrial biogenesis activity, influence mtDNA replication and repair, as well as influence the balance of mitochondrial fusion/fission proteins. Restoring mitochondrial function is expected to improve RV function in PAH.[100] However, limited studies exist on these mitochondrial genes and the associated molecular pathways as discussed in this review. Future studies are needed to determine the expressions of these mitochondrial genes at different RV stages in PAH. Transcriptomic and proteomic analysis are being used for profiling including mitochondria- and E2-related genes and proteins.[101,102] Mass spectrometry can be used to reveal the levels of E2 in the RV.[75,102] Further studies are also required to understand the exact mitochondrial genes, proteins, and signaling pathways by which E2 targets and the E2-mitochondria interaction in RV in PAH of both sexes in *in vitro* studies, *in vivo* preclinical studies, and clinical investigation to identify druggable targets and develop promising therapeutics. Finally, PAH patients are currently receiving the same treatments regardless of sex, even though studies have shown that male and female PAH patients respond to treatments differently.[103] Therefore, personalized treatments based on sex could be taken into consideration in the future.

Mitochondria play an important role in RV function, but the molecular mechanisms for sex-dependent mitochondrial function and the role of estrogen (especially E2) in RV in PAH are not well understood. In addition, understanding the roles of E2 and its targeting mitochondrial genes in RV function shall provide insight into therapeutics for RV in PAH. Future studies should focus on examining the expressions of these mitochondrial genes in both animal models of PH and PAH patients in both sexes at different RV stages (compensated and decompensated) through transcriptomic/proteomic analysis and/or mass spectrometry and their molecular mechanisms in *in vitro* and *in vivo* preclinical studies before the development of therapies for RV in PAH.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Chelbi Coyle, Lian Tian
Writing – original draft: Chelbi Coyle
Writing – review & editing: All authors

Ethics approval and consent to participate

Not applicable.

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