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The Role of Estrogen and Estrogen Receptors on Cardiomyocytes: An Overview

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Abstract

Sex differences in the onset and manifestation of cardiovascular diseases are well known, yet the mechanism behind this discrepancy remains obscure. Estrogen and its corresponding receptors have been studied for their positive salutary effects in females for decades. Estrogen protects the heart from various forms of stress, including cytotoxic, ischemic and hypertrophic stimuli. The postulated underlying mechanism is complex, and involves the actions of the hormone on the endothelium and myocardium. While the effects of estrogen on the coronary endothelium are well-described, delineation of the hormone's action on cardiomyocytes is still evolving. The focus of this article is to review the accumulated literature and latest data on the role of estrogen and its receptors on cardiomyocytes, the contractile cellular units of the myocardium.

Introduction

Gender is increasingly recognized as a major factor in the outcomes of patients with cardiovascular diseases (CVD). Findings from clinical studies suggest that premenopausal women have reduced incidence of CVD, compared to age-matched men. This observation has led to the postulation that estrogen, working via its receptors, may be cardioprotective, the conclusion supported by numerous controlled experiments at preclinical levels. However, trials testing the role of hormone replacement therapy (HRT) as a preventive measure have yielded mixed results,¹ with two such studies, Women's Health Initiative (WHI)² and Heart and Estrogen/progestin Replacement Study (HERS),³ showing no substantial benefit overall. The discrepancy between the initial findings of these trials and the cardioprotection by estrogen seen in experimental models is a complex topic that has been covered extensively elsewhere and continues to be debated to this date.⁴ Briefly, it includes the issues of the study design and subject characteristics, pharmacokinetics of the HRT used, statistical power to address cardiac risk, composite analyses of all outcomes of different diseases rather than focusing on cardiac end points.

Subsequent analyses of these trial data, however, showed significant cardiovascular benefits in younger patients who initiated HRT early in the perimenopausal period, raising a question of the "timing hypothesis" and leaving room for further debate on the role of HRT in CVD. More recently, the timing hypothesis was specifically addressed by the Kronos Early

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Estrogen Prevention Study (KEEPS) and the Early versus Late Intervention Trial with Estradiol (ELITE). The KEEPS showed that HRT started early in menopause improved markers of CVD risk.⁵ Results of the ELITE are yet to be published, but preliminary reports provide evidence directly supporting the timing hypothesis.^{6, 7} Thus, salutary relevance of HRT in CVD is far from being clearly defined, and remains a complex and controversial issue.

Cardiomyocytes and coronary endothelium are two principal units of the heart implicated in the pathophysiological processes underlying ischemic heart disease (IHD), a major component of CVD and the leading cause of death in developed countries. It is well-recognized that estrogen has potent impact on the vascular endothelium and the heart.^{8, 9} In this review, we will examine the molecular, cellular, and physiological evidence of estrogen's action on cardiomyocytes, the cells making up the largest part of the heart by mass and chiefly responsible for cardiac contraction and conduction, in order to further understand the intricate effects of the female hormone on the heart and IHD.

The source of estrogen

Estrogens are a group of sex steroids that exerts pleiotropic actions on multiple organs beyond the scope of its reproductive function. The primary sources of estrogens in premenopausal females are the ovaries. Estrogens are also produced in a number of extra-gonadal sites, including bone, breast, adipose tissue and the brain.¹⁰ Estrogens can be converted from testosterone by cytochrome p450 aromatase in the testis and prostate in men.^{11, 12}

Circulating estrogen

Three naturally occurring estrogens in circulation are estrone (E1), 17 β -estradiol (E2) and estriol (E3). Of these, E2 is the most potent. The secretion of E2 is pulsatile and its concentration fluctuating within a menstrual cycle (100 – 600 pg/ml). It can rise up to 17000 pg/ml during pregnancy.¹³ After menopause, serum E2 concentration precipitously drops to values similar to, or lower than, those in men of similar age (5 – 20 pg/ml).¹⁴ E2 is also the most commonly used bioactive estrogen in experiments, and for the remainder of this article, we will use E2 and estrogen interchangeably.

Cardiac estrogen

Local estrogen biosynthesis requires cytochrome P450 aromatase (CYP19A1), which metabolizes androstenedione and testosterone to their respective estrogens by aromatization.¹⁵ The enzyme activity of P450 aromatase was reported to be present in various extra-gonadal tissues more than two decades ago, and its transcript splicing shown to be tissue-specific,¹⁶ strongly suggesting an organ-dependent transcriptional regulation of the aromatase. There is now increasing evidence that the aromatase is expressed in the heart.^{17–19} These findings hint a local estrogen production in the heart. Given the relationship between sex hormones and aromatase expression *in situ* reported in other organs and association of aromatase polymorphism with CVD risk, these data provide functional

implications about local E2 activity and cardiac aromatase in E2-mediated cardioprotection.^{20–22}

Cardiac estrogen receptors

There are two classic nuclear estrogen receptors (ERs), ER α and ER β , encoded by ESR1 and ESR2, respectively. They are widely expressed in different tissue types, in addition to uterus and ovary. Both receptors are expressed in the adult and neonatal heart,^{23–25} and both in the ventricular and atrial cells from female and male mice.²⁶ The cardiac ERs are functionally active, participating in the regulation of gene expression and posttranslational modifications through genomic and non-genomic signaling pathways.²⁷ Evidence on the whole shows that ERs exert anti-apoptotic, pro-hypertrophic, anti-inflammatory, anti-atherosclerotic, vasodilatory, and angiogenic effects in the cardiovascular system.^{27–30}

The immunohistochemical analysis of the receptors indicates their subcellular localization to be distinct from each other. While both are seen in the cytosol of cardiac cells, the extent of nuclear localization is different between the two subtypes. ER α was found in the cardiomyocyte plasma membrane, while equivalent evidence for ER β is lacking.²⁶ Both receptor subtypes are found in the mitochondria of cardiomyocytes, and participate in the ER-initiated regulation of mitochondrial function.^{31, 32} Overall, the different and distinct subcellular localization pattern for each ER subtype may contribute to the diverse actions of estrogen in cardiac cells (Fig. 1).

In addition, GPR30, previously thought to be an orphan membrane receptor, is now recognized as a receptor for E2 involved in E2-mediated rapid, non-genomic signaling, and was re-named G-protein coupled estrogen receptor (GPER).³³ GPER is present in the heart and shown to be cardioprotective in its function under ischemic and hypertensive stress.^{34, 35} In sum, these estrogen receptors are expressed and functional in cardiomyocytes, and orchestrate a myriad of genomic and non-genomic effects in the heart.

ER polymorphism and cardiovascular disease

Some of the earlier studies involving ER α and ER β polymorphism in CVD linked the ER gene variants to the differences in the left ventricular (LV) mass and wall thickness.^{36, 37} While the underlying mechanism between the ER polymorphism and phenotypic variation may be complex, it is recognized that ER mutations leading to changes in downstream gene expression and signaling can alter the effects of E2 on the heart.³⁸ In fact, single nucleotide polymorphisms (SNPs) in both ER α and ER β have been shown to confer susceptibility for CVD. A polymorphism of ER α (minor allele -397T/C) was linked to coronary heart disease among Finnish men.³⁹ Another group of ER α variants, ESR1 c.454-397T and ESR1 c.454-351A, was associated with increased risk of myocardial infarction (MI) and IHD in Dutch women, but not in men.⁴⁰ The rs1271572 variant of ER β was associated with increased risk of MI in men in a Spanish population,⁴¹ while ER β rs1256049 was linked with reduced risk of CVD or MI in women.⁴² However, the latter study also noted the rs1271572 variant of ER β to be associated with increased risk of MI in women only, in contrast to the previous work showing the variant-specific risk limited to men.⁴¹ The reasons

for the reported inconsistency regarding this particular variant are unclear at this time, though differences in methodology and study population are acknowledged.

Thus, while emerging data indicate ER α and ER β polymorphism to have a role in gender-specific risk of CVD, further research is needed to determine the underlying mechanisms and the extent of phenotypic expression associated with these genetic variants. Data are currently lacking for GPER polymorphism and cardiac risk, though it has been implicated in cancer risk.⁴³

Estrogen and ERs in cardiac metabolism

Studies consistently indicate that estrogen plays a pivotal role in the control of energy balance and glucose homeostasis via a diverse set of mechanisms.^{44, 45} Postmenopausal women have altered metabolism manifested by increased abdominal adipose tissue and dyslipidemia.⁴⁶ Ovariectomized animals exhibit marked weight gain and dysregulated metabolic state, promptly reversed by E2 replacement.⁴⁷ Data, to date, support ER α at the center of the E2 actions on energy expenditure, as exemplified by ER α knockout (KO) mice which develop adipocyte hyperplasia, insulin resistance, and reduced energy expenditure.^{48, 49}

Until recently, most of the mechanistic studies examining E2-mediated effects in cellular energy metabolism have been conducted using non-myocardial cells. However, new data are now emerging to detail the role of E2 in the regulation of cardiac metabolism. E2 supplementation significantly improved myocardial ATP levels and mitochondrial respiratory function in the heart.⁵⁰ ER α and its specific agonist 16 α -LE2 were required to maintain physiological glucose uptake in the murine heart.⁵¹ Cardiomyocyte-specific deletion of ER α changed sex-dependent metabolic gene expression and diverse transcriptional networks in cardiomyocytes.⁵² Interestingly, one study reported regulation of GLUT4 expression by ER α in mouse gastrocnemius muscle, and this may provide an important clue to the underlying molecular mechanisms behind the E2-mediated regulation of glucose metabolism in cardiomyocytes.⁵³

Moreover, latest data implicate estrogen related receptor alpha (ERR α) to be a regulator of cellular metabolism via PGC-1 α .⁵⁴ Given the high homology of the DNA and ligand-binding domains between ER α and ERR α , and evidence of crosstalk between the two receptors, it is conceivable that ERR α may indirectly enhance E2/ER α signaling or vice versa.⁵⁵ This may serve as yet another mechanism by which E2/ER modulates pathways involved in cardiac bioenergetics.

The role of ER β in metabolic regulation is less evident, and available data conflicting. Deleting ER β did not affect total body fat or lipoprotein levels of mice,⁵⁶ and the selective ER β agonist diarylpropionitrile (DPN) did not alter food intake or body weight gain in rats.⁵⁷ This is in contrast to another study which showed that use of ER β ligands alleviated obesity induced by high fat diet and ovariectomy in mice, putatively by repressing several genes involved in the lipogenesis pathways and increasing energy expenditure through uncoupled respiration.⁵⁸ Other studies also support that ER β controls cellular metabolism

through mitochondria.^{32, 59} In an ischemia/reperfusion (I/R) injury model using cardiac-specific ER α and ER β KO mice, Gabel *et al.* showed that the expression of several fatty acid metabolism genes in the heart was differentially altered by ER β , when compared to ER α or wild-type mice.⁶⁰ ER β was reported to regulate mitochondrial respiratory complex IV activity in rat hearts after trauma-hemorrhage,⁶¹ with ER β -specific cardioprotection via upregulation of PGC-1 α .⁶²

In sum, while there is strong evidence to position E2/ERs as a major regulator of cardiomyocyte metabolism and energy balance, further work is needed to refine and integrate proposed working mechanisms.

Estrogen and ERs in myocardial apoptosis

The anti-apoptotic effects of estrogen on cardiomyocytes were first reported by Pelzer *et al.* in 2000.⁶³ Since then, several signaling pathways have been attributed to the E2/ER-mediated inhibition of myocyte apoptosis. They include inhibition of NF- κ B,⁶⁴ activation of phospho-inositide-3 kinase/Akt signaling,⁶⁵ inhibition of ASK1 activity,⁶⁶ upregulating corticotropin-releasing hormone receptor type 2,⁶⁷ and more recently, promotion of p38 β activity leading to inhibition of p53 and subsequent mitigation of mitochondrial redox response.^{68–70} Both Fas- and mitochondria-dependent apoptotic pathways are prevented by E2.⁷¹ Thus, cumulative data from both in-vitro and in-vivo experiments employing a variety of apoptotic triggers have consistently shown that E2 attenuates cardiac apoptosis.

What is not clear-cut is the extent of contribution by the ER subtypes to this anti-apoptotic action of E2. There are data available to support participation of both ER α and ER β in mitigating cell death. Both isoforms are present in cardiomyocytes.²³ Animal models testing either isoform led to positive results for the studied receptor subtype in offering cardioprotection.^{72, 73} One study did make a head-to-head comparison between ER α and ER β in this regard by using wild type (WT), ER α KO and ER β KO mice in an ex-vivo model of global ischemia/reperfusion.⁶⁰ In the study, ER β KO female hearts exhibited significantly less functional recovery than WT, while the extent of I/R injury was similar between ER α KO and WT female hearts, suggesting that ER β plays a larger protective role in the female heart.⁶⁰

Overall, literature to date yields no consensus on which ER receptor has a dominant role in cardioprotection. This may be due to variations in the experimental models, endpoints, and applied doses of ER agonists from one report to another, as well as selection bias. Mechanistic studies using cultured cardiomyocytes have yielded similarly mixed results.^{74, 75} To this end, a possibility of ERs working as heterodimers has been considered, as heterodimeric ER α/β was shown to be transcriptionally active in noncardiac cells.^{76, 77} Further investigation is needed, however, to confirm the role of ER heterodimer in cardioprotection *in vivo*.

Estrogen and ERs in cardiac regeneration

Along with the ability of E2 to protect cardiomyocytes from stress-induced cell death, another novel mechanism may be at hand for the hormone to preserve the integrity of

cardiac function: stimulation of myocyte regeneration. The latest evidence shows that the infusion of E2 treated-cardiac stem cells (CSCs) into the isolated mouse hearts leads to a more robust production of CSC-derived protective factors and improved cardiac function as well as better cardiomyocyte survival after acute I/R.⁷⁸ E2 promotes mouse embryonic stem cell proliferation.⁷⁹ Both ER α and ER β contribute to E2-mediated endothelial progenitor cell activation and tissue incorporation to the effect of preserving cardiac function after MI.⁸⁰ Of the ER subtypes, ER α may have more profound and direct effects on cardiac progenitor cells *in situ*, as the receptor was upregulated in post-infarct c-Kit⁺ precursor cells accumulating in peri-infarct myocardium, and supported proliferation of undifferentiated myoblast cells.⁸¹ ER α stimulation by E2 and PPT (ER α -specific agonist) reduced apoptosis and increased survival of adult myocytes co-cultured with post-infarct cardiac c-kit⁺ cells, while ER β -selective agonist DPN had no effect.⁸¹

These findings provide an exciting complementary mechanism of how E2 protects cardiomyocytes, and may prove a novel therapeutic strategy in the treatment and management of IHD.

Estrogen and ERs in myocardial hypertrophy

The involvement of E2/ERs in the LV hypertrophic response ranges from regulating normal physiological responses to exercise and postnatal cardiac growth to mitigating maladaptive ventricular hypertrophy.

As with the anti-apoptotic actions, both ER isoforms are implicated in the anti-hypertrophic effect of E2 against various pathological stimuli.^{82, 83} The purported molecular mechanisms involve calcineurin degradation,⁸⁴ mTOR signaling,⁸⁵ regulation of phosphorylated p38 MAPK pathways,⁸⁶ and regulation of cardiomyocyte histone deacetylases.⁸⁷ Under normal physiological conditions, ER β was shown to be responsible for sex differences in exercise-induced hypertrophy,⁸⁸ while estrogenic regulation for normal myocardial development was primarily via ER α .⁸⁹

One of the more recent developments in the study of cardiac hypertrophy is the identification of endonuclease G (EndoG) as a central link between maladaptive left ventricular hypertrophy (LVH) and mitochondrial processes.⁹⁰ As of this writing, evidence for a direct relationship between estrogen and EndoG is scant, and none pertaining to their interaction in the heart, though one study showed a correlation between E2 administration and EndoG release in the central nervous system.⁹¹ Given the strong physiological data on the role of E2 in LVH, probing the relationship between the E2/ER and EndoG may potentially be an exciting area of research to broaden current understanding of estrogen-mediated myocardial hypertrophic response.

Estrogen and ERs in cardiac electrophysiology and contraction

Sex differences in the electrophysiological properties of the heart have long been recognized. There are notable differences between surface electrocardiograms (ECG) of healthy men and women, with women more likely to have a longer QTc interval, faster

resting heart rate and shorter QRS duration.^{92, 93} Though QTc varies within a menstrual cycle, whether the hormonal fluctuation accounts for the change is not yet clear.

Nonetheless, a large body of evidence supports that estrogen directly impacts the electrical conduction properties of cardiomyocytes. In one of the earliest animal models of I/R-induced ventricular arrhythmia, dogs receiving conjugated equine estrogen had a significantly lower incidence of lethal ventricular tachyarrhythmias.⁹⁴ Subsequent mechanistic studies linked the antiarrhythmic effects of estrogen during I/R to the augmentation of endogenous nitric oxide (NO) release and opening of K_{Ca} channels, as well as Na^+/H^+ exchanger (NHE1) inhibition in the heart.^{95, 96}

Ovariectomy induces myocardial contractile dysfunction in female rats,⁹⁷ suggesting that estrogen plays an important role in cardiac contraction. Significant gender differences in the parameters of cardiac excitation-contraction (E-C) coupling exist between male and female hearts and cultured myocytes.⁹⁸ The underlying mechanism may be due to the regulation of calcium homeostasis by E2/ER in the heart. E2 inhibits L-type Ca^{2+} channels, and regulates the membrane density and expression of both L-type Ca^{2+} channels and low-voltage-activated $Ca(V)_{3.2}$ T-type calcium channels.^{99–101} Interestingly, one study showed that these effects were not mediated by $ER\alpha$ or $ER\beta$, while other reports support the ER involvement in regulating electrophysiological and contractile activities of cardiomyocytes.^{102–104} Together, they suggest E2 actions on cardiac conduction and contraction to be ER subtype- and target channel-specific.

There is currently limited evidence to suggest a role of GPER in cardiac contractility and ion channel activity.¹⁰⁵ Whether rapid signaling from GPER by E2 modulates cardiomyocyte E-C coupling needs further confirmation.

Conclusion

Sex differences in cardiovascular diseases have long eluded a satisfactory cohesive explanation. While research is ongoing to narrow this gap of knowledge, there is an abundance of accumulated clinical and experimental evidence to demonstrate the efficacy of E2/ERs in cardioprotection. E2 and its receptors play a critical role in an intricate network of genomic and non-genomic pathways to regulate cardiac metabolism, attenuate cardiomyocyte apoptosis, promote cardiac regeneration, modulate physiological and pathological LVH, and calibrate electrical and contractile function of the heart (Fig. 2). Yet, despite the well-known statistics on the differential outcomes and physiological distinctions between men and women with CVD, no gender-specific treatment strategies exist at this time. The collective body of knowledge from current and future research on estrogen and its role in the heart will be essential to developing much needed therapies that may be both personalized and gender-specific, and advance the field of cardiovascular medicine.

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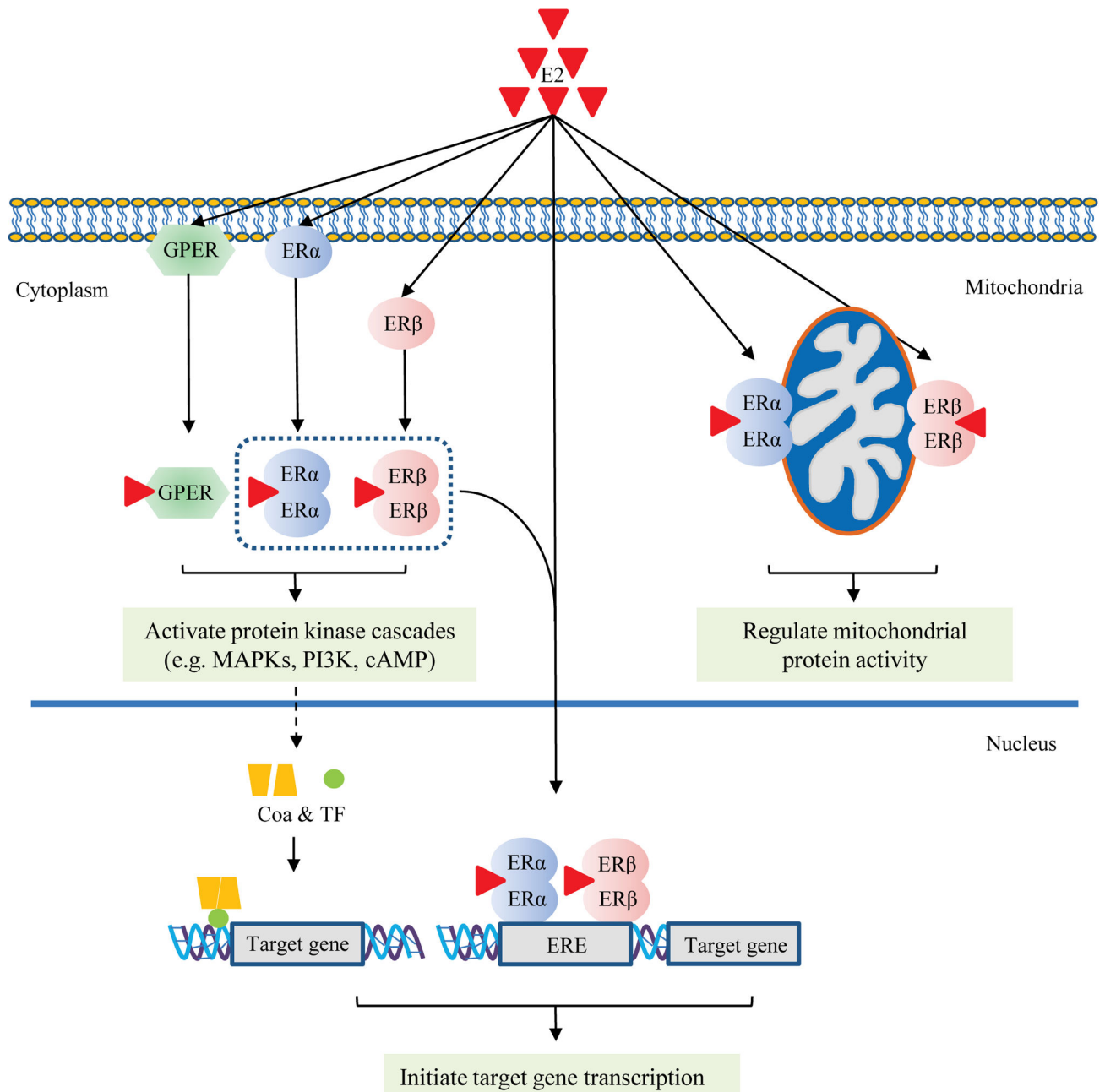


Fig. 1. Model of estrogen (E2) action via estrogen receptors (ERs) in the cardiomyocyte
 Genomic pathway (1): E2 directly activates ER α and ER β , which bind to the estrogen-response-element (ERE) of gene promoters and induce target gene transcription. Rapid or non-genomic pathway (2): E2 activates ERs (GPER, ER α and ER β) in the membrane and cytoplasm, leading to rapid changes in signaling pathways involving protein kinase cascades, which in turn affect downstream gene transcription via co-activators (CoA) and transcription factors (TF). In addition, E2 also activates ERs (ER α and ER β) located in mitochondria and regulates mitochondrial function and energy homeostasis.

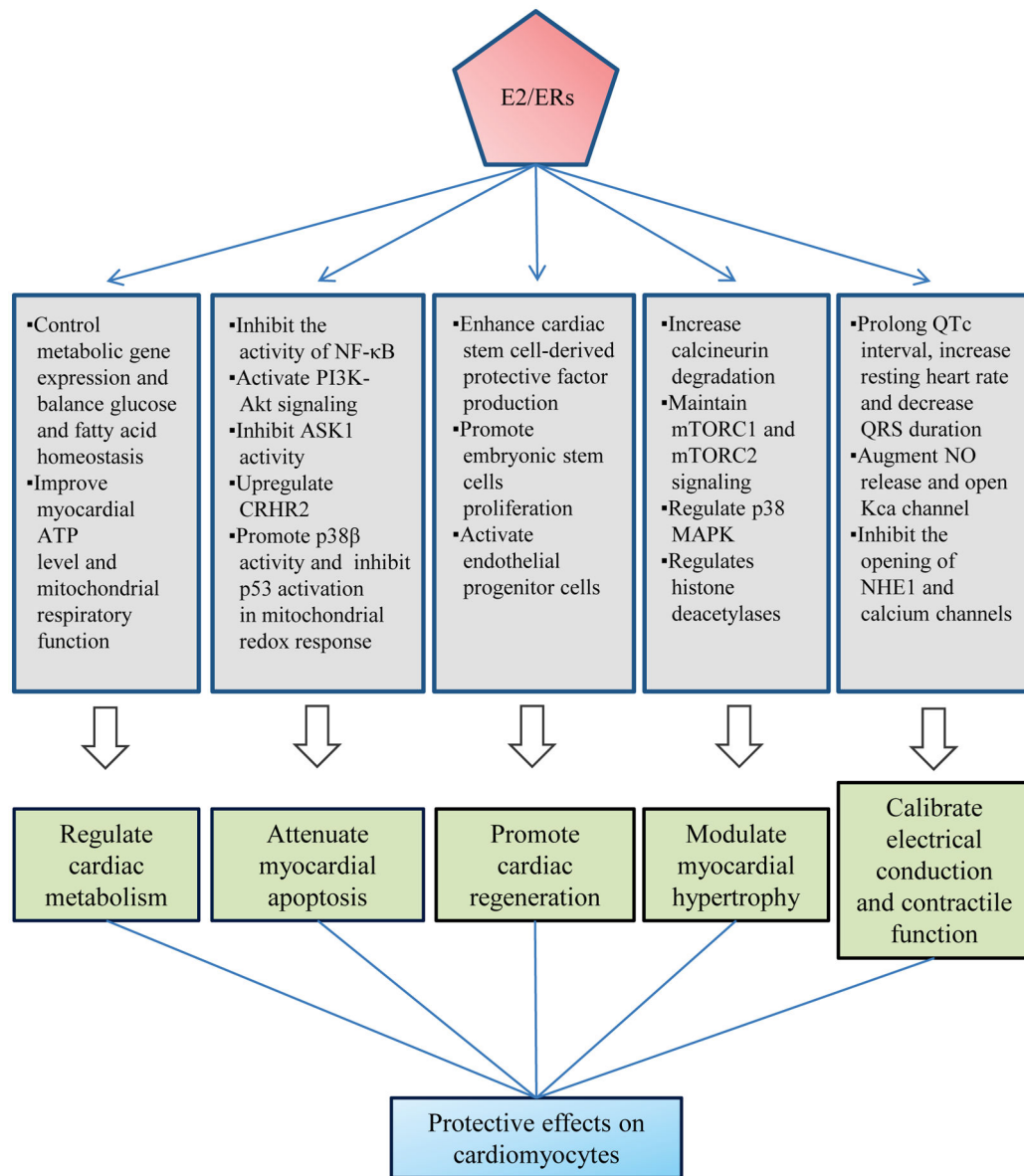


Fig. 2. Summary of key effects of E2/ER on cardiomyocytes.