1. Gender differences in heart failure

Project leader: Vera Regitz-Zagrosek  
Coworkers: Georgios Kararigas, Shokoufeh Mahmoodzadeh  
Funding: BMBF (DZHK: BER 3.2 HF)  
External collaboration: DZHK Partners Prof. K. Sipido (Leuven), Dr. F. Jaisser (INSERM, France)

Women with heart failure have better prognosis than men. Differences in the relative influence of risk factors and the response to therapy are also present. Goal of this project is to analyse these gender differences in heart failure in animal models as well as in clinical studies, identifying the existing mechanisms behind these differences and based on this knowledge develop new therapeutic strategies for both sexes. Clinical studies focus on sex differences in systolic and diastolic heart failure and its prevention. Basic research explores possible sources of gender differences. Sex-specific signals may be mediated by the estrogen and androgen receptors, which are functionally relevant in male and female cardiovascular cells. Based on previous results we postulate that 17b-Estradiol (E2), ERa or ERb regulate the calcium (Ca2+) metabolism in a sex-specific manner contributing to the gender differences in the cardiovascular system. We analyse the functional relevance and the involved mechanisms in order to detect appropriate therapeutic targets. In addition, we have already identified myocardial genes and proteins that are differentially regulated by E2 in both sexes. At least one of these proteins (Mylip) may be involved in the E2 mediated inhibition of contractility in the heart of older men but not of older women. This mechanism may explain why an elevated E2 level in older men is associated with increased cardiovascular complications and heart failure. We investigate the possibility to block this mechanism as a therapeutic approach in this patient group.

2. Estrogen receptor modulation of myocardial energy metabolism contributes to sex differences in myocardial hypertrophy

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Funding: DFG: FOR 1054 / TP1  
External collaboration: Jan-Ake Gustafsson (USA); Pierre Chambon (France), Bayer HealthCare (Germany); Renée Ventura-Clapier (France)

Females develop a more physiological myocardial hypertrophy (MH) than males. Male hearts have a higher incidence of cardiac dysfunction and heart failure in response to stress. Exercise leads to more physiological MH in female than in male mice. Female sex or estrogens may lead to an improved cardiac response to stress. Based on our previous findings, we hypothesize that estrogen (E2) and its receptors ERa and ERb improve the cardiac response to mechanical load by regulating mitochondrial function and energy metabolism. AKT signalling and key regulators of mitochondrial function and metabolic genes are probably involved in these processes. Therefore in this study, we investigate: 1) Sex differences in cardiac function in physiological (exercise) MH model. 2) Effect of E2 and pharmacological/mechanical load on cellular growth and metabolic genes. 3) Effect of E2-activated ERa and ERb on gene expression and biological activity of PGC1a and MEF2 transcription factors that control mitochondrial activity. The project will identify sex-specific MH mechanisms with therapeutic potential relevant to both genders.

3. Mechanisms of sex-specific extracellular matrix remodelling in pressure overload induced myocardial hypertrophy

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Funding: DFG: GK II/III 754 TP4, Re662/6-1, DW70/1-1  
External collaboration: German Heart Institute Berlin, Bayer HealthCare

Women develop under similar pathological conditions a more concentric form of myocardial hypertrophy (MH) with better preserved systolic function and less fibrosis than men. Goal of the study is to identify clinical parameters that determine MH, and to correlate underlying mechanisms of extracellular matrix (ECM) remodeling with clinical endpoints under sex specific aspects
in patients with aortic stenosis (AS). In order to investigate the role of the sex-hormone 17β-Estradiol (E2) and its estrogen receptors (ER) in ECM remodeling, we perform in-vitro studies in isolated adult rat cardiac fibroblasts. Our recent data show that women with AS adapt differently to pressure overload compared to men. Male patients had significant higher gene expression of ECM-associated genes, e.g. collagen I and III compared to females (Fig. 1A). We could show a sex-specific regulation of collagen I and III deposition and apoptosis of cardiomyocytes. A careful analysis of the influence of melusin on remodeling processes after myocardial infarction (MI) and sex differences therein is needed. We wanted to understand the protective effects of melusin against cardiac remodeling and early death after MI and sex differences therein. For that reason an animal model for MI was performed in male and female WT mice and in mice overexpressing the melusin gene (TG). Animals were killed 3 or 14 days after induction of MI. The experiments showed that melusin protects male animals against early cardiac rupture by decreasing MMP9 activity. In later maladaptive processes following MI the TG affects mainly females and protects them against heart failure, while in males it does not.

4. Melusin protects against cardiac rupture and maladaptive remodeling after myocardial infarction sex dependently

Projectleiter: Vera Regitz-Zagrosek, Carola Schubert
Mitarbeiter: Jenny Thomas
Funding: EC: EUGeneHeart EC-018833 (WP3.1)
Kooperationspartner: MD Bernhard Unsöld (Göttingen) Prof. Karin Sipido (Leuven) Prof. Guido Tarone (Turin)

The muscle specific b1 integrin interacting protein Melusin has been found to be upregulated during cardiac hypertrophy and protects against the transition into heart failure after pressure overload. Attenuation of cardiac hypertrophy was accompanied by lower tissue deposition and apoptosis of cardiomyocytes. A careful analysis of the influence of melusin on remodeling processes after myocardial infarction (MI) and sex differences therein is needed. We wanted to understand the protective effects of melusin against cardiac remodeling and early death after MI and sex differences therein. For that reason an animal model for MI was performed in male and female WT mice and in mice overexpressing the melusin gene (TG). Animals were killed 3 or 14 days after induction of MI. The experiments showed that melusin protects male animals against early cardiac rupture by decreasing MMP9 activity. In later maladaptive processes following MI the TG affects mainly females and protects them against heart failure, while in males it does not.

5. Identification and Functional Analysis of Protein Interaction Partners of the Estrogen Receptor alpha in the Human Heart

Project leader: Dr. Shokoufeh Mahmoodzadeh
Coworkers: Thi Hang Pham
Funding: DFG: GK754-II, Friede Springer Heart-Foundation
External collaboration: Prof. Ingo Morano (Germany); Prof. Lucas Brunsveld (Netherlands)

Estrogens (E2) play an important role in mammal's normal physiological functions and also in the pathology of several diseases. One important target organ for estrogen action is the cardiovascular system. Most of the biological actions of E2 are mediated by nuclear steroid receptors, the estrogen receptor alpha (ERα) and beta (ERβ), which function as hormone-inducible transcription factors. ERs act in concert with other regulatory elements to mediate estrogenic effects. So far, only few cofactors of ERs have been described in the human heart. To gain a better understanding of E2-mediated ERα action in the human heart, we identified and functionally analyzed the interaction partners of ERα. Yeast two hybrid screening of a human heart library revealed that ERα interacts with the atrial natriuretic peptide precursor A (NPPA) in an E2-dependent manner. The interaction of NPPA and ERα was also confirmed using proteins derived from human atrium by Co-IP. Further experiments will be performed to study the physiological/pathological significant of this interaction in the human heart.

6. Influence of different estrogen receptor modulators on pressure overload-induced myocardial hypertrophy

Project leader: Carola Schubert
Coworkers: Christina Westphal
Funding: DFG: GK 754 II/III TP2 EC: EuGeneHeart EC-018833 (WP19) Bayer Schering AG (Working group)
External collaboration: Dr. Katja Prelle (Bayer Schering AG (Working group))

Estrogens are implicated in the development or progression of cardiovascular diseases. In many of the diseases, 17b-estradiol (E2) mediates its effects through its estrogen receptors a and b (ERα and ERβ), which serves as the basis for many therapeutic interventions. Selective estrogen receptor modulators (SERMs) such as tamoxifen and raloxifen are examples of compounds that exhibit tissue-specific estrogenic activity. They may be useful for the reduction of cardiovascular risk. In the current project we wanted to identify the estrogen receptor, which is responsible for the protection of the heart against pressure-overload, which also occurs during aortic valve stenosis in patients. Ovariectomised (OVX) female mice were pressure loaded by transverse aortic constriction (TAC) and treated with E2 and specific estrogen receptor agonists (ERαA and ERβA) or raloxifen to prevent...
cardiac hypertrophy and heart failure. First results show the development of a significant left ventricular hypertrophy after TAC compared to the controls. The treatment with E2 and ERα-Agonist diminished the development of cardiac hypertrophy, slowed down the loss of cardiac contractility and reduced development of cardiac fibrosis in comparison to the untreated animals.

7. Analysis of the role of estrogen receptor in the heart in a transgenic mouse model

Project leader: Shokoufeh Mahmoodzadeh
Coworkers: Joachim Leber, Britta Fieltz, Arne Kühne
Funding: DFG: GK III 754 TP4 EC: EUGeneHeart EC-018833 (WP17)
External collaboration: Dr. F. Jaisser, INSERM, France

Estrogen exerts its beneficial effects on the myocardium during stress via estrogen receptors (ER) a and b. During and after myocardial infarct (MI), administration of ERα-selective agonist has protective effects in the heart. ERα-KO animals have a poorer outcome and abnormal mitochondrial morphology after MI. We hypothesize that ERα contributes to protection of ischemic myocardium and this differs in male and female. We therefore generate and evaluate the phenotype of a transgenic mouse model with an inducible, myocardial ERα-overexpression (ERα-OE). Furthermore, we perform functional and biochemical analysis of the transgenic mice in unstrressed condition and after induction of MI, in a sex-specific context. We expect major deviations from WT at base line conditions and after induction of MI, possibly leading to the discovery of major cellular pathways influenced by ERα and thereby to new therapeutic targets.

8. Sex differences and the influence of estrogen receptor β

Project leader: Daniela Fliegner, Carola Schubert
Funding: DFG: GK 754 II/III TP2 EC: EuGeneHeart EC-018833 (WP19)
Charité: Post-doctoral fellowship
External Collaborations: Prof. Dr. Ulrich Kintscher (CCR (Center for Cardiovascular Research)), Prof. Jan-Ake Gustafsson, Karolinska Institute Stockholm

Myocardial hypertrophy (MH) represents the cardiac response to different stimuli, such as pressure overload, hypertension, diet or aging and can result in heart failure (HF). The development of MH is associated with alterations in cardiac geometry (size and shape), which is characteristically referred to as ventricular remodeling and appears to be different between the sexes. Sex differences in the cardiovascular system have largely been attributed to the effects of sex steroid hormones such as estrogen, which are mainly mediated by their nuclear receptors: ERα, ERβ. The goal of this study was to investigate the molecular mechanisms underlying these sex differences. We used a mouse model of pressure overload induced myocardial hypertrophy in both sexes with or without deletion of ERβ. In this project our findings indicate that female sex offers protection against ventricular chamber dilation in the TAC model. Both female sex and ERβ contribute to the maintenance of energy homeostasis and attenuate the development of fibrosis and apoptosis, thus slowing the progression to heart failure. Current ongoing studies are focused now on the effects of sex and sexual hormones on the cardiac mitochondrial function in physiological and pathological conditions.

9. Effects of estrogen and estrogen receptors on cardiac mitochondrial function

Project leader: Daniela Fliegner
Coworkers: Julia Jost
Funding: DFG: FOR 1054; DHZB (Deutsches Herzzentrum Berlin); Bayer HealthCare (shared Post-doc position) External Collaborations: Prof. Renée Ventura-Clapier (INSERM; Paris) Dr. Heiko Bugger (Universitätsklinikum Freiburg)

Sex differences in the cardiovascular system have been attributed to the effects of sex steroid hormones such as estrogen (E2), which are mainly mediated by their nuclear receptors ERα and ERβ. Based on our own previous investigations, we hypothesize that E2 and its receptors mediate the response on stress sex-specifically and influences the mitochondrial function and the cardiac energy metabolism. Therefore the effects of sex and sex hormones on the cardiac mitochondrial function will be studied at physiological conditions ex vivo and in vivo in skinned heart and skeletal muscles fibers. For this approach the mitochondrial activity will be assessed in heart and muscles fibers using the Clark electrode (Fig.1).

First results of this study show that E2 enhances the activity of mitochondrial respiration accompanied with an increased oxidative phosphorylation in male and female mice (Fig. 2).

Further work will analyze the effects of sex hormones on proteins, involved in cardiac metabolism. This study could contribute to a better understanding of sex-differences and the effects of sex hormones in cardiac metabolic function and help to design sex-specific pharmacological interventions.

10. 17β-Oestradiol (E2) effects and mechanisms of sex-specific gene regulation in the heart
Cardiovascular disease (CVD) manifests differently in men and women. Sex steroid hormones are generally believed to play a major role in the occurrence of sex-related differences observed in the development of CVD. In particular, a large body of evidence from several observational and experimental studies indicates that E2 might be involved in reducing risk for cardiovascular disease. However, harmful effects of E2 have also been reported leading to the current controversy regarding the actions of E2. In addition, so far there has been no report of E2-dependent gene regulation in a sex-specific manner in the heart, while such effects have been described in other organs. The main goal here is to investigate the effect(s) of E2 in the heart. To achieve this, we are comparing the transcriptomes of human cardiac tissues and of hearts from mice treated with E2. We combine these analyses with data collected from in vivo studies and assays on cellular effects generated in vitro.

11. Sex specific differences in microRNA expression and their role in heart diseases

Project leader: Hugo Sanchez-Ruderisch
Coworkers: Claudia Eschen, Ana Queiros, Daniela Fliegner
Funding: DFG: GK 754 II/III

MicroRNAs (miRNAs) are small non-coding RNAs that control gene expression by duplex formation with 3’ untranslated regions of target mRNAs. Dysregulation of miRNAs has been reported in various cardiac diseases. Most of the miRNAs expressed in mouse cardiac tissue are regulated during cardiomyocytes hypertrophy. However, nothing is known about the regulation of expression of miRNAs considering the gender aspect. Gender differences in the development and progression of cardiovascular diseases such as myocardial infarction, hypertension, heart failure and sudden death have been described. In mouse model of cardiomyopathies, a more severe cardiovascular phenotype is observed in male animals and can be partially rescued by estrogen administration. Based on these data and on our previous results showing gender differences after TAC we hypothesise that an estradiol-dependent gender different expression of miRNAs is in part responsible for the observed gender different development of cardiac hypertrophy after pressure overload. The aims of this project are the identification of those miRNAs expressed in a gender-specific manner in the mice heart and elucidate their role in heart hypertrophy comparing male and female mice after TAC treatment. Preliminary results indicated sex different expression of various miRNAs. The influence of altered miRNAs expression on putative target genes involved in fibrosis is under current investigation.