Heart failure; role of the membrane proteoglycan syndecan-4 during mechanical stress of the myocardium

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Abstract

Heart failure is the most frequent cause of mortality and morbidity in industrialized nations. Prolonged pressure overload due to hypertension as well as valvular and ischemic heart diseases leads to compensatory hypertrophy and heart failure. The objective of this literature thesis is to provide detailed insight into a particular molecular mechanism important for development of hypertrophy and subsequent heart failure, which is an incompletely understood process. A hypothesis for development of hypertrophy and progression to failure is that the sarcomeric Z-disc proteins, among them the transmembrane proteoglycan syndecan-4, are essential molecular players. This hypothesis has been tested over the last ten years at the Institute for Experimental Medical Research (IEMR) at Oslo University Hospital Ullevål, and by a few other groups. Thus, syndecan-4 has been the focus of this thesis. By using genetically modified mice, a research group at the IEMR showed that mice lacking syndecan-4 did not develop concentric myocardial hypertrophy after being exposed to pressure overload, whereas in wild-type mice substantial hypertrophy was found, as expected. Moreover, following pressure overload, syndecan-$4^{-/-}$ mice showed a decreased activation of the pro-hypertrophic signaling molecule nuclear factor of activated T-cells (NFAT) compared to wild-type mice. NFAT is a central intracellular signaling molecule involved in development of hypertrophy. In cell culture experiments, cardiomyocytes lacking syndecan-4 displayed diminutive activation of NFAT in comparison to control cells, following 24 hours of mechanical stress. The results suggest that syndecan-4 acts as a mechanotransducer in the process of hypertrophy, activating pro-hypertrophic NFAT transcription factors in individual cardiomyocytes. Thus, syndecan-4 is thought to play an important role during development of heart failure in response to increased mechanical stress in the myocardium.

Preface

The tragic death of a young and successful Norwegian athlete got extensive media coverage earlier this year (2012). The cause of death was thrombosis in one of the coronary arteries, leading to myocardial infarction, a frequent disease of the heart. When thinking of themes for my thesis, it was this vital organ that caught my interest. Thus, I contacted Professor Geir Christensen at the Institute for Experimental Medical Research (IEMR), Oslo University Hospital Ullevål. At IEMR, cardiovascular research and in particular heart failure is the main area of interest. After having discussed several topics with Professor Geir Christensen, we decided on writing a literature thesis related to an ongoing project at the institute. This project aims at understanding molecular mechanisms underlying development of heart failure. I have used the original research article entitled "Syndecan-4 is essential for development of concentric myocardial hypertrophy via stretch-induced activation of the calcineurin-NFAT pathway" published December 2nd 2011 by Professor Christensen`s group at IEMR (1) as a basis for this thesis. In addition, I have included information from several publications on related topics, and these are cited in the reference section.

The aim of the thesis is to provide a detailed insight into one particular molecular mechanism which is important for hypertrophy and heart failure development.

In this thesis I start by describing the heart failure syndrome, the pathophysiological mechanisms underlying this condition, Z-disc proteins and syndecan-4. Subsequently, I describe some related methodological considerations. Finally, results and several central topics in relation to the central paper are discussed, before a conclusion is presented.

Writing this thesis would have been impossible without expert guidance from my supervisors, Geir Christensen and Ida Gjervold Lunde. I want to thank them for encouraging and believing in me, and for all their time and advice.

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Introduction

Heart failure – a syndrome

Heart failure is a major health problem in industrialized nations, representing the greatest cause of morbidity and mortality today (2). Heart failure affects almost 5 million people in the United States every year, and 300 000 die from it annually (3). Worldwide, an estimated 23 million people are living with heart failure and this creates large economic challenges (2). In 2010 in the United States, it was estimated that 1-2% of all health care costs were related to the care of patients with heart failure (4). Unfortunately, these costs do not seem to decrease, since the general population is aging and both the prevalence and incidence of heart failure rise with age (5).

Chronic heart failure develops over time, and may result from many pathological conditions. This progressive disease is often defined as a condition with a heart that is no longer capable of pumping blood to the peripheral tissues at a rate that meets the metabolic needs (3). Due to an inadequate cardiac output (the amount of blood being pumped out by the heart each minute), blood delivered to the heart (fig.1) piles up in the ventricles, leading to an increased end-diastolic pressure, which over time may cause increased end-diastolic volume. The increased end-diastolic pressure is transmitted backward to the venous circulation, causing an elevated venous pressure. In heart failure, the heart often has to work against a greater pressure (increased afterload), which causes a further reduction of cardiac function (3).

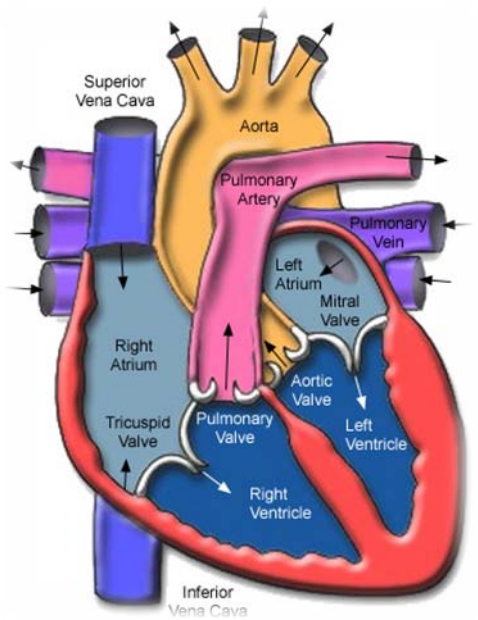

Fig.1: Illustration of the human heart with its four chambers. The arrows show direction of blood flow; into the right atrium from the systemic circulation and out of the left ventricle to the systemic circulation (illustration from reference (6)).

The New York Heart Association classifies heart failure based on a person's functional level during exercise. There are four classes, ranging from no symptoms during normal physical activity (class I), to experience of symptoms during all form for activities, and in class IV, the patients have symptoms of heart failure even when resting (7). Heart failure can be divided in two groups, left- and right-sided heart failure. The most common symptoms and signs of left-sided heart failure are due to pulmonary congestion. Symptoms are dyspnea (feeling of breathlessness), cough, orthopnea (shortness of breath when lying down), paroxysmal nocturnal dyspnea (nightly attacks of shortness of breath) and tachycardia. Right-sided heart failure is often a result of heart failure on the left side. However, right-sided heart failure may also be caused by lung diseases, and leads to congestion of the systemic circulation, e.g. peripheral edema, splenic and hepatic enlargement and ascites (3).

Causes of heart failure

The most frequent causes of left-sided heart failure are ischemic heart disease and hypertension, which frequently result in systolic dysfunction. In diastolic dysfunction, the heart is unable to relax adequately to allow for proper filling. Patients with diastolic dysfunction are often older and of female sex, suffering from either diabetes mellitus or hypertension (3). Other causes of heart failure are congenital heart disease, cardiomyopathies and valvular heart disease, which includes mitral- or aortic valve disease (stenosis or insufficiency) (3).

Aortic stenosis is the most common type of valvular disease. In this condition the heart is forced to work against a greater afterload because the opening between the left ventricle (LV) and aorta is narrower than normal. The narrow opening, the stenosis, normally arises because of calcification of the aortic valve. This is most common in patients older than 70 years, and it is more frequent among males than females. For patients younger than 70 years, the reason is in most cases congenital. The person is born with a bicuspid valve instead of the tricuspid valve, the bicuspid valve itself is usually not stenotic, but the possibility for calcification later in life is generally higher than normal (8).

Compensatory mechanisms

The cardiovascular system will do everything in its power to maintain cardiac output at any circumstance, and there are several compensatory mechanisms assisting the failing heart. The most important are:

1. Ventricular hypertrophy (fig.2); the amount and structure of contractile tissue are altered due to an increased workload. Because the ability of the adult myocardial cells to proliferate is limited, the response is characterized by hypertrophy of individual myocytes. We usually distinguish between two conditions which induce hypertrophy; pressure- and volume overload. In pressure overload (e.g., aortic stenosis and hypertension), the individual myocytes are increased in diameter and the ventricular wall is thicker than normal (fig.2). In volume overload (e.g., valvular insufficiency), the individual myocytes are longer, which results in a larger heart with increased chamber size.

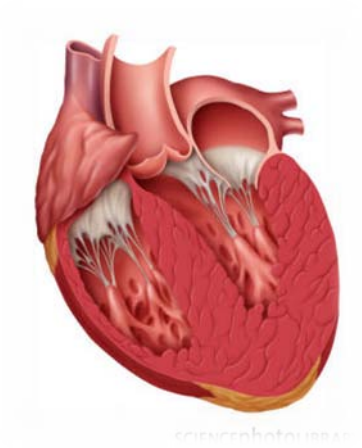

Fig. 2: Anterior view of the human heart, demonstrating concentric left ventricular hypertrophy. Please notice the thick wall and small lumen of the left ventricle (illustration from reference (9)).

2. The Frank-Starling law; as heart failure develops, the end-diastolic pressure increases and the ventricular muscle fibers are stretched. In accordance with the Frank-Starling mechanism, the ventricle will contract more forcibly because the individual muscle fibers are stretched, helping to maintain cardiac output. This is called the "compensated phase"; the cardiac output meets the tissue's need for blood supply, although the size of the ventricle is increased at this point. A larger ventricle requires more oxygen, so as time passes, entering of the "decompensated phase" is unavoidable. The weakened myocardium is no longer able to eject a sufficient amount of blood to meet the body's need for supply of oxygen.
3. The renin-angiotensin-aldosterone system; this is an important hormonal system which regulates blood pressure and blood volume. This system is activated due to the reduced cardiac output, and eventually causes increased hemodynamic burden, as the heart has to work against a higher peripheral pressure.
4. ANP (atrial natriuretic peptide); this hormone is secreted from the atria when distended. It eases the heart's workload by creating vasodilation, and by increasing the excretion of sodium in the urine, and thereby the urine output.
5. The sympathetic nervous system; release of norepinephrine leads to an increased heart rate, myocardial contractility and vascular resistance, and thus increases cardiac output. As for the renin-angiotensin-aldosterone system, activation of the sympathetic nervous system eventually results in the failing heart having to work against a higher afterload, which is why these two systems are inhibited in current heart failure therapy.

Due to these compensatory mechanisms, cardiac output is sustained for a while. In the long term, however, cardiac failure is most often unavoidable (3).

Current treatment of heart failure

Today, there is no cure for heart failure, and without transplantation the patient is going to die from this condition. Approximately half of the patients die within five years from time of diagnosis (2). Treatment today mainly focuses on relieving symptoms, in addition to prolonging lifetime and quality of life. When it comes to left-sided ventricular dysfunction, the most common pharmaceuticals used are angiotensin-converting-enzyme (ACE) inhibitors, beta-blockers and diuretics (7). Because the prognosis after being diagnosed with heart failure

is poor, today's treatment is evidently not optimal. Thus, to develop new therapeutic strategies, it is of great importance to understand the mechanisms involved in the cardiac remodeling process in greater detail.

Cardiac remodeling

Heart failure is a frequent result of cardiac remodeling, a process in which the compensative mechanisms are responsible for structural alterations within the heart. This process takes time and creates altered function, as well as geometrical changes in the heart (3). Changes at cellular and molecular levels are also present. An increased activation of the renin-angiotensin-aldosterone-system, increased levels of catecholamines (e.g., norepinephrine of the sympathetic nervous system) together with vasopressin, cytokines and other pro-inflammatory substances stimulate the remodeling process (7). The remodeling process, with LV hypertrophy being a central alteration, includes apoptosis of cardiomyocytes, induced inflammation, changes in extracellular matrix, and gene expression and patterns mimicking that of the fetal heart (2).

When LV is exposed to pressure overload, a number of biological processes are initiated. Alterations at the cellular level involve increased protein synthesis, creating new sarcomeres which in turn lead to increased myocyte size. For instance, the synthesis of myosin heavy chain is rising by approximately 35% during increased mechanical stress. In addition, the number of mitochondria in each cell is increased (10). Ventricular hypertrophy can be divided into concentric- and eccentric hypertrophy. Concentric hypertrophy occurs in pressure overload states, as described earlier, and because additional sarcomeres are being added in parallel, the myocytes get wider and the ventricular wall thickens. This is a contrast to eccentric hypertrophy, a result of volume overload, also described earlier. In eccentric hypertrophy, the cardiac muscle cells increase in size due to sarcomeres being added in series, and in this case we can see both ventricular dilation and increased muscle mass. The cardiac myocytes are increasing in size, and are generally believed not to increase in number (11). An imbalance between the number of capillaries, connective tissue and myocytes will develop. Thus, cardiomyocytes may suffer from insufficient blood supply (10).

In length a normal cardiac myocyte measures up to 100μm, and normal LV myocytes are 18μm in diameter (12). In concentric hypertrophy, however, the LV myocyte is 25μm or more. Looking at a histological section of a hypertrophied heart, myocyte nuclei are enlarged, and the shape of the nuclei is altered from oval to nearly rectangular (12). In addition to this, several hypertrophic myocytes are binucleated. The process of a slow myocyte turnover in a healthy heart, which includes renewal of myocytes from stem cells and myocyte death, seems to be accelerated in a hypertrophied heart. In addition, as part of the remodeling process, the loss of myocytes seems to exceed the generation of new ones, accelerating development of heart failure. Another alteration of the myocardium worth noticing is the density reduction of the myofibrils inside the myocytes (12).

The extracellular matrix is also altered in a hypertrophied heart, and changes in the interstitial space are crucial in progressive heart failure. The development of interstitial fibrosis is critical in this process. The insufficient blood supply to the myocardium, may be an important reason for myocyte necrosis, and thereby the development of fibrosis. Thus, during the remodeling process, there is a rise in collagen concentration and interstitial fibrosis.

In a histological section from a hypertrophied heart, the interstitial fibrosis makes up about 5%, most of the collagen is type I, and approximately 15% is type III. At a later stage, collagen may constitute as much as 25% of the area. There is also a greater degree of interstitial fibrosis in concentric hypertrophic growth, e.g., in aortic stenosis, compared to

eccentric hypertrophic growth. Studies show that there is a direct link between the degree of interstitial fibrosis in the myocardium, and a decreasing myocardial function (12).

It is not well known which molecules are sensing and transmitting the increased mechanical stress in the ventricular myocardium. However, during the ventricular remodeling process, more is known about the intracellular signaling pathways. One example is the calcineurin and calcium/calmodulin dependent kinase II (CaMKII) pathways, which are calcium-regulated pathways. These signaling pathways alter gene expression by transmitting their signals to the myocyte nucleus. A transcription factor family called "the nuclear factor of activated T-cells" (NFAT) shows great importance in the pathological process of cardiac remodeling downstream of calcineurin. Calcineurin is a protein phosphatase sensitive to calcium, which activates the NFAT transcription factors by dephosphorylating them. This leads to NFAT being translocated from the cytoplasm into the myocyte nucleus. After entering the nucleus, NFAT activates NFAT-responsive genes (2). For example, a paper under submission by authors at IEMR, concluded that hearts of aortic stenosis patients had a multi-leveled activation of the calcineurin-NFAT signaling cascade, as well as an increased activation and levels of all the four NFAT transcription factors (13). Thus, NFAT is established as one of the most important signaling molecules involved in cardiac hypertrophy and failure.

Hypertrophic growth is normally accompanied by a shift in gene expression patterns. The fetal gene program is re-expressed, so that genes that were active during the fetal development are reactivated as a result of prolonged mechanical stress (2). In particular, this leads to isoform switches in the metabolic enzymes and proteins of the contractile elements. Proteins involved in calcium handling may also be altered, impairing contraction and relaxation (10).

Another central process of pathological cardiac remodeling is myocardial inflammation. Inflammation influences not only myocardial hypertrophy and apoptosis, but certainly also the extracellular matrix architecture. Pathological regulation of the synthesis and degradation of collagen leads to loss of structural integrity of the heart. Central pro-inflammatory cytokines which are elevated in congestive heart failure are TNF-α, IL-1 and IL-6. IL-1, for instance, is an important factor for myocardial apoptosis and hypertrophy, and it has been shown to reduce the ability of the myocardium to contract properly. TNF-α has been demonstrated to contribute to myocardial hypertrophy as well as apoptosis, increased fibrosis, and activation of nitric oxide synthase (an enzymes catalyzing the production of NO, an important cellular signaling molecule) (14).

The cardiomyocyte Z-disc

As mentioned previously, the molecules sensing and acting as primary transmittors of the increased ventricular pressure, inducing LV hypertrophy and subsequent heart failure, are not well known. Proteins in the sarcomeric Z-disc (an area in the contractile unit of the cardiomyocyte) are believed to be involved in this so-called mechanotransduction (the Z-discs are illustrated as the blue vertical bands in fig.3). The Z-disc constitutes the dark lines of a longitudinal section viewed using electron microscopy. In cardiac myocytes these dense bands are varying in size from 100 to 140 nm (15). The Z-disc constitutes the lateral border of the myofibril sarcomere, and it represents a highly complex network containing multiple proteins, forming a dynamic and well-organized structure (16). The cardiomyocyte consists of many parallel myofibrils, which contain the "thick filaments"; the myosin filaments, and the "thin filaments"; the actin filaments (17) (fig.3). The area between two subsequent Z-discs; the sarcomere, represents the structural and functional unit of the myofibril (18). As much as 227 different proteins have been coupled to the Z-disc (15). The plus-ends of sarcomeric actin

filaments are localized here, and other central proteins are α-actinin, which crosslinks the plus-end of actin to the Z-disc (18) and CapZ, which "caps" the sarcomeric actin filaments and anchors them to the Z-discs (15). Titin, the largest known polypeptide of all, is stretching from the M-line (illustrated as the red and vertical band located in the middle of fig.3) to the Z-disc, anchoring the myosin filament to the Z-disc (18), with its amino-end being located to the Z-disc (15).

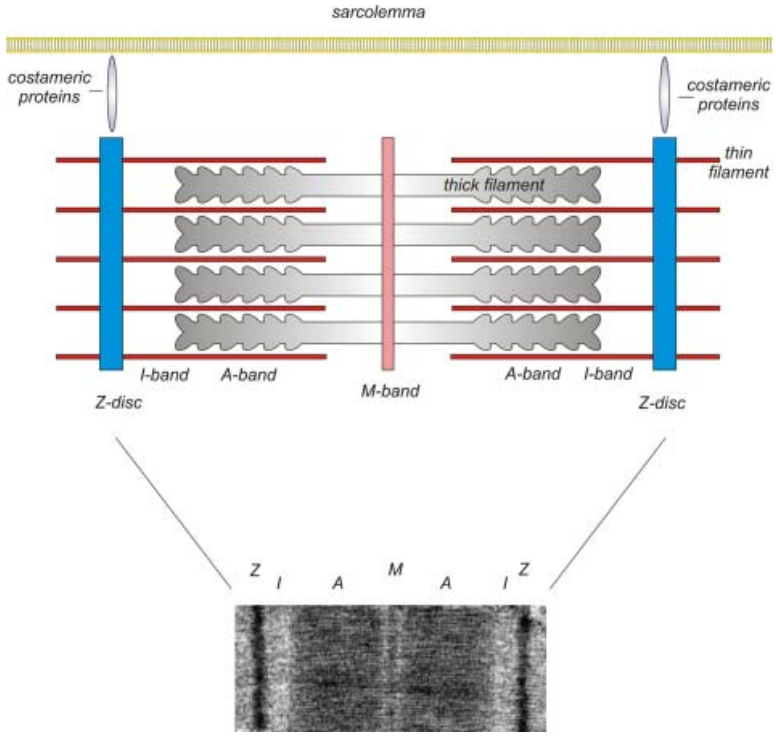

Fig. 3: Illustration showing the cardiac sarcomere. The blue pillars at the ends represent the Z-discs (illustration from reference (19)).

Desmin, an intermediate filament specific for muscle cells is wrapped around each Z-disc in a cyclic shape, ensuring Z-disc integrity (18). Telethonin is a small protein that binds to titin at the periphery of the Z-discs. Telethonin may influence the process of myocardial hypertrophy through an interaction with calsarcin-1 because calsarcin-1 further interacts with calcineurin (15) (the enzyme dephosphorylating NFAT in hypertrophic cardiomyocytes (2)). Moreover, protein kinase C and D is also localized to the Z-discs, important for pro-hypertrophic signaling (15).

Early studies suggested that the Z-disc and its proteins functioned mainly as mechanical stability, but the large number of Z-disc proteins that have been discovered indicate additional functions, such as intracellular signaling and mechanotransduction. Z-discs are today believed to play an important role in the development of hypertrophy and heart failure (16). Moreover, it has been identified that mutations in different Z-disc proteins lead to a diverse group of cardiac diseases (15), including cardiomyopathies and heart failure (16).

The proteoglycan syndecan-4

Syndecan-4 is a transmembrane heparan sulphate proteoglycan, located to the Z-disc of cardiomyocytes (2) (1) (fig.4). Its extracellular heparin sulphate chains interact with extracellular matrix compounds, and its cytoplasmic domain can signal through interaction with signaling molecules (20). In mammals there are four types of syndecans, all having the

three main domains; the cytoplasmic, the transmembrane, and the extracellular. The external ectodomain of syndecan-4 can be divided in two subdomains. An outer “heparan sulphate domain”, where heparan sulphate chains are attached and where interactions with growth factors and fibronectin are occurring, and a membrane proximal “cell-binding” domain, where integrin cell adhesion is mediated. The cytoplasmic domain contains three subdomains; C1, V and C2. The C1 subdomain, placed closest to the cell membrane, has a phosphorylation site regulating syndecan-4-mediated signaling. The medial V subdomain interacts with PKCα and α-actinin. The outermost C2 subdomain binds to numerous cytoskeletal proteins (21) (fig.4).

In cardiomyocytes, syndecan-4 is linking proteins of the extracellular matrix to the intracellular cytoskeleton, e.g. actin filaments. Furthermore, syndecan-4 was recently coupled to the calcineurin-NFAT signaling pathway by the study conducted at IEMR (1), an essential pathway in the progression of cardiac hypertrophy an failure (2).

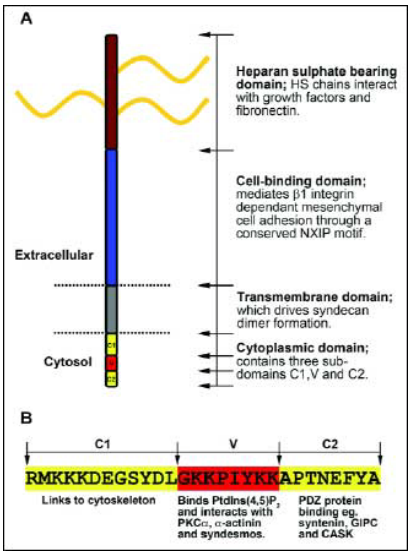

Fig. 4: Illustration of syndecan-4, showing the ectodomain above the upper stippled line, the transmembrane domain in the middle, and the cytoplasmic domain below the lower stippled line. (illustration from reference (21)).

Hypothesis

The hypothesis of this thesis is that syndecan-4 is important for sensing increased ventricular pressure, and transmitting signals leading to subsequent LV hypertrophy and heart failure.

Methods

Literature searches

The PubMed database has been the main source of articles for this thesis, and review articles have constituted the main type. Moreover, academic medical literature found in books has been used, and various internet searches have been performed, when searching for relevant literature.

During early summer 2012 several PubMed searches were performed, and this section describes how the searches were done. Search terms such as “left ventricular hypertrophy” and “cardiac remodeling” were used. In the beginning the searches were very broad, but as more detailed knowledge was needed, they got narrower. However, looking at the reference

list of the “main article” was great guidance for choosing relevant articles. In the introduction part, information about myocardial inflammation in the process of cardiac remodeling was needed. Thus, by using the search term “inflammatory heart disease AND remodeling AND heart failure”, a review article of interest was found. Searches for information about the Z-disc, what it is and what it consists of, using “sarcomeric AND Z-disc” and” Z-disc AND hypertrophy”, were also done. To find out more about syndecan-4, the “main article” was used in addition to other papers. “The main article” is (as mentioned in preface); “Syndecan-4 is essential for development of concentric myocardial hypertrophy via stretch-induced activation of the calcineurin-NFAT pathway” (1). This article has been used as a basis for this thesis, and in particular regarding the methods and results parts. To retrieve that article, the words “heart failure AND syndecan-4” were used. Another frequently used source is the PhD thesis by Ida G. Lunde, supervised by Professor Geir Christensen, and defended in May 2012 at the University of Oslo.

For the discussion part, however, searches for literature describing the link between syndecan-4 and cardiac hypertrophy were performed. The search terms “syndecan-4 AND heart failure” resulted in one article, and the search terms “syndecan-4 AND myocardial and hypertrophy” gave a second relevant article. By using the search terms “syndecan AND cardiac”, two additional articles regarding syndecan-4, cardiac remodeling and the cardiac healing process in light of myocardial infarction (MI) were found. Further, since I was particularly interested in more details regarding Z-disc proteins and their role in mechanotransduction, more articles using the words “cardiac Z-disc signaling” were searched for in PubMed. Finally, for the discussion part, searches using the terms “LVAD what is it?”, “shedding process extracellular molecule” were done.

Methodology used in the “main article”

The methodology described in this section, is a selection taken from the main paper “Syndecan-4 is essential for development of concentric myocardial hypertrophy via stretch-induced activation of the calcineurin-NFAT pathway”(1).

Experimental animals and echocardiography

For the studies, wild-type (WT) and syndecan-4$^{-/-}$ (syn-4$^{-/-}$) mice, were used as experimental animals (22). Syn-4$^{-/-}$ mice are mice in which the gene coding for syndecan-4 is “turned off”, resulting in mice lacking this proteoglycan. Pressure overload was induced by aortic banding, while the controls were sham-operated. Thus, there were four main groups for the study:

1. WT-SHAB mice = sham-operated WT mice
2. WT-AB mice = aortic banding-operated WT mice
3. Syn-4$^{-/-}$ SHAB mice = sham-operated syn-4$^{-/-}$ mice
4. Syn-4$^{-/-}$ AB mice = aortic banding-operated syn-4$^{-/-}$ mice

To induce LV pressure overload, banding of the ascending aorta of mice was carried out by making a split in the upper one third of sternum, and a silk band was tied around aorta ascendens. Reproducibility was ensured by tying against a 26G needle, which was removed after the procedure. The same surgical procedure was performed in sham-operated control mice, except for the actual tightening of the banding around the aorta. Both WT and syn-4$^{-/-}$ mice were seven weeks old, i.e. young adults, when the banding was done under deep anesthesia with isoflurane gas. After three weeks, echocardiography examinations were performed.

Echocardiography is an examination of the heart using ultrasound. M-mode is the traditional type of echocardiography. Its main purpose is to detect movements of the LV, such as movement and velocity of its walls and valves, but it is also great in evaluating morphology of cardiac structures. Echocardiography data are usually depicted in 2D, although information about the heart using this method can also be shown as 3D, but this technique is not fully utilized yet (23). The animals were lying on their back and breathing trough a mask with 2% isoflurane. M-mode echocardiography was used, and the tracings were recorded on two levels with 2D guidance; one at the valva aortae level, and the other at the level of musculus papillaris. During systole and diastole, both diameter and cavity dimensions were measured in the LV. The measurements of wall thickness were carried out at the thickest part of the wall. 2D echocardiography pictures were recorded in both long and short axis. Short axis was recorded at the level of musculus papillaris. To obtain a result as accurate as possible, three typical cycles were analyzed and averaged. To assess cardiac function, LV fractional shortening in percent was calculated from the diameter of the ventricle, during systole and diastole. Cardiac output was also calculated from echocardiographic recordings.

Only those mice were included that had a maximum flow velocity through the narrow opening of more than 3m/s and less than 4m/s twenty-four hours after the AB operation. Moreover, those with a maximum flow velocity of more than 4m/s and less than 6m/s twenty-one days after AB were included.

Measurements of isolated cardiomyocytes from WT and syn-4$^{-/-}$ mice

Three weeks after the AB and SHAB operations, cardiomyoctes were enzymatically isolated from WT and syn-4$^{-/-}$mice, respectively. Cardiomyocyte length and width were measured.

Luciferase reporter mice and luciferase activity

Two types of mice were used in the study to create syn-4$^{-/-}$-NFAT luciferase reporter mice; the NFAT-luciferase mice produced by Jeffery D. Molkentin, a collaborator on the IEMR study, and the syn-4$^{-/-}$ mice. NFAT-luciferase mice (24) were made by inserting nine copies of an NFAT-binding site from the interleukin-4 promoter upstream of the luciferase reporter gene. Thus, luciferase activity reflects the amount of NFAT binding to DNA, i.e. NFAT activity. The NFAT-luciferase mice were crossed with the syn-4$^{-/-}$ mice to produce syn-4$^{-/-}$-NFAT-luciferase reporter mice. Newborn mice from their offspring were used for isolation of cardiomyocytes. Quantification of the luciferase activity was done according to the Luciferase Assay System protocol from Promega, by adding the luciferin substrate after harvesting the cardiomyocytes and measuring luminescence.

Cardiomyocytes from neonatal mice

To isolate cardiomyocytes from a neonatal mice, four solutions were used; trypsin- and collagenase solutions, and so-called light and a dark media. Trypsin is an enzyme that digests proteins in duodenum, while collagenase is an enzyme that breaks the peptide bindings of collagen. Around 50 neonatal mice were decapitated, and the hearts were rapidly taken out and placed in a salt buffer. Then the LV was separated from the rest of the heart and sliced in smaller parts, before the collagenase solution was used to digest the small pieces. From this, the supernatants were placed in dark medium on ice. At this point, the digestion process was ended by adding serum. The cells were plated in cell culture flasks at 37 ˚C; non-cardiomyocytes attached to the flask, while the cardiomyocytes remained in the medium. The

medium was collected, centrifuged and the remaining cells, i.e. the cardiomyocytes, were allowed to attach to cell culture plates, coated with 0.2 % gelatin containing 0.1% fibronectin.

Cyclic mechanical stretch of isolated cardiomyocytes

Isolated cardiomyocytes, were plated on collagen-coated silicone membrane cell culture dishes. By using the FlexCell Tension system FX-4000, the cardiomyocytes were subjected to cyclic mechanical stretch for 10 min-24 hours (10%, 1 Hz). Cyclosporin A, an immunosuppressive drug used because of its ability to interfere with the T-cell function through inhibiting calcineurin, was used to inhibit calcineurin-NFAT signaling.

Assessment of protein synthesis in isolated cardiomyocytes

Protein synthesis during autonomous hypertrophy of isolated murine cardiomyocytes, was used to assess hypertrophic growth in culture. The isolated cardiomocytes were left in culture for two to five days, and on the second day, five μCi/ml 3^{H}-leucine was supplied to the medium. At day five, the cardiomyocytes were harvested for analyses of incorporated 3^{H}-leucine. As positive control, serum-stimulated cardiomyocytes were used. The incorporation of 3^{H}-leucine was measured by counts per minutes.

Immunoblotting

The standard molecular biology technique of immunoblotting, also called Western blotting, was used for analyses of proteins. Electrophoresis is used to separate the proteins based on molecular weight, and antibodies are used to detect the specific proteins. After separation by electrophoresis, the proteins are transferred to a membrane; a commonly used membrane is polyvinylidene difluoride (PVDF) (25). For this particular study, immunoprecipitates, cell- and tissue extract were analyzed on SDS/PAGE, and transferred onto PVDF membranes. To avoid unspecific binding, 5% non-fat dry milk or 1% casein were diluted in TBST. The blocking was done for one hour at room temperature. The membrane was further incubated with primary antibodies, either for 1-2 hours at room temperature, or at 4˚C overnight. For detection, the membrane was incubated with a secondary antibody, after being washed in TBST. ECL was used to develop blots. Las-1000 or Las-4000 was used for detection of the chemiluminescence signals from the secondary antibody. The specific antibodies, and various other molecular techniques that were used in the study, are detailed in the original paper.

Results

The results described in this section, including the figures, are based on the article “Syndecan-4 is essential for development of concentric myocardial hypertrophy via stretch-induced activation of the calcineurin-NFAT pathway”(1).

Syn-4 $^{-/-}$ mice did not develop concentric hypertrophy during pressure overload

Echocardiographic measurements, heart rates, LV pressure in addition to animal characteristics revealed that the hearts of untreated syn-$4^{-/-}$ mice were similar to WT mice. Thus, the lack of syndecan-4 did not severely affect cardiac development or normal function in adult mice. Aortic banding was performed, and after three weeks with pressure overload

WT mice had developed the expected LV hypertrophy, while interestingly, syn-4$^{-/-}$ mice had not. The hypertrophy was seen as a thicker posterior LV wall (fig.5A-C).

Syn-4$^{-/-}$ mice did not develop LV hypertrophy, but rather a prematurely dilated LV in response to pressure overload. LV cross-sections and longitudinal measurements showed that syn-4$^{-/-}$AB mice compared to syn-4$^{-/-}$ SHAB mice, and WT-AB mice, had developed a significant increase in diameter (fig.5D). Accordingly, syn-4$^{-/-}$ AB mice had a reduced LV fractional shortening, and thereby a reduced cardiac function compared to WT-AB mice (fig.5E). Neurohormonal measurements revealed that syn-4$^{-/-}$AB mice had higher levels of angiotensin II in serum, compared to WT-AB mice. Further comparison also showed that syn-4$^{-/-}$AB mice had increased lung weight and left atrial diameter, suggesting congestive heart failure.

In accordance with missing concentric hypertrophy observed by echocardiography, the cardiomyocyte width was the same in syn-4$^{-/-}$ AB mice, and in syn-4$^{-/-}$ SHAB mice (fig.5F). In contrast, the WT-AB mice cardiomyocyte width had increased 25% compared to the WT-SHAB mice, which were in accordance with the echocardiography findings. The cardiomyocyte length, however, was increased in both AB groups; 10% in the case of syn-4$^{-/-}$ mice and 8% in the case of WT mice, in comparison to their controls.

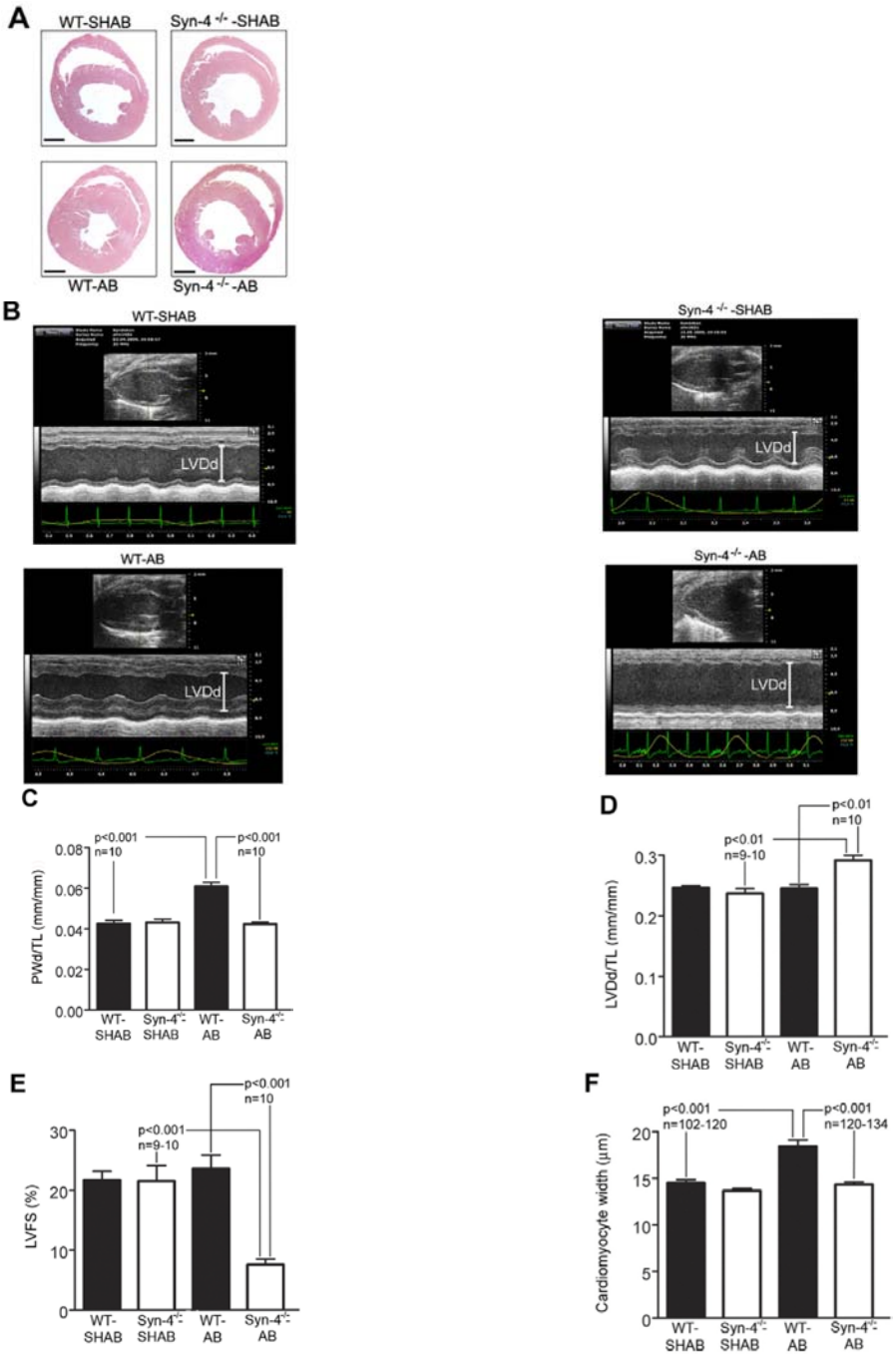

Figure 5A-F: There are four different groups; wild-type (WT)-sham-operated (SHAB) mice, WT-aortic banding (AB) mice, syndecan-4 $^{-/-}$ (syn-4$^{-/-}$) SHAB mice, syn-4$^{-/-}$ AB mice. A) Cross sectional view of the heart showing wall thickness and ventricular dimensions. B) 2D and M-mode tracings (echocardiography) from the left ventricle (LV) in diastole. C) PWD= posterior wall thickness during diastole;TL= Tibia length. D) LVDd= LV diameter during diastole. Notice how dilated the hearts of the syn-4$^{-/-}$AB mice are compared to the rest of the groups. E) Figure shows LVFS= LV fractional shortening. F) This figure demonstrates the cardiomyocyte width meassured in µm. Notice the difference in width between WT-AB and syn-4$^{-/-}$ AB, and the similarity between syn-4$^{-/-}$ -SHAB and syn-4$^{-/-}$ AB. Figures and text are taken from reference (1).

Inhibition of cardiac NFAT signaling in mice lacking syndecan-4

Mouse hearts and isolated cardiomyocytes exposed to aortic banding and mechanical stress, respectively, were studied to understand the role of syndecan-4 in pro-hypertrophic calcineurin-NFAT signaling. NFAT activation was assessed as dephosphorylation (pNFAT, i.e. inactive NFAT in the cytoplasm) and NFAT luciferase reporter activity (i.e. active NFAT bound to the promoter of NFAT-responsive genes in the nucleus). Activation of the isoform NFATc4 in the hypertrophic human myocardium was investigated in particular, because this isoform has been proposed to be of importance for cardiac hypertrophy. The NFAT transcription factor family consists of four isoforms; NFATc1-c4, and in cardiomyocytes all four isoforms are expressed (2). The results showed that following AB, syn-$4^{-/-}$ mice had a significantly lower activation of NFATc4 than WT mice. This was demonstrated as increased pNFATc4 levels (fig.6A).

One central and exciting research question was whether syndecan-4 acts as a mechanotransducer in the process of calcineurin-NFAT activation. To study this, isolated cardiomyocytes were exposed to cyclic mechanical stretch. Twenty-four hours of mechanical stretch significantly increased calcineurin-NFAT activation in neonatal cardiomyocytes from NFAT-luciferase reporter mice. Interestingly, syn-$4^{-/-}$ -NFAT luciferase cardiomyocytes displayed diminutive activation of NFAT (1.6 fold) in comparison to NFAT–luciferase control cells (5.8 fold) (fig.6B). Likewise, phosphorylated (i.e. inactive) NFATc4 levels were significantly higher in cardiomyocytes lacking syndecan-4, compared to WT cardiomyocytes after mechanical stretch (fig.6C). Finally, syndecans-4`s role in activating calcineurin-NFAT in cardiomyocytes exposed to autonomous hypertrophy was studied. Neonatal cardiomyocytes undergoing autonomous hypertrophy showed a significantly higher level of pNFATc4 (inactive NFAT) when lacking syndecan-4 (fig.6D). This fitted well with a higher level of NFAT-luciferase activity in neonatal cardiomyocytes from NFAT-luciferase mice subjected to autonomous hypertrophy, compared to neonatal cardiomyocytes from syn-$4^{-/-}$ mice (fig.6E). In accordance with lower pro-hypertrophic NFAT signaling in cardiomyocytes lacking syndecan-4, protein synthesis was lower in syn-$4^{-/-}$ cells subjected to autonomous hypertrophy for five days. Thus, these result showed a reduced hypertrophic response in syn-$4^{-/-}$ mice, compared to WT mice (fig.6F). Conclusively, all these results showed that the pro-hypertrophic NFAT signaling pathway was inhibited in cardiomyocytes missing the transmembrane heparan sulphate proteoglycan syndecan-4.

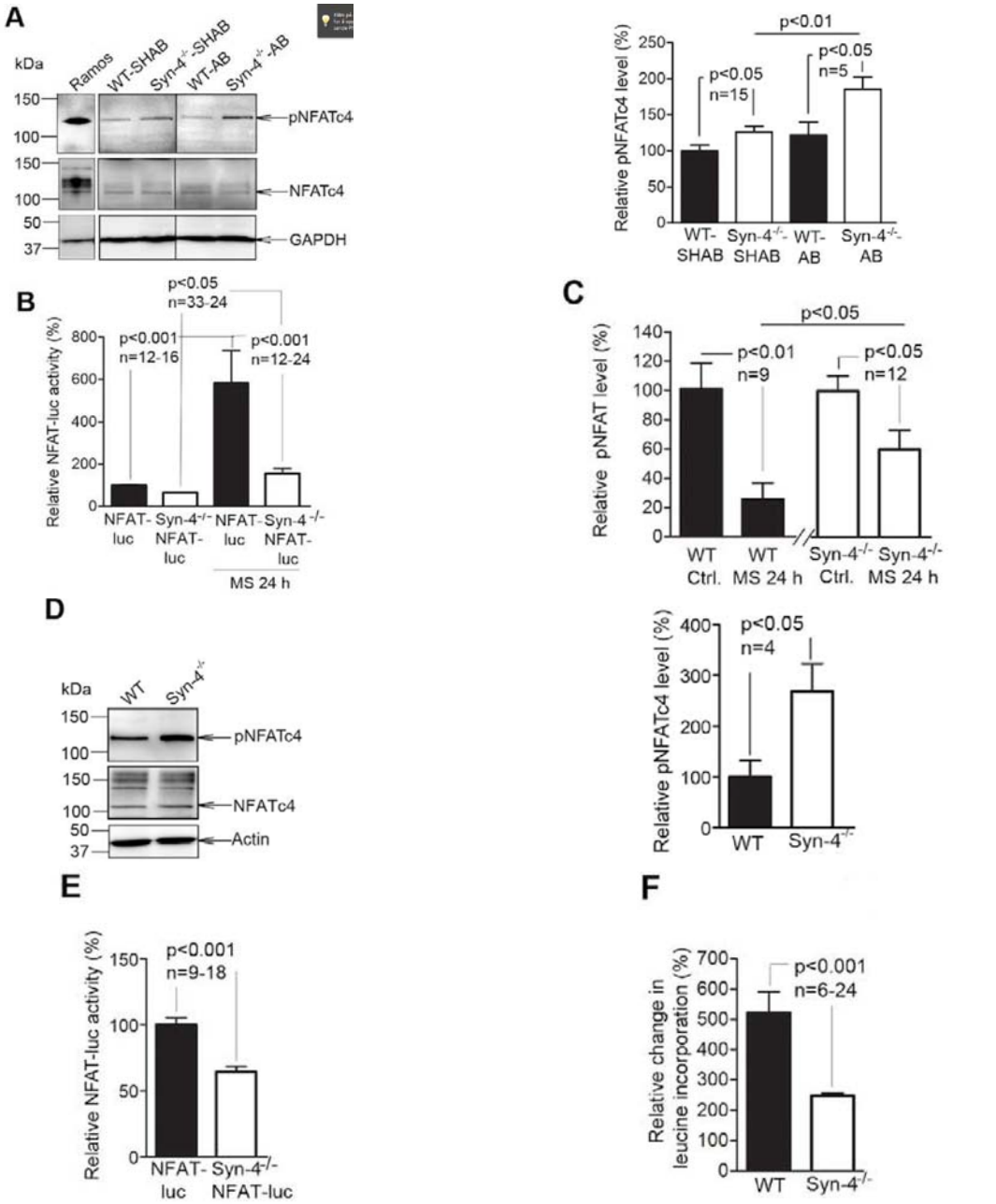

Figure 6A-F: A) In syndecan-4 $^{-/-}$ (syn-4$^{-/-}$mice as a response to aortic banding (AB), reduced activation of the calcineurin-nuclear factor of activated T-cells (NFAT) pathway was shown. The left panel; representative immunoblotting showing the level of phosphorylated NFATc4 (pNFATc4) in wild-type (WT) sham-operated (SHAB) mice, WT-AB mice, syn-4$^{-/-}$ SHAB mice, and syn-4$^{-/-}$AB mice. Ramos= NFAT rich control used for NFAT-positive protein bands. The right figure shows the relative level of pNFATc4. Notice the high level of pNFATc4 in syn-4$^{-/-}$ AB mice compared to the other groups, illustrating that less NFATc4 was activated in syn-4$^{-/-}$ AB mice. B) Relative NFAT-luciferase activity in neonatal cardiomyocytes after 24hours of mechanical stress (MS), in cells isolated from NFAT-luciferase and syn-4$^{-/-}$ NFAT- luciferase mice. C) The relative level of pNFAT in WT- and syn-4$^{-/-}$ cardiomyocytes that have been exposed to MS, compared to controls. Notice the higher level in syn-4$^{-/-}$-MS compared to WT-MS. D) The relative pNFATc4 level shown as a representative immunoblot, was significantly higher in syn-4$^{-/-}$ neonatal cardiomyocytes compared to WT neonatal cardiomyocytes, following autonomous growth E) Relative luciferase activity in neonatal cardiomyocytes from NFAT-luciferase mice and syn-4$^{-/-}$-NFAT-luciferase mice, exposed to autonomous hypertrophy. F) Relative leucine incorporation in WT- and syn-4$^{-/-}$ mice, followed by autonomous hypertrophy for five days. This was assessed to quantify protein synthesis in WT- and syn-4$^{-/-}$ cardiomyocytes. Figures and text are taken from reference (1).

Discussion

This thesis is a literature-based study on the role the heparan sulphate proteoglycan syndecan-4 (fig.7) in heart failure development. In the study that this thesis is based on (1), it was shown that after being exposed to pressure overload, syn-$4^{-/-}$ mice did not develop concentric LV hypertrophy as WT mice. However, syn-$4^{-/-}$ mice exhibited a reduced cardiac function, shown as decreased LV fractional shortening and a prematurely dilated LV. In accordance with the hypertrophy, WT mice had increased cardiomyocyte width (25%), while syn-$4^{-/-}$ mice showed no change in cell width, demonstrating the lack of cardiomyocyte hypertrophy in mice without syndecan-4, compared to mice with normal expression of syndecan-4. Reduced activation of NFAT in syn-$4^{-/-}$ mice after they had been subjected to pressure overload was central in explaining the reduced hypertrophy of syn-$4^{-/-}$ mice. Syn-$4^{-/-}$ mice demonstrated increased levels of pNFATc4, and thus reduced activation of the transcription factor NFATc4 which has been shown to be sufficient for cardiac hypertrophy. In comparison, WT mice showed increased activation of NFATc4 and reduced levels of pNFATc4. Other main findings were that in isolated WT cardiomyocytes subjected to cyclic mechanical stretch, there was significant calcineurin-dependent NFAT activation after 24hours. However, in isolated cardiomyocytes lacking syndecan-4, there was only a minimal activation of this important transcription factor. Correlating with the in vivo studies in mice, pNFATc4 levels were higher in syn-$4^{-/-}$ cardiomyocytes than in cardiomyocytes with normal syndecan-4 expression, reflecting that more NFATc4 was activated in cardiomyocytes with normal syndecan-4 expression. Conclusively, the results indicate that syndecan-4 indeed has a role as a mechanotransducer for activation of calcineurin-NFAT signaling in hearts exposed to pressure overload.

These findings indicate that there is a link between syndecan-4 and the calcineurin-NFAT pathway. There are a few other studies that suggest a relationship between syndecan-4 and myocardial disease. For instance, one study found that serum syndecan-4 concentration was increased in patients suffering from heart failure compared to healthy people, and thereby concluded that, in the failing heart, syndecan-4 may be a biomarker of LV remodeling (26). In another study, Echtermeyer et al. (22) investigated the effects of syndecan-4 insufficiency in mouse models with MI and ischemia. They concluded that prevention of syndecan-4 signaling enhanced the LV remodeling process and thereby LV function. In addition, the results demonstrated that activation of NFAT signaling was amplified in syn-$4^{-/-}$ mice, and that syndecan-4 signaling controlled the activity of NFAT during the process of myocardial remodeling (27). However, Matsui et al. showed the opposite; after MI the syn-$4^{-/-}$ mice showed a worsening of cardiac function and an increased mortality rate. In essence, their results suggested that syndecan-4 was preventing cardiac rupture and dysfunction after MI (28). Xie et al. examined syndecan-4 overexpression by inserting adenovirus containing the syndecan-4 gene in rats direct after inducing MI. They wanted to find out if prolonged over-expression of syndecan-4 could prevent cardiac remodeling. Overall, they concluded that cardiac remodeling was prevented. These data are in line with the findings of Matsui et al., and indicate that syndecan-4 was enhancing cardiac function and remodeling (29). All of the three above listed studies were performed in mice/rats with MI, leading to cardiac remodeling and hypertrophy. Only Echtermeyer et al. examined the link between syndecan-4 and NFAT signaling. Thus, no information was available in the literature elucidating the connection between syndecan-4 and concentric hypertrophy as a result of pressure overload.

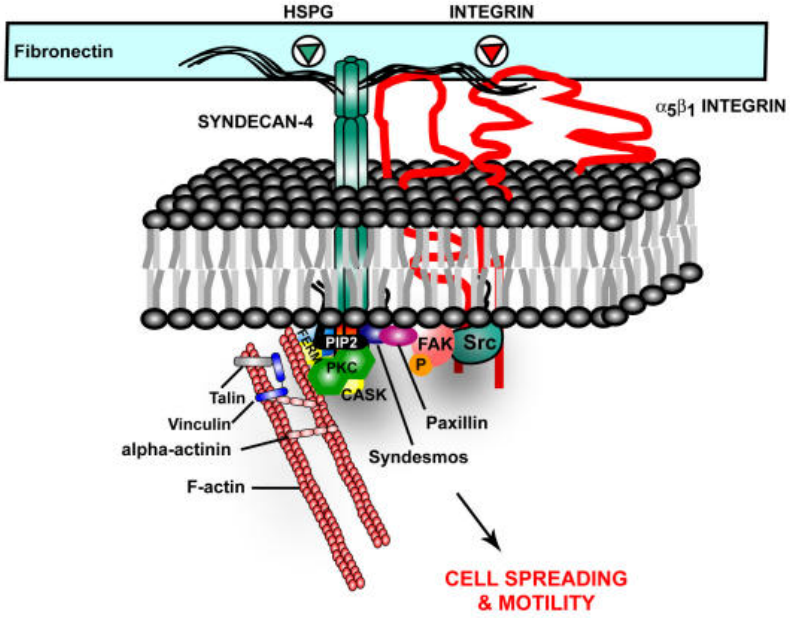

Fig. 7: Picture of the transmembrane proteoglycan syndecan-4 showing its interaction with integrin and other molecules. The illustration is taken from reference (30).

It is well established that cardiac hypertrophy can be initiated by NFAT dephosphorylation by calcineurin, and that by inhibiting calcineurin pharmacologically prevention of hypertrophy in vivo and in vitro may be achieved (31). For instance, one study showed that the calcineurin/NFAT pathway was activated early in the hypertrophic human myocardium, and that there was a shift from the phosphorylated NFAT, which is located in the cytoplasm, to the dephosphorylated NFAT in the nucleus (32). Thus, given that the calcineurin/NFAT pathway is as important for development of hypertrophy and heart failure as currently believed in the international research community, combined with the results present in the main paper that this thesis is based on, syndecan-4 might have a previously unrecognized role in sensing and transmitting upstream signals for this pathway. But before jumping to a hasty conclusion, recall the results regarding syndecan-4 in the paper from Cardiovascular Research (2011): “Its role for cardiac function and disease needs yet to be investigated” (27).

Z-disc proteins

Previous data has shown that syndecan-4 is a component of the cardiomyocyte Z-disc (fig.8). The Z-disc is believed to be involved in mechanotransduction, which is the sensing and perceiving of increased pressure overload, leading to a transcriptional response favoring pro-hypertrophic genes. In addition to syndecan-4, several of the Z-disc proteins have been suggested to have a critical role in the mechanotransduction process within the cardiomyocyte. Some of these proteins are involved in protein-protein interactions, and some act as transcriptional modulators having the capacity to translocate to the nucleus, or they are localized in the nucleus, as well as in the Z-disc. One example is a group of proteins called LIM proteins. Even though the Z-disc proteins are believed to have a great diversity, proteins with some specific molecular domains are more frequently represented. The LIM domains are rich in the amino acid cysteine, and they are viewed as sensors of pressure overload and

transmitters of downstream signals, creating alterations in gene expression. Examples of such proteins with LIM domains are; muscle LIM protein (MLP) (fig.8) (19) and actinin-binding LIM proteins (ALP) (15).

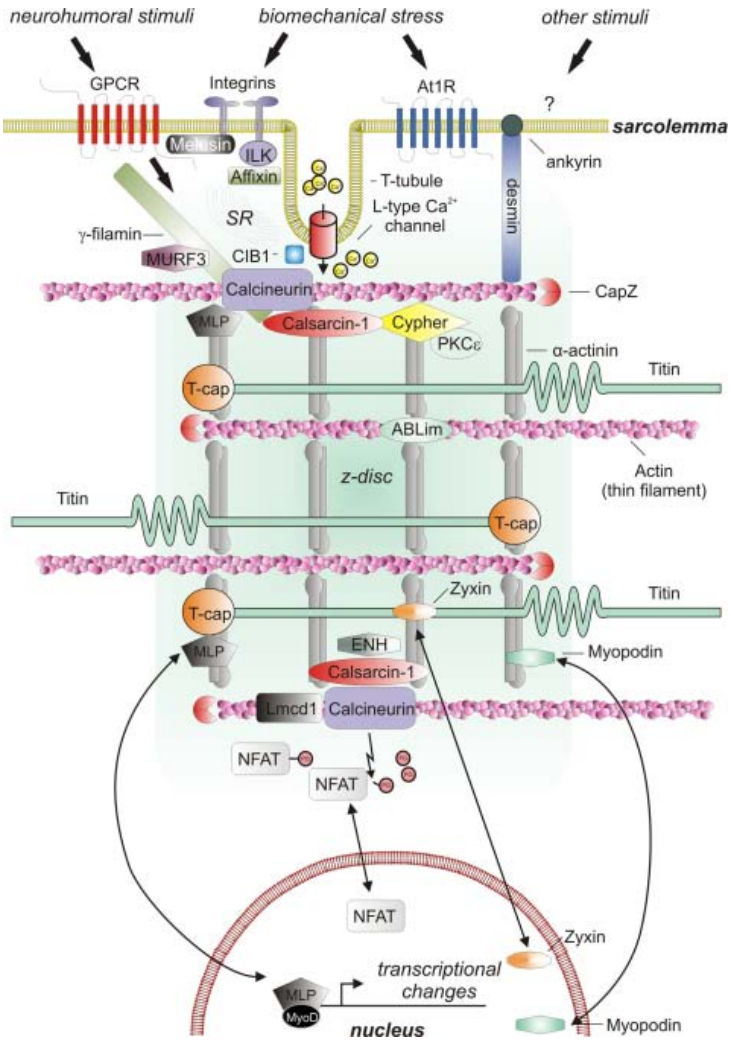

Fig. 8: The complex structure of the sarcomeric Z-disc with some of its associated proteins, showing also pro-hypertrophic stimuli. The illustration is taken from reference (19).

Muscle LIM protein (MLP) is located in the Z-disc as well as in the nucleus (fig.8). MLP is thought to interact with several other Z-disc proteins such as α-actinin, calcineurin and telethonin (TCAP) (fig.8). It is also said to interact with proteins in the nucleus such as myoD (fig.8), myogenin and MRF4, as well as proteins located to the costameres, e.g. integrin linked kinase (ILK) (fig.8), zyxin and β1 spectrin. The costameres create a link between the sarcomeres and the sarcolemma, and have an interesting position regarding signal reception and transmission. Importantly, MLP is involved in a number of signaling pathways, including mechanotransduction (15), and the protein is said to be up-regulated in many cardiac hypertrophy and remodeling models (19). Furthermore, humans with mutations in the MLP-gene develop either dilated (DCM), or hypertrophic cardiomyopathy (HCM), and MLP-deficient mice develop heart failure although a rare type. Finally, yet another interesting feature of MLP is its close interaction with calcineurin (fig.8). MLP can bind calcineurin directly, and may thereby interfere with calcineurin`s activity (19).

Near the Z-disc, at costameres, a muscle-specific protein called melusin is located (15) (fig.8). Melusin attaches to integrins, a type of receptors important in signal transduction (15;33). The integrins, spanning the plasma membrane, transduces information from the cell`s outer to the interior, and in addition they inform the cell exterior of the inside condition. They are heterodimers and consist of α- and β-subunits (33). Melusin binds to the intracellular β-subunit (β1) (15) (fig 8). A genetic deletion of the melusin gene in mice demonstrated a healthy phenotype under normal conditions, but when inducing pressure overload by aortic banding, the mice suffered from dilated cardiomyopathy as well as contractile dysfunction (19). Also humans have been investigated, and in patients suffering from heart failure three different melusin mutations were identified, but translating genotype to phenotype was difficult (15). Because of melusin`s association with integrins, this Z-disc protein could possibly have a specific role in the process of mechanotransduction (19). It may be potentially important for development of new therapeutic strategies (15).

ILK is another protein coupled to integrins (15) (fig.8). ILK binds to β1-integrin and connects extracellular matrix interactions of integrins, to intracellular processes, e.g. cytoskeleton protein remodeling and growth (19). In addition to being an integrin-binding protein, ILK is localized to the Z-disc and at costameres. ILK consists of a triple complex called PINCH and parvin (IPP), and it is this complex that interacts with the β1-integrin cytoplasmic domain and connects them to α-actinin. ILK is attached to α-actinin via IPP (15;19). In the Z-disc ILK interacts with several proteins, as for instance MLP. In zebrafish an ILK-nonsense mutation has been found underlying a rare condition; cells lost their attachment with surrounding cells. This phenomenon affected cardiomyocytes and endothelial cells in particular (15). Moreover, $ILK^{-/-}$ mice developed DCM and underwent sudden cardiac death. During a screening of the ILK gene in zebrafish, a mutation was identified leading to a progressive weakening of cardiac contractility. Thus, it has been proposed that the presence of this protein is essential for having a functional heart, and importantly, that ILK may be involved in sensing mechanical stress. Moreover, overexpression of ILK leads to cardiac hypertrophy, and thus, ILK is proposed to act as a signaling activator in hypertrophic hearts (19).

At the periphery of the Z-disc, a small protein named telethonin (TCAP; titin cap) is positioned (fig.8). With its special β-sheet structure, it binds in antiparallel to titin (another protein in the Z-disc) (fig. 8), and makes sure that the N-termini of two neighboring titin molecules are held together. Actually, the telethonin-titin interaction represents the strongest protein-protein interaction we know of today. Because of this interaction, the function of telethonin is looked upon with great interest in relation to mechanical stress. Telethonin is said to possibly be part of the regulation of G-protein-coupled receptor (GPCR) signaling, because of its ability to be phosphorylated by protein kinase D (PKD). Moreover, telethonin has been suggested to be important during myofibril growth, because the protein is a substrate of titin kinase in vitro.

Overall, there seems to be several interesting interactions between telethonin and other proteins in the cardiomyocyte, but most of them still need to be elucidated. For instance, its interaction with a protein named minK (the β-subunit of a potassium channel) connects it to the t-tubular system, which is an interaction of interest. Unfortunately the exact interaction between telethonin and the t-tubular system is not fully discovered. Importantly, telethonin is through its interaction with calsarcin-1 (FATZ2, myozenin2) (fig.8), believed to regulate myocardial hypertrophy (calsarcin-1 is another Z-disc protein that interacts with calcineurin). In addition, telethonin interacts with a pro-hypertrophic regulator (BMP100). Another important interaction is its regulation of myostatin secretion; a protein that negatively regulates muscle growth. Thus, telethonin is involved in both inhibiting and promoting factors

of hypertrophy. Unfortunately, at this point, final conclusions cannot be drawn, and this theory needs more investigation.

Patients with a mutation in the telethonin gene suffer from a variety of diseases, such as limb-grindle muscular dystrophy type 2G, intestinal pseudo obstruction, in addition to DCM and HCM. In a mouse model, deficiency in telethonin did not lead to heart failure without inducing mechanical stress. A theory that may explain some of the illnesses coupled to telethonin mutations is based on the effect of telethonin on the pro-apoptotic protein p53, and that during mechanical stress, the amount of telethonin is decreasing while p53 is increasing, and more cells die by apoptosis (15).

Desmin, the most abundant type of intermediate filament in cardiomyocytes (19) (fig.8), surrounds each Z-disc and links the Z-disc to the core membrane, to each other, to the costameres and to the cardiomyocyte cell membrane, the sarcolemma (16;19). Given its widespread distribution, it is understandable that this protein is involved in multiple protein-protein interactions, in the cellular force transmission and in mechanical signaling. Desmin$^{-/-}$ mice suffer from disorders in all three muscle types. The heart was the most severely affected, with cardiomyopathy in addition to extensive fibrosis and calcification. Furthermore, desminopathy is a form of skeletal- and cardiac myopathy that originates from mutations in the desmin gene, and today approximately 45 mutations in this gene has been identified in patients (19).

Future work

Desmin and the other described Z-disc proteins seem to have a function in development of hypertrophy and failure. Future work regarding the role of syndecan-4 and the other Z-disc proteins as key players in hypertrophy must include both experimental and clinical studies. Experimental studies are necessary to identify the underlying mechanisms and functions of Z-disc proteins in hypertrophy and heart failure.

We know that mechanical forces affect cardiomyocytes (2), but how do mechanical forces convert into extracellular- and intracellular signals leading to myocardial hypertrophy? It has been shown in mice without syndecan-4 that there is less activation of pro-hypertrophic calcineurin-NFAT signaling (1). This suggests that syndecan-4 transfers mechanical stimuli into a signal, which again is affecting the activation of calcineurin-NFAT signaling inside the cell, but what happens on the outside of the cardiomyocyte? When pressure overload is a fact, how does syndecan-4 sense this? Is there another extracellular molecule that transfers a signal to syndecan-4, and how does syndecan-4 transduce this signal over the membrane, to the cardiomyocyte interior, and further to the nucleus?

The role of syndecan-4 in myocardial hypertrophy still needs to be investigated in greater detail. What is known today is very interesting in light of future therapeutic strategies, which maybe could give heart failure patients a better prognosis. As mentioned previously, syndecan-4 consists of three major domains; the ectodomain, the transmembrane and the cytoplasmic (fig.4) (21). Maybe the making of transgenic mice, by removing one by one of the domains, could give a clearer view of syndecan-4`s role in sensing extracellular signals, and the conversion into intracellular signals to other Z-disc proteins and the calcineurin-NFAT pathway. The model used for the studies that have been completed regarding cardiac function has been a mouse model where the whole proteoglycan is knocked out in every cell in the body. More information about syndecan-4`s role in hypertrophy may be gained by knocking out both integrin β1 and syndecan-4, just syndecan-4, just integrin β1 and compare these results to WT mice. It is known that syndecan-4 works partly through integrin β1 (2), and it would be interesting to examine what role syndecan-4 would have without integrin β1, to maybe get a clearer picture of its signaling role, and its interaction with integrin β1.

Overexpression of syndecan-4 in cardiomyocytes specifically for gain of function studies is underway at IEMR. The goal is to test this model in vivo.

It is also important to investigate the role of syndecan-4 in patients, and to see how syndecan-4 is altered in various etiologies of heart failure. This can for instance be done by taking a biopsy during open-heart surgery, such as in aortic stenosis patients, when the aortic valve is replaced. A biopsy can also be taken using a catheter, or during heart transplantation, and under a LV assist device (LVAD) insertion in patients with end-stage heart failure (34). Syndecan-4 is currently investigated at IEMR in such patient biopsies.

Also the use of syndecan-4 as a diagnostic tool, a biomarker, could be a future area of investigation. Shedding of the ectodomain is a process in which the extracellular part of the transmembrane molecule is cut off (35). The article "Serum syndecan-4 is a novel biomarker for patients with chronic heart failure" (26) suggests that syndecan-4 could be used as such biomarker in patients suffering from heart failure. This makes it possible to use syndecan-4 as a diagnostic tool, a biomarker, in the future. But before being able to use syndecan-4 as a biomarker, we need to understand what alterations in its concentrations in blood reflect.

Overall, the general aim of heart failure research is to develop new therapeutic treatments, and diagnostic strategies. Maybe by modifying syndecan-4, we could alter the signal leading to hypertrophy in a way that leads to prevention of hypertrophy and heart failure. To do this, possible targets on syndecan-4 should be identified, using for instance peptide technology or small molecules. A solid foundation is built in current research concerning underlying mechanisms for heart failure, but many questions remain unanswered and among them, several questions about how to target syndecan-4 therapeutically. Moreover, as more research is done, maybe in the future, unfortunate events like the death of the young athlete described in the introduction could be avoided.

Conclusion

The objective of this thesis was to provide a detailed insight into particular molecular mechanisms important for the progression of hypertrophy and heart failure. The hypothesis is that syndecan-4 is important in sensing and transmitting the initial response to increased ventricular pressure, leading to LV hypertrophy and subsequent heart failure. In essence, the results of this thesis indicates that syndecan-4 does participate in the sensing and transmitting of pro-hypertrophic signals in the process of hypertrophy, thus making syndecan-4 a component of importance in the development of heart failure.

Throughout this thesis, the complexity of underlying mechanisms of heart failure is shown, especially related to the cardiomyocyte Z-disc. This thesis points out one protein at a time, emphasizing the reductionist ways of current basic cardiac research. Thinking of all the cardiac proteins together, it is interesting to see how much impact one protein such as syndecan-4 may have on a major health issue that heart failure is representing. Thus, it will be interesting to see where future research is leading us, and most importantly, whether today`s troubling number of heart failure patients is altered.

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