Three fingers on the brake: Kruppel-like factor 15, a repressor of cardiac gene expression
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SUMMARY

The heart is a crucial organ in the human body. It is a four chambered pump that is vital for the circulation of blood through the body. A healthy heart ensures that all organs and tissues are provided with enough oxygen and nutrients that enables them to function properly. Due to cardiac diseases or changing biological conditions, the heart is capable to adapt in such a way that cardiac output is maintained. When this situation lasts too long, the heart cannot cope with the continues stress anymore and the function of the heart deteriorates resulting in heart failure. This is a condition in which the heart fails to supply the body with sufficient blood. Many cardiac conditions can give rise to heart failure. Myocardial infarctions, left ventricular hypertrophy due to hypotension, but also genetic cardiac diseases like hypertrophic cardiomyopathy are among the most common causes leading to heart failure.

To be able to prevent the onset of heart failure and treat established heart failure it is important to obtain more knowledge on the different etiologies of the disease. Like every disease, also heart failure is the result of molecular changes that occur due to changing biological conditions. To study which molecular changes underlie heart failure or processes leading towards heart failure we compared the molecular status of the failing heart to the normal situation. This allowed us to distillate molecules and processes that differ between the two.

Not every hypertrophied heart will develop heart failure in the same way. To study whether there are genes that make a hypertrophied heart susceptible to rapid development of heart failure we performed a microarray analysis on left ventricular biopsies taken from hypertrophied left ventricles of rats. These animals were then followed in time to evaluate to progression towards heart failure (Chapter 1 and 3). Among the many genes that were differentially expressed between the failing and non failing group, two were studied in greater depth: lysosomal integral membrane protein 2 (LIMP2) and kruppel-like factor 15 (KLF15).

The cardiac expression of LIMP2 is increased in the hypertrophied heart. We studied the role of LIMP2 in the heart and we found that LIMP2 is present on the intercalated disc, an important structure involved in cell-cell communication. Mice lacking LIMP2 have a normal heart but when we stressed the hearts they failed to develop hypertrophy, but they rather develop dilation. It turned out that LIMP2 binds to essential components of the intercalated disc thereby increasing the important N-cadhering/β-catenin interaction.
Gene expression is mainly regulated by transcription factors and transcriptional regulators. A tight interplay between repressors and activators of transcription closely determines the expression of a gene. Only lately the important role of repressors in cardiac hypertrophy and failure has been acknowledged. In Chapter 2 the currently known regulators of hypertrophy have been summarized and categorized in functional groups: repressors of signal transduction or repressors of transcription.

KLF15 is one of these repressors of transcription. The expression of KLF15 shows the opposite behavior of LIMP2 expression. Its expression decreases during hypertrophy, but more in rats that will rapidly develop heart failure (Chapter 1). When we studied the role of KLF15 in the healthy and diseased heart we identified transforming growth factor beta (TGFβ) as one of the most powerful inhibitors of KLF15 expression. TGFβ is a cytokine (signaling protein) that is released into the circulation during hypertrophy. When it binds to transforming growth factor beta receptors that are present on the surface of cardiac muscle cells and fibroblasts, molecular pathways that govern cardiac growth and fibrosis are activated. One of these pathways is the MAPK pathway leading to phosphorylation of the hypertrophic protein p38. We found that activation of this pathway leads to a decreased expression of KLF15 (Chapter 4).

But what are the functional consequences of this loss of KLF15? To answer this we looked at the effect of KLF15 on proteins that are known to promote cardiac growth. We found that KLF15 has a strong repressive effect on the cardiac transcriptional regulator myocardin (Chapter 4). Myocardin promotes cardiac growth by binding to serum response factor (SRF). By doing this, genes that contain srf-binding elements (CArG boxes) in their regulatory region will be activated and transcribed. Our studies show that in the healthy heart, when ample KLF15 is available, KLF15 binds myocardin in the same region that is needed for association with SRF. The competition between KLF15 and SRF results in a decreased activity of SRF/myocardin regulated genes and thus results in reduced cardiac growth. In addition to this we also discovered that KLF15 also represses the pro-hypertrophic factors myocardin related transcription factor A and B (MRTF-A and MRTF-B) and the transcriptional regulator GATA4 (Chapter 5). KLF15 binds to the DNA binding part of GATA4, thereby hindering its interaction with GATA elements in the enhancers of many cardiac genes like BNP. Surprisingly, GATA4 activity can also be enhanced by myocardin, which makes the repression by KLF15 dual. GATA4 responsive reporters can be activated by GATA4 and enhanced by myocardin. When KLF15 is present, both the myocardin-GATA and the GATA-DNA interaction are blunted because of an interaction with KLF15 (Chapter 5).

Given the important role of KLF15 in cardiac biology and hypertrophy we wondered

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whether KLF15 could also play a role in hereditary forms or hypertrophy. Hypertrophic cardiomyopathy (HCM) is a common heritable cardiac disease with a prevalence of 1 in 500. HCM is commonly caused by mutations in genes that encode the contractile proteins of the heart such as MHC and MyBPC3. We hypothesized that mutations in transcriptional regulators could also underlie or modify HCM. For the first time we identified mutation in the coding region of KLF15 in patients with HCM. A total of six new mutations were identified that are not present in healthy controls. Functional studies showed a small but significant effect of these mutations on GATA4 function that could suggest that KLF15 might play a role in the etiology of HCM (Chapter 6). Further studies (in larger families) should be performed to see whether there is a causal relation between KLF15 mutations and the development of HCM.

Despite years of elaborative and expensive research, heart failure is still a non-curable disease. We speculated that if we could prevent the loss of KLF15 as is seen during hypertrophy, we could actually prevent the de onset of hypertrophy. To study this we made use of a adeno-associated virus (AAV) for overexpression of KLF15 in the cardiomyocytes of mice. We then induced hypertrophy by using the pro-hypertrophic hormone Angiotensin-II. It turned out that when the loss of KLF15 was prevented, the mice developed less hypertrophy. This implies that KLF15 might be used as a therapeutic target for the treatment of hypertrophy and thereby the prevention of heart failure (Chapter 7).

Taken together, this thesis shows the role of the transcriptional repressor KLF15 in the heart. We show how the expression of KLF15 is regulated, what the mechanism is behind KLF15 mediated cardiac hypertrophy. We showed that genetic variations are present in patients with a hereditary form of hypertrophy. Finally we show that KLF15 might play a role in preventing hypertrophy and heart failure.