Chapter 7

Summary and Perspectives
Summary

Heart failure (HF) is a syndrome characterized by high mortality, frequent hospitalization, poor quality of life and multiple co-morbidities attributable largely to cardiac structural changes with associated cardiac dysfunction [1, 2]. Pathological changes of the myocardium is the primary cause of cardiac remodeling, which includes structural changes such as myocyte loss, apoptosis, hypertrophy and myofilament disorganization, as well as disturbance of extracellular matrix homeostasis, collagen synthesis and deposition [3-14]. Such changes therefore lead to cardiac dysfunction.

Recent pharmacological therapy for HF focuses on angiotensin converting enzyme inhibitors and beta blockers which provide stability and even reverse adverse cardiac remodeling [15-19]. However, HF appears to be not only the result of myocardial injury or hemodynamic overload as commonly perceived, it is also the result of an interaction among genetics, neurohormonal, inflammatory and biochemical factors – some of the latter being referred to as biomarkers. Some biomarkers are not only indicative of an increased risk, but also serve as potential targets for therapeutic intervention. One such novel biomarker is galectin-3 (Gal-3) [20]. Macrophage derived Gal-3 is associated with activated myofibroblasts and increased synthesis and deposition of collagen. The role of Gal-3 in cardiac remodeling has been intensively discussed in recent years [21-26], whereas little attention has been given to genetically- and pharmacologically-targeted therapy for Gal-3 in cardiac remodeling and renal dysfunction.

In this thesis we have employed several models of cardiac remodeling and chronic heart failure to investigate the role of Gal-3 under pathophysiological conditions. Chapter 1 discusses the characteristics of Gal-3 and its therapeutic implication. In chapter 2 and 3, we provided an overview of Gal-3 in cardiac remodeling and heart failure, aiming to systemically describe the chemical characteristics of Gal-3 and its biological role including expression levels of Gal-3 in physiologically normal and pathologically-altered tissue specimens. Furthermore, we also enumerate the potential utility of Gal-3 in experimental models of cardiac remodeling and clinical HF patients. Finally, we discussed the role of Gal-3 in modulating the fibrosis pathway and further point out Gal-3 as “culprit biomarker” that involved in the pathophysiology circle of cardiac remodeling and HF. In chapter 4 we evaluated whether Gal-3 is a relevant therapeutic target in cardiac remodeling and HF. In this study we firstly employed Gal-3 knock out (Gal3-KO) and wild-type (WT) mice which were subjected to angiotensin II or transverse aortic constriction (TAC) to provoke cardiac remodeling. Secondly, the Gal-3 inhibitor, N-acetyllactosamine (Gal3i), was administered to hypertensive TGR (mREN2)27 (REN2) rats and WT mice with TAC. The homozygous REN2 rat model is a well-defined model for progressive cardiac remodeling. We demonstrated that the inhibition of Gal-3 by genetic disruption or pharmacological intervention halted the progression of cardiac remodeling, attenuated myocardial fibrogenesis and preserved LV function. These beneficial effects can be explained, at least in part, by the lower number of myofibroblasts in combination with diminished collagen synthesis, processing and cross-
linking. Finally, to elucidate the beneficial effects of Gal-3 inhibition on myocardial fibrogenesis, cultured fibroblasts were treated with Gal-3 in the absence or presence of Gal3i. We found that inhibition of Gal-3 was associated with a down-regulation of collagen production (collagen I and III), collagen processing, cleavage, cross-linking and deposition. Collectively, we confirm that genetic disruption and pharmacological inhibition of Gal-3 attenuates cardiac fibrosis, LV dysfunction and subsequent HF development, suggesting that the drugs binding to Gal-3 may be potential therapeutic candidates for the prevention or reversal of HF with extensive fibrosis.

Renal dysfunction is frequently observed in cardiovascular disease [27, 28], and is one of the strongest predictors in heart failure prognosis, playing an important role in the pathophysiological process [29]. We therefore performed a subsequent study in the well-established REN2 rat model with hypertensive end-organ damage (chapter 5), and showed that targeted inhibition of Gal-3 attenuates progressive hypertensive nephropathy. Over a 6-week time course, the untreated REN2 rats developed substantial proteinuria, a typical marker of glomerulosclerosis. We observed that treatment with Gal3i completely prevented the development of proteinuria and the associated histological markers of kidney damage. Interestingly, this pathological conversion was apparently without effects on blood pressure in REN2 rats treated with the Gal3i. We concluded that Gal-3 exerts its protective effects probably by directly acting on the renal glomeruli, parenchyma and tubuli. This new finding offers a novel insight to new therapies for HF associated with chronic kidney disease (CKD). Taken together, we reveal that drugs binding to Gal-3 may be potential therapeutic candidates in cardiac remodeling or the related renal dysfunction.

To further investigate the important role of Gal-3 in chronic heart failure (CHF), we examined the correlation of plasma Gal-3 levels with cardiopulmonary aerobic capacity and cardiac function in patients with CHF (chapter 6). In this clinical cohort, we measured plasma levels of Gal-3 in 99 patients with stable CHF with NYHA class II-IV. All patients had left ventricular ejection fraction (LVEF) ≤0.45 and ability capacity to undergo cardiopulmonary exercise testing. Analysis of the results showed that high Gal-3 levels were associated with poor renal functional: creatinine (Creat), blood urea nitrogen (BUN), estimated glomerular filtration rate (eGFR), high levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) and lower peak VO$_2$. Additionally, linear regression analysis predicted significant correlations between the plasma Gal-3 level and aerobic capacity (VO$_2$ max) and renal function (eGFR). However, Gal-3 did not further predict the peak oxygen uptake (VO$_2$ max) and renal function after adjustment for age and gender. Moreover, no significant relations were observed between the plasma Gal-3 and diastolic function (E/A, and E/E') or systolic function (Simpson LVEF%). Finally, we demonstrated that high plasma Gal-3 levels were associated with poor renal function and lower aerobic capacity in patients with CHF. From all these experimental and clinical studies, we draw the conclusion that macrophage-derived Gal-3 is associated with myofibroblast-induced collagen synthesis and deposition, a process involved in the pathophysiological cardiac remodeling and heart failure.
Future perspectives

Gal-3 as a multifunctional biomarker participates in fibrogenesis and inflammatory responses [21-26][30]. In our present studies, we further confirmed that the increased expression of Gal-3 enhanced myofibroblast activation with subsequent increases in collagen synthesis and deposition in cardiac remodeling and progressive heart failure, which were in turn attenuated by gal3i treatment. However, progressive cardiac remodeling is a complex process which is not only the result of the generation of adverse fibrosis, but also because of chronic inflammatory responses. Therefore, during the cardiac remodeling process, the interaction between fibrosis and inflammation cannot be ignored. In the early stage of the inflammatory response, resident macrophages induce neutrophil activation and peripheral monocytes or macrophages are attracted to the injury area which then activates the release of macrophage-derived Gal-3 into the extracellular matrix compartment. The released Gal-3 then takes part in the inflammatory response or fibrogenesis process [31-36]. In our in vitro experiment on human dermal fibroblasts, the mechanistic effect of Gal-3 in fibrogenesis has been clearly defined herein, however, the underlining mechanism of Gal-3 in the inflammatory response in cardiac remodeling should be addressed in future studies.

Additionally, myocyte hypertrophy, apoptosis or necrosis is one of the main causes leading to cardiac remodeling. As myocytes are stretched and become hypertrophied, there is an increase in the local production or release of angiotensin II and endothelin. Subsequently, increased angiotensin II and cytokines stimulate collagen synthesis, leading to fibrosis and remodeling of the extracellular matrix [37-40]. From our group, analysis of gene array data of neonatal cardiomyocyte hypertrophy shows that Gal-3 expression is up-regulated approximately 2-fold [41]. The above evidence proves that Gal-3 is most likely also involved in cardiomyocyte hypertrophy in the cardiac remodeling process. Nevertheless, the regulating pathway and mechanism linking hypertrophic cardiomyocytes and Gal-3 is still unclear, providing a challenge for future study.

Thirdly, previous experimental and clinical studies demonstrated that (ACEi) alleviated cardiovascular disease under different pathological conditions such as hypertension, acute myocardial infarction, chronic systolic heart failure, stroke and diabetic renal disease. However, ACEi is also accompanied with more side-effects, in particular hypotension, hyperkalaemia and renal impairment [30, 42-49]. In our present study, chapter 4 and 5 shows that pharmacological inhibition of Gal-3 not only attenuated cardiac fibrosis, LV dysfunction and subsequent HF development, but also attenuated progressed hypertensive nephropathy in an established REN2 rat model. We observed that the treatment with Gal-3 inhibitor prevented the development of cardiac remodeling, proteinuria and the associated fibrosis markers. Interestingly, this pathological conversion occurred without apparent effects on blood pressure in the REN2 rat treated with the Gal3i. Therefore, we conclude that ACEi and Gal3i combination should be considered as a viable therapeutic strategy in severe chronic heart failure that is accompanied with impaired renal function.
Interestingly, it has been recognized that Gal-3 plays a key role in many pathological processes via carbohydrate-dependent (and some independent) mechanisms [50-52] that are amenable to therapy. The intriguing findings related to galectin-3 biology encourage researchers to utilize a number of ways to block or inhibit galectin-3 and its signal transduction. The ongoing research includes galectin-3 knock-out mice (mice deficient for the gene that encodes galectin-3), the use of small interfering RNA (siRNA) that silence galectin-3 gene activity, and the use of certain complex carbohydrate molecules, e.g. pectins. The studies with carbohydrate molecules takes advantage of the fact that galectin-3 has a built-in carbohydrate “switch” that can activate and de-activate galectin-3’s activity. These carbohydrates, such as lactose (Lac), N-acetyllactosamine (N-lac) and modified citrus pectins (MCP) are complex oligosaccharides that are present in a variety of foods. The present thesis has investigated the therapeutic efficiency of N-lac in pathophysiological cardiac remodeling and heart failure. However, when considering economic and health benefits, the advantage of pectins is that it is easily and commercially available for anti-galectin-3 therapy. Pectins are the complex large carbohydrate molecules and a natural source of galectin-3 inhibition. Their structure consists of an important part of cell walls and material that can be found in between plant cells, providing structure to fruits and vegetables. Furthermore, recent research found that the effect of MCP on kidney fibrosis [53]. Moreover, our unpublished data involving a galectin-3 chemotaxis assay also shows that recombinant galectin-3 significantly induces monocyte migration, which could be markedly attenuated after treatment with MCP. Therefore, we consider pectins are viable low-cost and natural source of galectin-3 inhibition, and should be tested in experimental cardiac remodeling and heart failure models in future studies.
References


