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REVIEW

Growth differentiation factor 15 in adverse cardiac remodelling: from biomarker to causal player

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Abstract

Heart failure is a growing health issue as a negative consequence of improved survival upon myocardial infarction, unhealthy lifestyle, and the ageing of our population. The large and complex pathology underlying heart failure makes diagnosis and especially treatment very difficult. There is an urgent demand for discriminative biomarkers to aid disease management of heart failure. Studying cellular pathways and pathophysiological mechanisms contributing to disease initiation and progression is crucial for understanding the disease process and will aid to identification of novel biomarkers and potential therapeutic targets. Growth differentiation factor 15 (GDF15) is a proven valuable biomarker for different pathologies, including cancer, type 2 diabetes, and cardiovascular diseases. Although the prognostic value of GDF15 in heart failure is robust, the biological function of GDF15 in adverse cardiac remodelling is not fully understood. GDF15 is a distant member of the transforming growth factor-β family and involved in various biological processes including inflammation, cell cycle, and apoptosis. However, more research is suggesting a role in fibrosis, hypertrophy, and endothelial dysfunction. As GDF15 is a pleiotropic protein, elucidating the exact role of GDF15 in complex disease processes has proven to be a challenge. In this review, we provide an overview of the role GDF15 plays in various intracellular and extracellular processes underlying heart failure, and we touch upon crucial points that need consideration before GDF15 can be integrated as a biomarker in standard care or when considering GDF15 for therapeutic intervention.

Keywords Adverse cardiac remodelling; GDF-15; Biomarker; Fibrosis; Hypertrophy

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Introduction

The mortality rates related to cardiovascular disease (CVD) have increased worldwide; since 2015, one in three deaths worldwide is a consequence of a CVD.[1] Heart failure is a growing health issue as a negative consequence of improved survival upon myocardial infarction (MI), unhealthy lifestyle, and the ageing of our population.[2] Therefore, the European Society of Cardiology recently updated their criteria defining heart failure by including extra-cardiac organ co-morbidities like diabetes, hypertension, and kidney dysfunction.[3,4] These new criteria show the complexity of heart failure throughout the patient population.

Heart failure cannot be classified as a single disease; multiple underlying causes, including hypertension, vascular calcification, or MI, show that heart failure better fits the description of a syndrome rather than a disease.[5,6] Furthermore, apart from underlying cardiac pathologies, extra-cardiac pathologies such as cardiorenal syndrome and anaemia contribute to the development of heart failure.[7,8] Disease progression is further accelerated by ageing, diabetes, and hypertension as they cause endothelial dysfunction, left ventricular hypertrophy, and vascular disease.[9–11]

A key process underlying heart failure is cardiac remodelling as response to injury, like inflammation, volume, and pressure overload. In response to injury, the heart compensates for the loss of cardiac output by remodelling of the myocardium. Cardiac remodelling is characterized by molecular, cellular, and structural changes that manifest in morphological changes of heart size, shape, and function.[12,13] The

mechanisms underlying cardiac remodelling are not fully understood, as they vary from apoptosis, oxidative stress, and inflammation to changes in energy metabolism and contractile proteins.[5,12,13] Severe remodelling of cardiac tissue associates with progressive worsening of cardiac function eventually increasing mortality risk in patients, this highlights the need for assessment of cardiac remodelling to monitor disease and therapy adjustment where needed.

The demand for biomarkers that improve disease management of heart failure patients is increasing.[14] Although no curative therapy for heart failure is available, co-morbidities influence disease progression and contribute to worsening of cardiac function. Proper biomarkers would allow to routinely assess disease progression and, in case of heart failure, which includes many co-morbidities, inform on their presence to combine this information and maintain optimal treatment for the patient.

Elevated protein expression of circulating growth differentiation factor 15 (GDF15) is correlated to many pathological conditions, mainly being different types of cancer and also metabolic diseases such as obesity and diabetes.[15–18] GDF15 is easily detectable in the blood; however, concentrations vary with age and gender.[15,19–21] For instance, we have shown that circulating levels of GDF15 can serve as strong independent predictor for cardiovascular events in women but not in men.[22] Although GDF15 has a sex-dependent prognostic value in heart failure patients,[23] the prognostic value of GDF15 is not standardly analysed for men and women separately to increase accuracy.[24–26] Elevated serum levels of GDF15 were also associated with enhanced CVD development, progression, and mortality in both disease and general population.[17,27–31] In line, experimental murine ischaemia/reperfusion injury models show an rapid increase in circulating and tissue GDF15 levels upon cardiac injury that remained elevated for several days.[27,32] Moreover, GDF15 has been proven to be a valuable biomarker for heart failure, apart from the existing cardiac markers such as natriuretic peptides, ST2, high-sensitivity troponin, and procalcitonin,[19,33,34] as it can serve as independent biomarker for survival and outcome.[35,36] This accounts for both heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF) and heart failure with mild reduced ejection fraction (HFmrEF), where GDF15 levels are reported to be similar.[37,38] Based on the current lack of knowledge on the function of GDF15, there is no significant evidence regarding a clinical advantage of GDF15 in diagnosis or classification of HFpEF and HFmrEF compared with HFrEF.[39–41] Nevertheless, GDF15 has been linked to the incidence, progression, and prognosis of heart failure as biomarker for acute and chronic cellular stress.[28,35] In line, a commercial assay that provided robust data of GDF15 levels in serum and plasma under routine conditions is currently developed.[21] To implement GDF15 as biomarker in standard clinical practice, we need to understand which pathophysiological processes are associated with increased levels in order to adjusted disease management accordingly.

Besides its biomarker function, GDF15 may have a causal role in heart failure, something we need to elucidate before GDF15 can become the new discovered target for therapeutic therapy. As GDF15 is an active player in many pathophysiological processes,[16,42] understanding its molecular basis, biological mechanism, and receptor activity in heart tissue could help elucidating its role in the onset and progression of heart failure. Therefore, the aim of this review is to summarize current literature regarding biomarker function and causal role of GDF15 relevant in heart function and adverse cardiac remodelling. We describe the molecular background of GDF15, followed by an overview of effects on intracellular and extracellular processes associated with pathophysiological mechanisms driving heart failure. Lastly, based on all this information, we will touch upon future perspectives and current needs in the GDF15 cardiac research field.

Growth differentiation factor 15

Growth differentiation factor 15, also termed macrophage inhibitory cytokine 1, is a divergent member of the transforming growth factor (TGF)-β family.[42,43] The TGF-β family consists of TGF-β isoforms, activins, and bone morphogenetic proteins (BMPs) and are best known for their effects on tissue homeostasis and cell proliferation and differentiation.[44] Although GDF15 belongs to this TGF-β superfamily and shares homology with BMPs, its major functions are not completely identical. GDF15 is robustly expressed by placenta and prostate tissue, while in other tissues, expression is very low.[15,17,42,45] However, under pathophysiological conditions like cellular stress and tissue injury, GDF15 can be produced and secreted by many various cell types like macrophages, vascular smooth muscle cells, endothelial cells, and cardiomyocytes[15–18] in organs such as the kidney, heart, and liver.

Growth differentiation factor 15 receptor identification

Knowing GDF15 is rapidly produced and secreted by various tissue and cells, one of the most urgent questions is to which receptor GDF15 binds and which intracellular signalling cascades are activated. It was recently established that GDF15 can bind with high affinity to the GDNF family receptor α-like (GFRAL) receptor.[46–49] GFRAL is mainly locate in the central nervous system,[50] and binding and signalling of GDF15/GFRAL axis lead to a decreased food intake and subsequent weight loss.[51,52] This discovery helped unravel a role for GDF15 on activation of certain metabolic pathways and

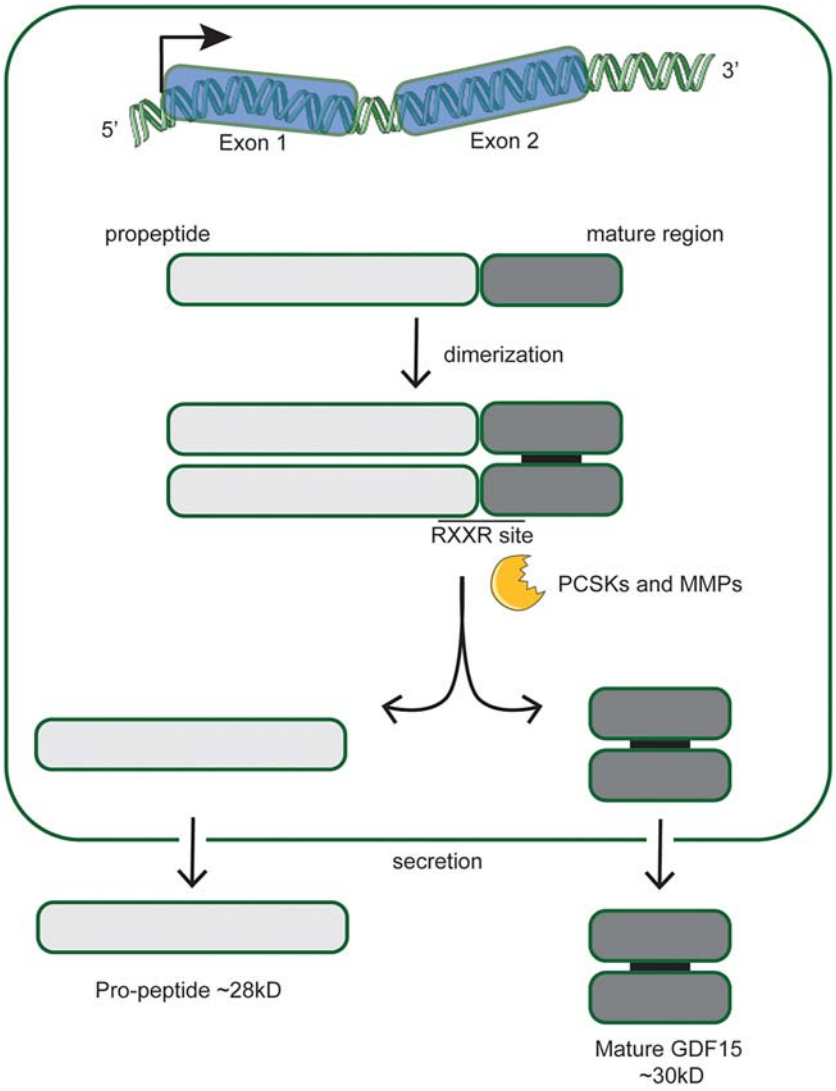
increases knowledge about possible therapeutic use of GDF15 in obesity and weight loss.[48]

To our knowledge, expression of the GFRAL receptor has only been found in the central nervous system, leaving the question to which primary receptor GDF15 binds in the periphery still open.[53] So far, studies have reported GDF15 binding to the TGF-β II receptor[54] and ALK receptors,[17,54,55] and some indicate binding to tyrosine or serine/threonine receptors.[56] The discovery of GFRAL provided important insight in the signalling capabilities of GDF15 via non TGF-β-related receptors and suggests that signalling of GDF15 beyond the TGF-β receptor family may be very important in the periphery as well. As such, exploring the possible cardiac signalling receptors of GDF15 in cardiomyocytes, fibroblasts, and endothelial cells may provide more insights in mechanistic effects and possible therapeutic targeting of GDF15 during progressive heart failure.

Regulation of growth differentiation factor 15 on the genetic level

Growth differentiation factor 15 is located on chromosome 19p12-13.1, with a length of 2.746 base pairs containing two exons separated by an intron[15,57] (*Figure 1*). Various gene polymorphisms [single nucleotide polymorphisms

Figure 1 Growth differentiation factor 15 (GDF15) transcription and maturation. Originating from two exons, GDF15 is synthesized as polypeptide consisting of a propeptide and a mature region. Between two mature regions, a homodimer is formed by a interchain disulfide bond. The propeptide plays an important role intracellular trafficking and secretion. Pro-protein convertase subtilisin/kexin types (PCSKs) and matrix metalloproteinases (MMPs) are able to cleave the pro-GDF15 polypeptide at the RXXR cleave site, thereby forming a biological active mature GDF15. After cleavage, both the propeptide and a mature GDF15 are secreted (figure adapted from Servier Medical Art, https://smart.servier.com/).



(SNPs)] are suggested to affect GDF15 expression; for example, rs888663 and rs1054564 located upstream of the GDF15 gene[58–60] are associated with CVDs.[61–63] Contradictory, no effect of SNPs increasing GDF15 transcription activity[62] in CVDs is also present.[60,64] In addition, SNPs in the miRNAs (miR) regulating GDF15 expression are suggested to be important as well; an example is miRSNP rs1054564 in the 3′ UTR of the GDF15 transcript, which causes allele-specific translational repression via has-miR-1233-3p.[65] Furthermore, miRSNP rs1054564 is associated with reduced levels of circulating GDF15 in a Taiwanese CVD population.[66] They suggest GDF15 to be a major genetic determinant of the GDF15 concentration.[66]

Production of growth differentiation factor 15

Originating from two exons, GDF15 is synthesized as a polypeptide (pre-pro-GDF15), which consists of a signal peptide, a propeptide, and a mature region[47,67] (*Figure 1*). The GDF15 polypeptide is biologically inactive and forms a homodimers through an interchain disulfide bond at the C-terminus in the endoplasmic reticulum.[68] The N-terminal side contains the signalling peptide important for secretion and intracellular trafficking.[47] Once located in the endoplasmic reticulum, the polypeptide is cleaved by the serine proteinases pro-protein convertase subtilisin/kexin types (PCSKs).[69] PCSK3, PCSK5, and PCSK6 are able to recognize and remove the signalling peptide of the GDF15 polypeptide and therefore essential in the formation of a biologically active GDF15.[70] In addition to PCSKs, GDF15 can also be processed by matrix metalloproteinase (MMP)-26[71] (*Figure 1*). The presence of GDF15 and MMP-26 in placental development suggests that MMP-26 is just as important as PCSKs in the processing and maturation of GDF15. Besides serine and MMPs, there are also cysteine proteinases,[72] all involved in extracellular proteolysis,[73] and further research should elucidate their possible contribution to GDF15 maturation. After cleavage of the pro-GDF15 domain, both a mature GDF15 protein and the remaining propeptide are secreted (*Figure 1*). Pro-GDF15 is secreted into the extracellular matrix and is stored in latent stromal extracellular matrix stores.[68] Under stress conditions, latent pro-GDF15 from the storage pools is cleaved to its active mature form. Bauskin *et al*. [68,74] found that the propeptide of pro-GDF15 is responsible for this cleaving and signalling to increase circulating serum levels of GDF15 upon demand. Whether these storage pools are present or activated in cardiac tissue during the progression of heart failure has not been clarified, but it may contribute to increased GDF15 secretion into the circulation during heart failure. As previously reported that an increase in these stromal stores of GDF15 associates with disease outcome of prostate cancer patients, it could be very relevant to investigate the presence of stores in cardiac tissue.[68] Therefore, histopathological assessment of GDF15 in cardiac tissue of heart failure patients could indicate the increased GDF15 production and storage, possibly predictive of disease severity and outcome.

Growth differentiation factor 15 as non-cardiac specific biomarker in heart failure

The heart failure population is diverse as multiple causes and co-morbidities affect disease progression and prognosis.[75] Underlying risk factors like diabetes, hypertension, and inflammatory responses predict the onset of future CVDs including heart failure.[76] This exemplifies the urgency for methods to distinguish between heart failure subpopulations based on the underlying processes aside from a functional classification.[77] Current biomarkers like natriuretic peptides and cardiac troponins are especially strong in reflecting the degree of acute cardiac injury and mostly represent systolic heart failure or HFrEF (*Table 1*). We are currently lacking biomarkers reflecting the more chronic type of cardiac remodelling, which is mostly observed in patients with heart failure of non-ischaemic origin.[78] New and promising biomarkers like soluble ST2, galectin-3, and GDF15 are currently evaluated for their contribution to diagnosis or prognosis of heart failure as they reflect underlying pathophysiological pathways related to chronic cardiac remodelling (*Table 1*). Non-cardiac-specific biomarkers have a potential use as diagnostic tool in heart failure patients as they report on the different biological processes involved in the systemic consequences or causes of heart failure.[78] In heart failure patients, GDF15 levels increased with disease severity in various tissues and cells particularly during pathological inflammatory conditions.[78] We propose that GDF15 levels represent underlying mechanisms of disease that would inform clinicians about the patients' general state of disease progression. In relation to treatment of co-morbidities to reduce disease progression, GDF15 may also provide information on treatment responsiveness. This would especially help patients with chronic heart failure or HFpEF, which are difficult to diagnose and often affected by several co-morbidities contributing to the disease. Nevertheless, we feel that all patients independent on their heart failure classification would benefit from a general marker of disease to evaluate the patients' systemic conditions.

Growth differentiation factor 15 as a causal player in adverse remodelling

Growth differentiation factor 15 can be produced by almost every cell type in the periphery under stress conditions and

Table 1 Advantages and disadvantages of current heart failure biomarkers

Biomarker	Source	Reflective of	Biomarker properties for HF	Advantage	Disadvantage	Reference
Natriuretic peptides (NT-proBNP)	Cardiomyocytes	LV systolic dysfunction and cardiac wall stress	Diagnosis of HF, prognosis of HF, and mortality	Useful in risk stratification of patients with acute HF	Less prognostic in HFpEF and stable HF Not discriminative between HFrEF and HFpEF	Berezin and de Lemos *et al.*[40,81]
Cardiac troponins (TnT and TnI)	Cardiomyocytes	Reflects myocardial injury	Diagnosis of HF, prognosis of HF, and mortality	Useful in risk assessment of outcome and disease severity in HF patients	Not discriminative between HFrEF and HFpEF	Several studies[82–84]
Soluble ST2	Enhanced cardiac strain increases production by cardiomyocytes and cardiac fibroblasts	Cardiac fibrosis, hypertrophy, and ventricular remodelling	Diagnosis of HF, prognosis of HF, and mortality	Good prognostic marker beyond risk factors Less affected by age, BMI, and eGFR	Unclear if it could be superior for HFpEF compared with HFrEF	Berezin and McCarthy and Januzzi[40,85]
Galectin-3	Produced upon inflammatory responses by inflammatory cells	Fibrosis and inflammation in development and progression of HF	Diagnosis of HF, prognosis of HF, and mortality	Combined with NT-proBNP reflecting a worse prognosis in suspected and proven HF	Not discriminative between HFrEF and HFpEF	Dong *et al*. and van Kimmenade *et al*.[86,87]
GDF15	Cardiac cells: cardiomyocytes, cardiac fibroblasts, endothelial cells, inflammatory cells, etc.	n.d.	Prognosis of HF and mortality	Independently prognostic in both HFpEF and HFrEF	Not discriminative for early HFpEF Not discriminative in HF diagnosis	Several studies [37,88,89]

BMI, body mass index; eGFR, estimated glomerular filtration rate; GDF15, growth differentiation factor 15; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LV, left ventricular; NT-proBNP, N-terminal pro-brain natriuretic peptide.

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can have an influence on numerous cell types.[79,80] Depending on the state of cells and the micro-environment present, GDF15 can have both beneficial and adverse effects on several different cellular processes.[16,49] Most pathophysiological and mechanistic effects of GDF15 are observed in cancers; however, also inflammation, hypertrophy, and fibrosis in organ dysfunction are under direct influence of GDF15.[16,49] In the succeeding text, we describe the most important cellular mechanisms that can be influenced in cardiac cells by GDF15 and are related to the onset and progression of heart failure.

Effect of growth differentiation factor 15 on cardiomyocytes

The loss of cardiomyocyte as a consequence of apoptosis and the very low proliferation rate of cardiomyocyte are highly important mechanisms in the development of heart failure. Studying interactions of GDF15 with cell cycle processes has so far gained most insights from the cancer research field. Elevation of circulating GDF15 levels has been associated with increased apoptosis and reduced cell proliferation in solid tumours.[90,91] Multiple oncogenic studies propose GDF15 to play a role in cell growth arrest and apoptosis, via either p53-dependent or p53-independent mechanisms.[92–94] In line, as mentioned earlier, Jones *et al*. [95] identified a p53-regulated miR embedded in the GDF15 intron gene able to reduce cell proliferation and desensitize cells to DNA damage-induced apoptosis in a human colorectal cancer cells line. Moreover, as GDF15 is also a downstream target of p53, early growth response 1, and Akt/GSK-3β, there is a feedback loop for the effect of GDF15 plays in cell growth arrest and apoptosis.[90,92,93,96,97] Nevertheless, knowing that GDF15 is pleotropic, opposing studies showed that an increase in GDF15 is able to induce proliferation of cervical and malignant glioma cancer cells.[98,99] Relating to cardiomyocytes, GDF15 is associated with protection against ischaemia reperfusion and angiotensin II, nitric oxide (NO), or TGF-β_1 induced apoptosis.[32,100] Even more interesting, GDF15 is associated with ERBB2 and cyclin D1 in cervical cancer cell proliferation,[98] both known factors to induce cardiomyocyte proliferation.[101–103] In line, recently, the Hippo–YAP pathway gained special attention in regard to cardiac regeneration as potential therapeutic target.[104,105] Moreover, interplay between Hippo–YAP and TGF-β pathways is known to be involved in tissue homeostasis.[106–108] This suggests that GDF15, as TGF-β family member, may affect the Hippo–YAP pathway, thereby possibly targeting cardiomyocyte proliferation. To conclude, the effect of GDF15 on proliferation and apoptosis is relevant to study in cardiomyocytes to maintain high number of viable and functional cardiomyocytes in order to maintain cardiac output.

Cardiac hypertrophy is characterized by an increase in heart size and a loss of sufficient cardiac output as cardiomyocytes enlarge as consequence of pathophysiological stimuli.[109] Elevated circulating GDF15 levels positively correlate with thickness of the posterior wall of the left ventricle, interventricular septum, and left ventricular mass.[56,110,111] Mechanistically, GDF15 is reported to have a pro-hypertrophic effect on cardiomyocytes that attenuates cardiac hypertrophy via phosphoinositide 3-kinase and extracellular signal-regulated kinase signalling pathways, thereby affecting transcription via the Smad1 pathway.[100,112,113] However, it has also been described that GDF15 can protect against hypertrophy through Smad-dependent pathways.[112] Moreover, it has been shown that GDF15 can inhibit the activation of endothelial growth factor receptor, thereby attenuating hypertrophic responses in a Smad-independent manner.[111] Furthermore, in animal models, mesenchymal stem cell treatment showed beneficial paracrine effects via induction of GDF15 secretion, thereby reducing hypertrophy and left ventricular remodelling.[114,115] Concluding, both pro-hypertrophic and anti-hypertrophic effects of GDF15 are described, suggesting a mediating role of GDF15 in cardiac hypertrophic responses dependent on the environmental circumstances. It remains unclear if Smad-dependent signalling pathways dominate other pathways in GDF15-mediated hypertrophic responses.

Effect of growth differentiation factor 15 on endothelial cells

Endothelial dysfunction is crucial mediator of impaired coronary and systemic perfusion and reduced cardiac capacity via directly negatively affecting cardiac remodelling and cardiomyocyte function.[116,117] Endothelial dysfunction in patients with chronic heart failure is associated with increased mortality.[118] Increased adhesion molecule expression, reduced anticoagulant properties, and imbalanced production of vasodilating and vasoconstriction substances all lead to endothelial dysfunction.[119] There are sufficient indications that GDF15 causes endothelial dysfunction by impairing vascular contraction and relaxation, which consequently could have a large impact on the function of the heart, by inducing not only large artery disease but also microvascular disease, which is associated with a deteriorating cardiac function.[120] Mechanistically, Mazagova *et al*. [119] showed that the vascular contractility in response to vasoconstrictor agents was repressed under presence of GDF15, suggesting that GDF15 affects the NO system in endothelial cells. Indeed, others show that increased levels of GDF15 are important for NO release in endothelial cells that will result in reduced vasodilation.[121] Furthermore, it has been shown that GDF15 can induce proliferation of endothelial cells during angiogenesis[122] and also endothelial senescence via reactive oxygen species pathway activation, implicating endothelial function loss.[123,124]

Recently, various studies have addressed the contribution of epithelial–mesenchymal transition and endothelial–mesenchymal transition (EndMT) to the inflammation and fibrosis response in tissue repair, implicated to play a role in pathological processes of heart failure.[125,126] It is well established that the TGF-β pathway plays an important role in EndMT and thereby cell migration and fibrosis as expression of respectively MMPs and collagens is up-regulated during this process. It has been shown that GDF15 inhibits TGF-β I target genes, thereby diminishing cell migration as a result of suppressed epithelial–mesenchymal transition in bone tumour epithelial cells.[127] These data support the notion that GDF15 has a potential anti-migratory effect on endothelial cells.[128] However, contradictory results are found that display EndMT progression and increased cell migration promoted by GDF15, through activation of the TGF-β pathway in a paracrine and autocrine signalling manner.[93,129,130]

Effect of growth differentiation factor 15 on fibroblasts

In cardiac pathologies, during repair and regenerative processes following upon tissue injury, an excessive amount of fibrous connective tissue is formed consisting of extracellular matrix deposition, including collagen, fibronectin, and laminin.[131,132] This myocardial fibrosis is an integral component leading to both functional impairment and arythmogenesis.[133–136] Various studies show associations between GDF15 and cardiac fibrosis, collagen turnover, and collagen depositions in respectively heart failure, MI, and atherosclerosis.[54,137,138] However, the exact source of this increased GDF15 production has not been clearly identified. Lok *et al.*[137] showed that cardiac tissue itself was not the main source of GDF15 production in cardiac fibrosis but suggest systemic oxidative stress to increase GDF15 in different cells and organs, while Kempf *et al.* [32] show that GDF15 is expressed and secreted in cardiomyocytes subjected to ischaemia/reperfusion injury, through a nitrosative stress-dependent signalling pathway. GDF15 is recently identified as a possible inhibitor of fibroblast growth via repression of TGF-β signalling and oncogenic protein N-Myc, reducing fibroblast activation and fibrosis in chronic kidney disease and pulmonary fibrosis.[139,140] These results suggest the possibility of using GDF15 as therapeutic to delay progression of fibrosis.[139] However, contrary results have also been found in gastric cancer, suggesting that GDF15 stimulates the activation and proliferation of fibroblasts and therefore playing an important role in fibrosis progression.[141] Considering the anti-fibrotic and pro-fibrotic effects of GDF15 described, using GDF15 as possible therapeutic target for cardiac fibrosis relies on further research to discover specific effects of GDF15 on cardiac-related fibrosis.

Effect of growth differentiation factor 15 on resident and infiltrating inflammatory cells

As GDF15 is a family member of TGF-β and an inflammatory cytokine secreted upon injury, it is opposed to be a mediator of tissue inflammation.[142] The balance in resident and infiltrating inflammatory cells varies depending on acute and chronic heart failure, with respectively monocytes and macrophages and later reparative monocytes and T-cell infiltration.[143,144] In acute heart failure upon MI, the necrotic area is controlled by inflammatory cells like neutrophils, monocytes, and macrophages, thereby prone to cardiac rupture.[145,146] Kempf *et al.*[147] showed an anti-inflammatory role of GDF15 after an MI, as the infarct border zone increased GDF15 expression, thereby inhibiting myeloid cell recruitment and protecting the myocardium from cardiac rupture. In chronic heart failure, for example, HFpEF, the increase in GDF15 is thought to reflect the inflammatory response as systemic low-grade inflammation is a central pathophysiological mechanism.[120] In chronic heart failure, an increasing amount of infiltrating inflammatory cells is present in cardiac tissue; the same accounts for GDF15 levels with progression of the disease. For example, macrophages express GDF15 during inflammatory responses contributing to the inflammatory activity of activated macrophages.[148] In line, a lack of GDF15 resulted in impaired macrophage migration and monocyte recruitment and a down-regulation of pro-inflammatory cytokines such as interferon-γ.[54,149] This suggests that circulating GDF15 reflects the inflammatory status of the patient, and reduction of GDF15 as therapeutic intervention may be useful to attenuate macrophage inflammation in CVD.

Discussion and future perspective

With this review, we aimed to summarize the current knowledge about GDF15 in heart failure and define the most vital questions that should be addressed in the coming years. Over the last years, GDF15 gained more and more interest in the cardiovascular field as it hold promise as a valuable biomarker. It has been shown that GDF15 has cardioprotective properties mostly through anti-apoptotic, anti-hypertrophic, anti-fibrotic, and anti-inflammatory actions. However, an increase in GDF15 concentrations has also been associated with pro-apoptotic, pro-hypertrophic, pro-fibrotic, and pro-inflammatory responses including a worse prognosis and higher mortality rates among heart failure patients. However, a causal role for GDF15 in adverse cardiac remodelling remains to be elucidated; whether GDF15 plays an adaptive or maladaptive role in heart failure patients is still poorly understood. Summarizing on the data included in this review, we propose that GDF15 may be a valuable therapeutic target

in heart failure as it is involved in several key processes in the pathobiology of heart failure.

Added value of growth differentiation factor 15 as a heart failure biomarker?

Currently, it has been well established that GDF15 level is increased during CVD development and progression and can prognosticate disease progression.[31] However, the availability of a reliable diagnostic test for routine clinical use and the complementary relevant cut-off values are lacking. With the recent development of a diagnostic GDF15 kit, the first steps towards a clinical biomarker approach are made.[21] However, it remains unclear what the specific implications are when heart failure patients have increased levels of GDF15, as we cannot connect the level to a specific pathophysiological contributor to disease progression, like cardiac fibrosis. Therefore, we need more information on the causal role of GDF15 before the specific biomarker function of GDF15 in clinical care can be established. For example, it has been reported that after left ventricular assist device implantation in patients with advanced heart failure, GDF15 levels decrease.[137,150] This indicates that the elevation of GDF15 in heart failure patients is reversible upon treatment. A pharmacological treatment with vasodilator hormone human relaxin-2 (Serelaxin) was able to lower to GDF15 levels in patients with acute heart failure.[151,152] This indicates that improving heart function by reduction of cardiac stress due to treatment consequently lead to down-regulation of GDF15 levels and insinuates GDF15 may be an interesting biomarker for treatment responsiveness. Furthermore, treatment of co-morbidities could strongly benefit the heart failure prognosis; however, determining if patients are treated optimally remains very difficult as this is poorly reflected by current biomarkers.[77] Current biomarkers, like N-terminal pro-brain natriuretic peptide, provide information on the cardiac function in HFrEF, where critical information on disease state and progression for HFpEF are lacking from current biomarkers. HFpEF patients will most likely benefit most from non-cardiac biomarkers like GDF15, specifying the general disease state, as they provide information on the systemic and chronic cardiac stress induced by multiple co-morbidities. If treatment focuses on the co-morbidities in HFpEF patients, we can use GDF15 as a marker for treatment responsiveness, as the general state of disease in HFpEF patients should improve upon therapy. To assess within the diverse heart population which patients would benefit most from GDF15 as biomarker for diagnosis or treatment responsiveness, we suggest that levels of GDF15 should be thoroughly assessed in patients with severe non-ischaemic heart failure, which would benefit most. Nonetheless, because this is a very heterogeneous patient population, the relation between GDF15 and specific co-morbidities and their underlying pathophysiology should be thoroughly addressed. Besides patient-based research, the molecular insights should be studies in experimental disease models (*in vivo* and *in vitro*) that reflect the specific patient population as best as possible.

When looking for future therapeutic intervention options or clinical discriminative biomarkers to aid to prediction and guide treatment, it is of crucial importance to gain more insight in the specific signalling effects of GDF15 within cardiac tissue. Elucidating the balance in GDF15 concentration needed for normal pathophysiological function, thereby needing to either increasing or decreasing the GDF15 levels, is needed to provide a beneficial effect on cardiac function.

Growth differentiation factor 15 as therapeutic intervention for heart failure

Before GDF15 can become a therapeutic option, we need to elucidate on the possible options for intervention, for example, inhibiting or enhancing GDF15 production, post-transcriptional regulation, receptor ligand binding, and protein interactions. Before clinical application of therapeutic interventions with GDF15, we need to understand these processes through thorough basic research into the function of GDF15. This includes receptor identification and unravelling the specific effects of GDF15 on cardiac cell types both *in vitro* and *in vivo*. Furthermore, we have to establish the contribution of GDF15 to adverse processes of cardiac dysfunction like fibrosis and cardiac remodelling to find a specific cellular target for GDF15. Although cardiomyocytes are the functional cellular cardiac component, these cells have proven to be difficult targets[153] as endothelial cells form the functional barrier between the circulating levels and cardiac tissue. Therefore, a more relevant cell type for targeting via receptor interaction would be endothelial cells, especially as endothelial dysfunction can be reversible.[154] In this manner, modulation of fibrotic responses could be made possible. GDF15 receptor inhibition is where potential lies, as shown with the GFRAL receptor in the blood–brain barrier, which yields beneficial treatment potential for obesity.[47,48] A logical druggable target are receptors, as they are easily accessible for biologicals; however, for heart failure, this will remain complex because of the lack of known cardiac receptor for GDF15. Therefore, emphasizing more research into the specific cardiac receptor for GDF15 is crucial.

Microvascular intervention

To the best of our knowledge, no research has been performed into the role of GDF15 on cardiac tissue calcification, something less prevalent but nevertheless interesting as it plays a major role in conduction disturbances in cardiac tissue.[155] In line, HFpEF is associated with microvascular

stiffness and microvascular calcification.[156,157] Well established are coronary artery calcifications associated with heart failure as they increase the risk for cardiovascular events.[158,159] Until known preventive treatment for calcification is not possible because of lack of knowledge about the underlying mechanism,[160–162] GDF15 is associated with the presence of carotid artery calcification,[22] increased expression resulted in reduced atherosclerotic lesion formation,[163] and absence of GDF15 in leukocytes resulted in stable lesion formation.[54] From patients and animal studies, we know that endothelial dysfunction leads to increased vascular calcification via BMP pathway activation,[164,165] addressing endothelial cells as possible target to reduce calcification. Therefore, the role of GDF15 in vascular calcification and stiffness could give valuable information towards unravelling the mechanism behind heart failure.

Conclusions

With this review, we aimed to display the potential behind GDF15 beyond a biomarker function as it is involved in many pathophysiological processes in heart failure. The future of GDF15 as therapeutic target lies in additional cardiac specific research unravelling the causal effect of GDF15 in cardiac dysfunction on a cellular and molecular level.

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