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REVIEW ARTICLE

Growth differentiation factor 15 in cardiovascular diseases: from bench to bedside

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Abstract

Context: Growth differentiation factor 15 (GDF-15) is a novel cytokine showing close association with cardiovascular diseases. The biological mechanism and clinical use of GDF-15 in cardiovascular diseases have been well demonstrated. We review recent investigations from both basic research and clinical trials into the biological role of GDF-15.

Methods: The data were obtained mainly from MedLine via PubMed and from our own investigations.

Results: Laboratory investigations revealed that GDF-15 has biphasic effects on cellular survival by several signaling pathways. GDF-15 participates in several cardiovascular pathological processes such as cardiac remodeling, ischemia/reperfusion injury and atherosclerotic plaque formation. As well, GDF-15 was found a prognostic biomarker of heart failure and acute coronary syndrome. The evidence for diagnostic or therapeutic utility is poor.

Conclusion: GDF-15 has great potential as a biomarker in cardiovascular diseases, especially for prognosis, and is seen as a myocardial protective cytokine, but the exact mechanism of GDF-15 in cardiovascular diseases remains unknown.

Keywords: GDF-15, cardiovascular disease, myocardial protection

Introduction

Growth differentiation factor 15 (GDF-15), also known as PTGF-h, PLAB, GDF-15, PDF, NAG-1, and PL74, is a member of the transforming growth factor β (TGF-β) superfamily (Bootcov et al. 1997). Normally, GDF-15 expresses in only a few normal tissues such as the placenta and the central nervous system tissues, with lower levels in some other tissues such as colon, kidney, and prostate. The expression can be significantly upregulated in injured organs such as liver, kidney, heart, and lung. GDF-15 participates in the development of malignant diseases, especially prostate and rectocolonic tumors (Cheung et al. 2004; Xu et al. 2006; Bauskin et al. 2006; Karan et al. 2009), and is involved in the process of some benign diseases. It has been identified as a hepcidin-suppression factor expressed at high levels in patients with ineffective erythropoiesis (Ashby et al. 2010). GDF-15 level was also found increased in systemic sclerosis-associated pulmonary arterial hypertension (Meadows et al. 2010). In recent years, increasing investigation has focused on the association of GDF-15 and cardiovascular diseases. GDF-15 is increasingly being found involved in many pathological cardiovascular disease processes such as ventricular remodeling in heart failure and coronary heart disease (CHD) and seems to play a role as a prognostic indicator.

In this article, we review the recent approaches to study of GDF-15 in basic research and clinical trials to review the association of GDF-15 and cardiovascular diseases. The data were obtained mainly from MedLine via PubMed and from our own investigations.

Basic research investigations of GDF-15

Basic information about GDF-15

GDF-15 is found on chromosome 19p12–13.1 and consists of two exons and 1 intron. Exon I has 309 base pairs, including 71 base pairs of 58 untranslated sequences.

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Exon II has 647 base pairs, including 244 base pairs of 38 untranslated sequences. The exons are separated by an intron sequence of about 1800 base pairs (Böttner et al. 1999).

GDF-15 is a secretory protein that becomes a mature production of the dimeric peptide of 224 amino acids after the cleavage of an RXXR motif in amino acids 193–196 (Tetsuro et al. 2006). It is secreted as a 25-kDa dimeric protein produced from a 62-kDa intracellular dimer. Interestingly, the pro-domain of GDF-15 selectively binds to an extracellular matrix (Bauskin et al. 2005). Therefore, both the mature domain and pro-domain likely play central roles in modulating the biological activity of GDF-15.

Table 1. Agents inducing GDF-15 expression. Selected from *J Biochem Mol Biol* 2006 39:649–655.

	Concentration (μM)	NAG-1 expression
Cyclooxygenasc inhibitors		
Aspirin	1000–10,000	↑
Sulindac	10–40	+/–
Sulindac Sulfone	100–400	+/–
Indomethacin	10–100	↑
Sulindac Sulfide	1–50	↑↑
Piroxicam	200–1000	↑
Diclofenac	50–200	↑
Acetaminophen	10–100	+/–
lbuprofen	100–1000	↑
Sodium salicylate	1000–5000	↑
SC-5SI25	10–100	↑
Cclccoxib*	0.01–0.1	Toxic
Dietary agents		
Resveratrol	10–100	↑
Capsaicin	10–100	↑
EGCG	10–100	↑
ECG	50	↑
Indol lndol-3-carbinol	50	↑
DIM	1–10	↑
Genistein	10–100	↑
DADS	50–100	↑
Lycopene	10–100	+/–
Sulforaphane	10–100	+/–
Trail-10,*cis*-12 conjugated linoleic acid	25	↑
PPAR agonist		
Troglitazone	5	↑↑
15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2	1–10	↑
MCC-555	5	↑
AHPN	1–10	↑
Anti-cancer drugs		
5F-203†	0.01–5	↑

*Treatment of mice increased NAG-1 expression in intestinal tissue.
†Treatment of mice inireased NAG-1 expression in breast tumors.
AHPN, mantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid; DADS, diallyl disulfide; DIM, diindolylmethane; EGCG, epigallocatechin gallate; ECG, epicatechin 3-gallate; PPAR, peroxisome proliferator-activated receptors

Regulatory mechanism of the synthesis of GDF-15

GDF-15 expresses in only a few tissues or organs under normal conditions. Its expression increases with induction by exogenous agents or stress. Several agents have been found to induce the expression of GDF-15 (Table 1) through different signaling pathways. Some agents, such as sulindac, milkvetch, conjugated linoleic acid, capsaicin and antitumor drug Celecoxib, etc, induce the expression of GDF-15 by activating GSK3β (Yamaguchi et al. 2004; Lee et al. 2006; Pang et al. 2007; Lee et al. 2010). Sulindac may also induce the expression of GDF-15 by activating the extracellular signal-regulated kinase 1/2 (ERK1/2) and early growth response gene 1 (EGR1) pathway. Other agents, such as the peroxisome proliferator-activated receptor agonist troglitazone, radix bupleuri, and quercetin, may activate the ERK1/2 and EGR1 pathways to induce the expression of GDF-15 (Baek et al. 2004; Baek et al. 2005; Chen et al. 2007; Lim et al. 2007). The expression of GDF-15 could also be induced by vitamin E, anisomycin, and trachycarpus by activating the p38-ATF3 pathway (Kim et al. 2008b; Minsub et al. 2008). GDF-15 can be induced by berberine through the protein kinase C (PKC) pathway. However, some studies also showed that the inhibition of the PKC pathway by PKC antagonists can upregulate the expression of GDF-15 (Shim & Eling et al. 2005; Piyanuch et al. 2007). In addition, p53 is considered an important way to induce GDF-15 expression. Hypoxia increases the secretion of GDF-15 through a p53 or HIF-1-independent pathway (Albertoni et al. 2002). As one of the important downstream products of p53, GDF-15 can be used as a marker of p53 activation (Yang et al. 2003).

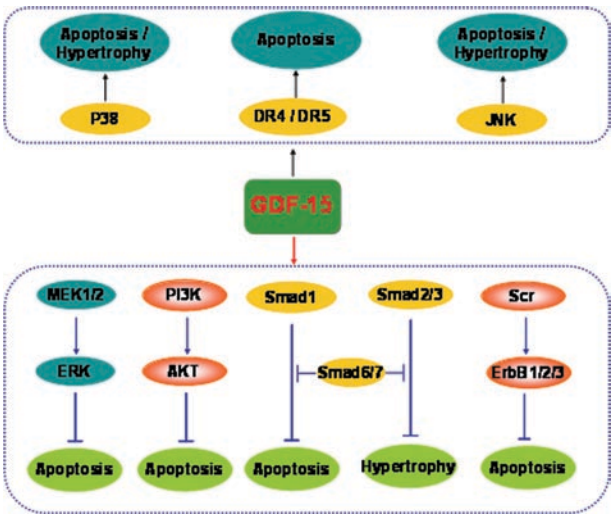

Figure 1. Biological functions and signal transduction pathways of GDF-15.

The biological functions and signal transduction pathways of GDF-15

The biological functions of GDF-15 are still unclear. The effect of GDF-15 in cell survival is like a double-edged sword—a two-way regulation mechanism. The basic biological functions and signal transduction pathways are summarized in Figure 1.

The pro-apoptosis role of GDF-15 Most of the current evidence demonstrates that GDF-15 promotes apoptosis in cancer cells. Apoptosis was increased in GDF-15-overexpressing human colorectal cancer (HCT-116) cells (Kim et al. 2002). GDF-15 expression was increased in the colonic surface epithelium where cells undergo apoptosis. The authors also observed an increased expression of GDF-15 in the normal surface epithelium than in that of most of the tumors, which indicates that GDF-15 might be a cancer suppressor in the normal human body. Another investigation demonstrated that GDF-15 could make the prostate cancer cells DU-145 detach from their substrate and undergo caspase-dependent apoptosis (Liu et al. 2003). The intensity of GDF-15 expression is inversely associated with tumor differentiation and T- and N-stage status (Park et al. 2008), so GDF-15 is closely related to cell apoptosis in cancers with different genesis. Further studies demonstrated that GDF-15 plays a crucial role in cancer cell apoptosis induced by different agents with varied pathways.

In AMC-HN5 human sinonasal carcinoma cells, indomethacin, a member of the non-steroidal anti-inflammatory drugs (NSAIDS), a potent GDF-15 inducer, caused GDF-15 expression in a time- and dose-dependent manner (Kim et al. 2008c). In intestinal neoplasia, sulindac chemoprotection activity, although potent in GDF-15$^{+/+}$ mice was abolished in GDF-15$^{-/-}$ mice (Zimmer et al. 2009). Another investigation found GDF-15 expression strongly induced in GDF-15-cDNA—transfected gastric cancer cells, SNU601, which lack endogenous cyclooxygenase 2, by sulindac sulfide, and that this was closely related with increased apoptosis and decreased cell viability. Forced GDF-15 expression significantly induced apoptosis and DR-4 and DR-5 expression (Jang et al. 2004). GDF-15 was also found to play a key role in apoptosis induced by sodium salicylate treatment in A549 human lung adenocarcinoma cells (Kim et al. 2008d). Therefore, GDF-15 plays a crucial role in NSAID-induced cancer cell apoptosis, with DR-4 and DR-5 a main pathway. We summarize the main signaling pathways in Figure 1.

GDF-15 promotes apoptosis in other pathways such as the p38 mitogen-activated protein kinase (MAPK) pathway. GDF-15 inhibits urethane-induced lung tumor formation, probably by inhibiting phosphorylation of the p38 MAPK pathway (Cekanova et al. 2009). In colon cells, ribotoxic anisomycin induced GDF-15 expression via the p38-ATF3 pathway and subsequent apoptosis (Yang et al. 2009). Vitamin E succinate treatment in PC-3 human prostate carcinoma cells resulted in greater than three-fold increase in the half-life of NAG-1 mRNA in a p38 kinase-dependent manner (Shim & Eling et al. 2008). GDF-15 expression was upregulated by 12-*O*-tetradecanoylphorbol-13-acetate in LNCaP cells through a PKC-dependent pathway involving the activation of nuclear factor-κB and was independent of p38 MAPK (Shim & Eling et al. 2005).

Some evidence shows that Jun *N*-terminal kinase (JNK) activation is another probable pathway of GDF-15 inducing apoptosis. Fenretinide-induced GDF-15 upregulation occurs downstream of JNK activation and mediates the fenretinide-induced apoptosis (Appierto et al. 2009).

In cardiovascular diseases, the function of pro-apoptosis is mainly revealed in atheromatous plaque formation. GDF-15 participates in oxidized low-density lipoprotein (LDL)-mediated macrophage apoptosis in atheromatous plaque and in the formation of plaque (Schlittenhardt et al. 2004). Recently, GDF-15 deletion was found to have a beneficial effect both in early and later atherosclerosis by inhibiting CCR2-mediated chemotaxis and modulating cell death. Plaque stability seems to increase with hematopoietic GDF-15 deficiency in LDL receptor$^{-/-}$ mice (de Jager et al. 2011).

Anti-apoptosis and pro-proliferation role of GDF-15

GDF-15 participates in cell protection and proliferation in some other circumstances. The PI3K/Akt pathway seems essential for GDF-15 in protecting cells against apoptosis. In cerebellar granular neurons in mice, GDF-15 could activate glycogen synthase kinase and Akt by PI3K and in turn inhibit the hypokalemia-induced neuron apoptosis (Subramaniam et al. 2003). GDF-15$^{-/-}$ mice showed progressive postnatal loss of spinal, facial, and trigeminal motoneurons (Strelau et al. 2009). As well, GDF-15 could induce resistance to oxaliplatin, 5-fluorouracil and SN38 in HCT-113 cells by activating the PI3K/Akt pathway (Huang et al. 2007).

GDF-15 overexpression could benefit metastasis of tumors. GDF-15 may lead to cytotactin recombination by activating the FAK-RhoA pathway in PC-3 and LNPCa cell lines, which may explain in part the relation between GDF-15 and increased invasiveness of tumor cells by promoting the motility of these cells (Senapati et al. 2010). In the breast carcinoma cell line SK-BR-3, GDF-15 induced significant phosphorylation of epidermal growth factor receptor at Tyr845, ErbB2 at Tyr877, and ErbB3 at Tyr1289, as well as Akt, which increased the phosphorylation of Src at Tyr416 and induced invasiveness of those cells (Park et al. 2010).

Pro-angiogenesis could be another way for GDF-15 to benefit tumor growth. The expression of GDF-15 in these cells led to the expression of hypoxia inducible factor 1a and its target genes such as vascular endothelial growth factor (VEGF) by activating the mammalian target of rapamycin pathway, which led to increased angiogenesis (Kim et al. 2008a). GDF-15 secreted from melanoma cells, together with VEGF, promoted vascular

development mediated by (V600E)B-Raf signaling (Huh et al. 2010). The increased angiogenesis would retain the tumor growth. An opposite finding of the function of pro-angiogenesis was demonstrated by Ferrari et al. (2005), whereby increased GDF-15 level during new vessel growth was repressed by fenritimide.

In cardiovascular diseases, GDF-15 used to be considered a cardioprotective cytokine with functions of anti-inflammation, anti-apoptosis, and anti-myocardial remodeling. Findings of its role in myocardial hypertrophy are contradictory. GDF-15 mRNA and the precursor protein levels were found increased in myocardial cells from ischemia-reperfusion mice models (Kempf et al. 2006). Furthermore, *in vivo* investigation revealed that the serum concentration of GDF-15 was related to the size of the myocardial infarction area and that the precursor of GDF-15 was increased in level in the infarction area. GDF-15$^{-/-}$ mice showed an increased infarcted area and increased myocardial apoptosis around the infarction. The survival-promoting effect of GDF-15 disappeared on treatment with an antagonist of PI3K or transfection with an Akt1 dominant-negative adenovirus. Thus, GDF-15 may have a crucial role in anti-apoptosis through the PI3K/Akt1 pathway (Kempf et al. 2006). Also, GDF-15 was found to inhibit myocardial hypertrophy through a Smad2/3 pathway in a pressure-induced hypertrophy model, whereas the activation of Smad6/7 led to an inverse effect (Xu et al. 2006). Thus, the myocardial protective effect of GDF-15 is by anti-apoptosis and anti-hypertrophy. Recently, a similar anti-apoptosis effect of GDF-15 was found in rat cardiomyocytes by the activation of Smad1. Interestingly, GDF-15 might promote hypertrophy (Heger et al. 2010), which is opposite to the previous opinions. Therefore, different Smad pathways may lead to a different effect of GDF-15 on cardiomyocytes.

Data from our own investigations showed that some pro-fibrotic agents such as AngII, ET-1, NE, and TGF-β1 might upregulate the GDF-15 expression in cultured mouse fibroblasts. High-dose rhGDF-15 (150 pg/mL)-treated myocardial fibroblasts showed better growth than did the control group. As well, we found that GDF-15 promotes collagen secretion in fibroblasts (Wang et al. 2010).

In conclusion, GDF-15 has different biological functions by different pathways and functions in different circumstances (Figure 2). The expression of GDF-15 is decreased in tumors but increased with bodily stress such as inflammation and ischemia. The increased expression of GDF-15 is mostly beneficial to our body, for example, in cancer treatment and myocardial protection. However, GDF-15 also participates in the progress of some diseases. The factor's activity is like a double-edged sword in cell survival or apoptosis and in the human body. These novel mechanisms associates to varied function in cardiovascular diseases, not only in myocardial protection, but also in myocardial hypertrophy, angiogenesis and fibrosis.

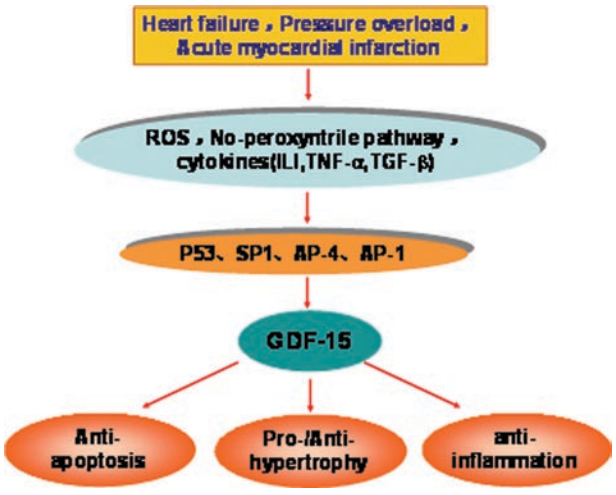

Figure 2. Biological function of GDF-15 in cardiovascular diseases.

Clinical investigations of GDF-15 in cardiovascular diseases

The expression of GDF-15 in cardiovascular diseases

Clinical trials of GDF-15 in cardiovascular diseases are relatively recent (Table 2). Kempf et al. (2007) first linked GDF-15 to cardiovascular disease and found the mean expression of GDF-15 to be 762 ng/L in healthy elderly individuals as seen on radioimmunoassay; further investigation showed the concentration of GDF-15 significantly increased and positively associated with disease progression in patients with chronic heart failure, different types of CHD and acute chest pain (Kempt et al. 2007c). In the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study, which mainly focused on people older than 70 years, the serum concentration of GDF-15 was negatively associated with vascular endothelium function; function and end-diastolic diameter of the left ventricle; and media thickness of carotid arteries. The elevated level of GDF-15 was also related to several clinical concomitant conditions such as diabetes mellitus, low glomerular filtration rate, smoking, elevated level of C-reactive protein, high LDL concentration and low high-density lipoprotein concentration. Adjusting for these concomitant conditions showed increasing levels of GDF-15 significantly related to previous angina pectoris, hospital-treated myocardial infarction, previous coronary revascularization, history of heart failure, and hospital-treated stroke (Lind et al. 2009).

In conclusion, the concentration of GDF-15 is elevated in patients with CHD or heart failure and is related to several causative factors and clinical complications. Table 2 describes the association of GDF-15 and individual heart diseases.

GDF-15 and CHD

GDF-15, a biomarker for risk stratification in CHD Clinical trials show that serum concentration of GDF-15 is elevated in patients with CHD. In a series of clinical trials

Table 2. Clinical trials of GDF-15 in cardiovascular diseases.

Year	Disease	Sample capacity	Cut-off	Result	Trial name	Reference
2007	CHF	455	>2729 ng/L	Mortality of 1 year increases		Khan et al. 2009
2007	NSTEMI	2081	>1800 ng/L	Mortality of 1 year increases	(GUSTO)-IV	Kim et al. 2002
2007	STEMI	741	>1800 ng/L	Mortality of 1 year increases	ASSENT-2 ASSENT-plus	Kim et al. 2008a
2007	NSTEMI	2079	<1200 ng/L	Cannot benefit from emergent PCI	FRISC-II	Kim et al. 2008b
2008	Aortic valve stenosis (post-valve replacement)	24	Increase	Reverse ventricular remodeling		Kim et al. 2008c
2009	>70 Years old	1004	Increase	1. diastolic functions of endothelial-related resistance vessel decreases 2. Increased plaque burden 3. Increase in the mass and hypertrophy of left venricular	PIVUS	Kim et al. 2008d
2009	Dyspnea	124	>444.5 ng/L	The sensitivity and specificity of cardiac dyspnea was 100 and 89.3%		Lankeit et al. 2008
2009	Primary hypertension	1524		Reverse ventricular remodeling		Lee et al. 2006
2009	STEMI	1142	Increase	Mortality of 1 year and the incidence of heart failure increase		Lee et al. 2010

CHF, congestive heart failure; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous transluminal coronary intervention; pivus, Prospective Investigation of the Vasculature in Uppsala Seniors study.

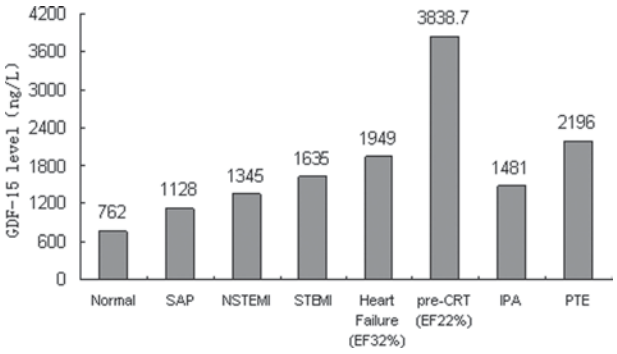

Figure 3. Mean concentrations of GDF-15 of cardiovascular patients in random clinical test SAP stable angina; NSTEMI, non-ST segment elevation myocardial infarction; STEMI, ST-segment elevation acute myocardial infarction; pre-CRT, pre cardiac resynchronization therapy; IPA idiopathic pulmonary artery hypertension; PTE, pulmonary thromboembolism.

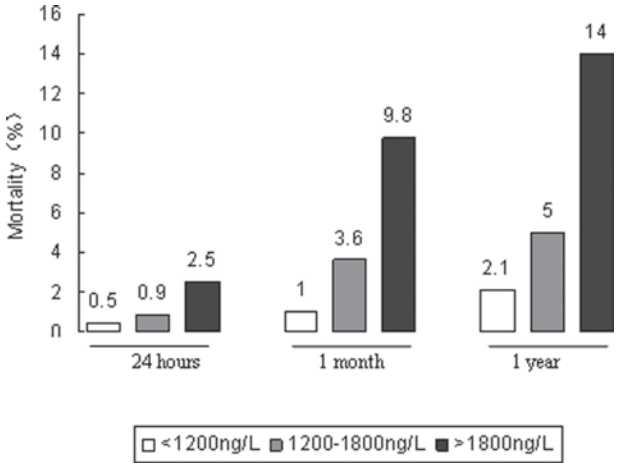

Figure 4. The relation between prognosis and GDF-15 in acute ST-segment elevation acute myocardial infarction.

of patients with CHD, the concentration of GDF-15 was elevated significantly and was even higher in patients with ST-segment elevation myocardial infarction (STEMI). GDF-15 was independently associated with level of NT-proBNP ($P < 0.001$) and >0.1 μg/L cardiac troponin T(cTnT) level at presentation ($P = 0.011$) in STEMI patients. It was elevated in patients with non-STEMI (NSTEMI) and stable angina pectoris (Wollert et al. 2007a; Kempf et al. 2007b; Wollert et al. 2007b; Khan et al. 2009). A summary of the mean level of GDF-15 at presentation in these trials (Figure 3) revealed the level related to severity of myocardial injury in acute coronary syndrome (ACS). The GDF-15 level was positively related to mortality of STEMI patients at 24 h (Figure 4). GDF-15 remained elevated at 5 days and peaked at 90 min after STEMI in patients with or without successful reperfusion treatment. The remaining high level and peak level of GDF-15 may relate to reperfusion injury. Other studies revealed that GDF-15 could be induced by several inflammatory factors, and its concentration was positively associated with the number of abnormal coronary arteries. Further, GDF-15 was found to participate in atheromatous plaque formation and its level was increased in severe arterial lesions and systemic inflammation (Sun et al. 2010). Increased GDF-15 level was also associated with many causative factors of CHD

such as obesity and diabetes mellitus (Dostálová et al. 2009; Ding et al. 2009). GDF-15 seems associated with severity of CHD, especially in ACS. It may be linked to severe myocardial injury, increased plaque burden and intense inflammation.

GDF-15, a prognostic biomarker in CHD GDF-15 may be a prognostic biomarker of CHD. Elevated GDF-15 level is related to poor long-term outcomes. Many clinical trials have investigated the prognostic value of GDF-15 in CHD. In NSTEMI and acute STEMI, the increased serum concentration of GDF-15 was independently associated with poor prognosis. The cardiogenic and overall mortality at 1 year and 5 years and the incidence of re-infarction 6 months after NSTEMI were significantly increased in cases with serum GDF-15 > 1800 ng/L. After a follow-up of 3.6 years, on average, the readmission rate and mortality in patients with stable angina pectoris was also increased with baseline level of GDF-15 > 1800 ng/L. Figures 4 and 5 show the prognostic value of GDF-15 for both stable angina pectoris and ACS indicating GDF-15's link to long-term outcomes of CHD and that it might be used as a prognostic biomarker (Wollert et al. 2007a; Kempf et al. 2007b; Kempf et al. 2009a; Eggers et al. 2010).

GDF-15, a diagnostic biomarker? Little evidence supports GDF-15 as a diagnostic biomarker. Investigators have attempted to use GDF-15 in the differential diagnosis of acute chest pain. The mean concentration of GDF-15 in patients with confirmed ACS and arrhythmia was significantly higher than in patients with atypical chest pain, and the sensitivity reached 80%. However, the specificity was much lower with increasing GDF-15 level in many other diseases as well (Eggers et al. 2008). Although the risk stratification for ACS based on cTnT and NT-proBNP levels on admission was significantly improved by adding GDF-15, no study has compared the diagnostic value of GDF-15 and cTnT in CHD or ACS.

GDF-15, a biomarker guiding treatment? Few data support GDF-15 as a biomarker to guide treatment. The only study is from the FRISC-II trial, which revealed that emergent percutaneous transluminal coronary intervention (PCI) may reduce the mixed endpoint in NSTEMI patients with GDF-15 > 1200 ng/L but has no benefit for patients with GDF-15 < 1200 ng/L. This result adds more significance to cTnT level for emergent PCI treatment of NSTEMI (Wollert et al. 2007b).

GDF-15 and heart failure

GDF-15 as a diagnostic biomarker? A clinical trial of GDF-15 as a diagnostic biomarker was conducted in 2009 with 124 participants (Stejskal et al. 2009). The sensitivity and specificity in diagnosing cardiogenic dyspnea was 100 and 89.3%, respectively, with 444.5 ng/L as the cut-off value. Thus, GDF-15 is a heart-specific biomarker in some situations and is less associated with pulmonary diseases. Unfortunately, the sample size of the study was small, and patients with severe pulmonary disease, infection or acid-base disturbance were not included. Therefore, the clinical significance is still uncertain. Nevertheless, these results still demonstrate a strong association of GDF-15 and other factors in evaluating left ventricular function such as left ventricular ejection fraction (LVEF) and brain natriuretic peptide (BNP).

Our research evaluated concentrations of GDF-15 at different stages of heart failure and its potential screening value. Patients were selected from the department of cardiology at the Peking University Third Hospital (Beijing, China) over the period July 2007 to June 2010. Total 208 participants were divided into four groups which comprised different stages of heart failure. Plasma GDF-15 was measured by ELISA. Plasma GDF-15 was increased gradually from stage A (697.5 ± 324.3 ng/L), stage B (978.9 ± 278.5 ng/L) to stage C (1302.3 ± 324.4 ng/L) compared with control group (245.2 ± 101.7 ng/L), and had a significantly positive correlation ($r = 0.802$, $P < 0.001$). In distinguishing patients with stage B heart failure, the area under the receiver operating characteristic curve was 0.873 ($P < 0.001$). Thus, GDF-15 concentration was elevated with increasing stage of heart failure and might have potential screening implications for stage B heart failure (Wang et al. 2010).

GDF-15 concentration was lower in patients with increased cardiac load of the right ventricle than with left ventricular failure. These data are from investigations of acute pulmonary thromboembolism and idiopathic pulmonary hypertension (Kempf et al. 2007c; Nickel et al. 2008; Kempf et al. 2009b).

GDF-15, a prognostic biomarker? Increased serum GDF-15 concentration is associated with worse prognosis in heart failure. In an investigation of 455 participants (mean age 64 years, mean LVEF 32%), the mean concentration of GDF-15 was 1949 ng/L, which was much higher

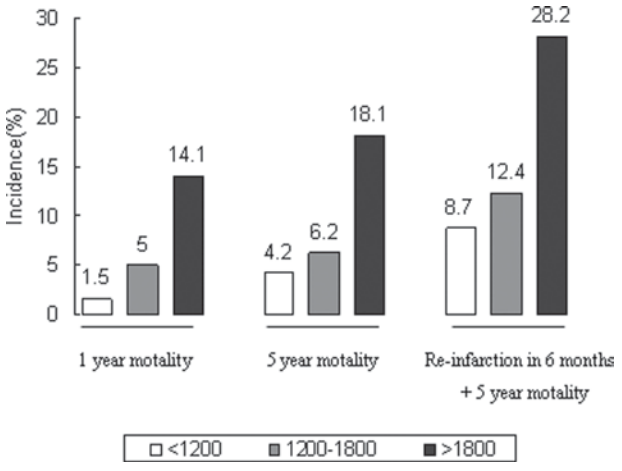

Figure 5. The relation between prognosis and GDF-15 in non-ST segment elevation myocardial infarction.

than the first quartile limit of 1200 ng/L. The mortality at 1 year and 2 and 4 years was 9.9, 16.5 and 24.6%, respectively. The 2-year mortality increased with increasing GDF-15 concentration. With GDF-15 > 2729 ng/L as the cut-off, the sensitivity and specificity in predicting 1-year mortality were 75 and 70.9%, respectively (Kempf et al. 2007a). This cut-off is similar to the result from an investigation of the prognosis of cardiac resynchronization therapy (CRT) (2720 ng/L, mean follow-up 950 days) in which pre-implant GDF-15 was found a strong predictor of mortality and morbidity after CRT, independent of NT pro-BNP level. The predictive value was enhanced by combined measurement (Foley et al. 2009). Thus, GDF-15 has important prognostic value for heart failure patients. Elevated GDF-15 concentration at presentation mainly relates to worse long-term outcome.

Increased GDF-15 level may also lead to better outcomes in some situations. In patients with aortic valve stenosis, early elevation of GDF-15 level after artificial metal valve replacement was associated with cardiac remodeling (Bjørnstad et al. 2008). A study of 1527 primary hypertension patients with three GDF-15 phenotypes revealed that with tag variation on 3148C > G (rs4808793), the elevated GDF-15 level had independent prognostic value on decreased end-diastolic diameter, end-systolic diameter and weight of the left ventricle (Wang et al. 2010).

GDF-15 and other biomarkers in heart failure Serum concentration of GDF-15 is increased with decreased mean LVEF and New York Heart Association score in patients with left heart failure. In an investigation of outcomes of patients with cardiac resynchronization treatment, the mean LVEF of selected patients was 23.1%, and the mean GDF-15 concentration reached 3838.7 ng/L, which is the highest among investigations so far. This trial showed a negative relation between GDF-15 level and LVEF and indicated that GDF-15 level was related to ventricular pressure and cardiac remodeling (Kempf et al. 2007a; Foley et al. 2009). However, most patients with low LVEF are elderly. They have more clinical complications, which may also relate to higher GDF-15 concentration. This observation may explain in part why low LVEF level is associated with increased GDF-15 concentration. A recent investigation showed the serum concentration of GDF-15 elevated in heart failure patients with normal ejection fraction to a similar degree as in systolic heart failure (Raoul et al. 2010), so the level of GDF-15 may be linked to systolic dysfunction and diastolic dysfunction.

Data also shows a tight relation between GDF-15 level and other biomarkers such as NT-proBNP in heart failure patients. It has similar prognostic value to NT-proBNP for 1-year mortality in these patients (Figure 6). This result is also demonstrated in NSTEMI patients. Furthermore, the combined use of GDF-15 and NT-proBNP to predict prognosis significantly improves the accuracy (Kempf et al. 2007a; Foley et al. 2009). Whether GDF-15 is a better prognostic factor or just supplemental and the intrinsic mechanism are under investigation.

Conclusions and perspectives

The factor GDF-15, first found a decade ago, has varied biological mechanisms. GDF-15 participates in many pathological processes, most malignant. In the last 10 years, investigators have attempted to elucidate the association of GDF-15 and cardiovascular diseases. Most evidence supports that GDF-15 is a myocardial protective cytokine, but the mechanism is still not well elucidated.

Clinical trials have revealed that GDF-15 could protect the heart against hypertrophy, but it is linked to poor outcomes in heart failure as well. The mechanism of GDF-15 may vary in different stages of heart remodeling and have varied biological functions. Unfortunately, laboratory results are insufficient to explain the mechanism. Although GDF-15 may protect the heart function by reducing myocardial apoptosis with ACS and ischemia-reperfusion injury, its effect in myocardial hypertrophy, fibration, energy metabolism, and myocardial apoptosis is still uncertain. Whether GDF-15 targets other organs such as the kidney or arteries, or has a role in systemic inflammation in heart failure, needs to be determined. Some investigations showed that GDF-15 is closely related to angiogenesis in malignant diseases, which suggests further investigation of an association of GDF-15 and collateral circulation formation of coronary arteries. GDF-15 was first found as macrophage inhibiting factor 1, and it may inhibit tumor necrosis factor α (TNF-α) secreting from activated macrophages. TNF-α is important for recruiting macrophages to change to foam cells, a crucial step in atherosclerotic plaque formation. Whether GDF-15 may reverse this process needs further investigation.

The prognostic value of GDF-15 is additive to that of other biomarkers in several cardiovascular diseases. Most results support that GDF-15 is related to worse long-term outcomes in patients with heart failure, and few results

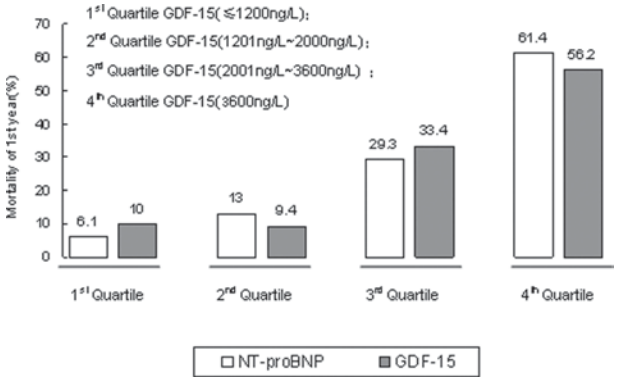

Figure 6. The relation between NT-proBNP,GDF-15 and one year mortality of heart failure.

indicate that GDF-15 may reverse cardiac remodeling. GDF-15 also showed considerable value as a biomarker for CHD, in evaluating severity, especially in ACS, or prognosis. The long-term alteration in the concentration and its role in ventricular remodeling, which contribute to long-term outcomes, are unclear. The diagnostic value of GDF-15 is limited; however, its use as a sentinel marker is worthy of investigation.

Future studies should determine whether the myocardial protective effect of GDF-15 demonstrated from laboratory investigations could be used in treatment of early-stage ischemia events by preventing myocardial infarction or protecting cardiac function. Otherwise, most reports of medicine-induced GDF-15 expression are based on oncological investigation. Few data exist of the association of GDF-15 and cardiovascular medicine. The PROVE IT-TIMI 22 investigation showed no significant interaction between GDF-15 and intensive statin therapy for risk of death or myocardial infarction in ACS patients (Bonaca et al. 2011). The association of GDF-15 and other cardiovascular medicines such as aspirin, adrenergic receptor blockers or ACEI/ARB remains to be determined. Other GDF-15-inducing drugs, which are not for cardiovascular use, could be studied for new medicine treatment in cardiovascular diseases.

Declaration of interest

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References

Albertoni M, Shaw PH, Nozaki M, Godard S, Tenan M, Hamou MF, Fairlie DW, Breit SN, Paralkar VM, de Tribolet N, Van Meir EG, Hegi ME. (2002). Anoxia induces macrophage inhibitory cytokine-1 (MIC-1) in glioblastoma cells independently of p53 and HIF-1. *Oncogene* 21:4212–4219.

Appierto V, Tiberio P, Villani MG, Cavadini E, Formelli F. (2009). PLAB induction in fenretinide-induced apoptosis of ovarian cancer cells occurs via a ROS-dependent mechanism involving ER stress and JNK activation. *Carcinogenesis* 30:824–831.

Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, Taube DH, Bloom SR, Tam FW, Chapman R, Maxwell PH, Choi P. (2010). Erythropoietin administration in humans causes a marked and prolonged reduction in circulating hepcidin. *Haematologica* 95:505–508.

Baek SJ, Kim JS, Nixon JB, DiAugustine RP, Eling TE. (2004). Expression of NAG-1, a transforming growth factor-beta superfamily member, by troglitazone requires the early growth response gene EGR-1. *J Biol Chem* 279:6883–6892.

Baek SJ, Kim JS, Moore SM, Lee SH, Martinez J, Eling TE. (2005). Cyclooxygenase inhibitors induce the expression of the tumor suppressor gene EGR-1, which results in the up-regulation of NAG-1, an antitumorigenic protein. *Mol Pharmacol* 67:356–364.

Bauskin AR, Brown DA, Junankar S, Rasiah KK, Eggleton S, Hunter M, Liu T, Smith D, Kuffner T, Pankhurst GJ, Johnen H, Russell PJ, Barret W, Stricker PD, Grygiel JJ, Kench JG, Henshall SM, Sutherland RL, Breit SN. (2005). The propeptide mediates formation of stromal stores of PROMIC-1: role in determining prostate cancer outcome. *Cancer Res* 65:2330–2336.

Bauskin AR, Brown DA, Kuffner T, Johnen H, Luo XW, Hunter M, Breit SN. (2006). Role of macrophage inhibitory cytokine-1 in tumorigenesis and diagnosis of cancer. *Cancer Res* 66:4983–4986.

Bjørnstad JL, Neverdal NO, Vengen OA, Knudsen CW, Husebye T, Pepper J, Lie M, Christensen G, Tønnessen T. (2008). Alterations in circulating activin A, GDF-15, TGF-beta3 and MMP-2, -3, and -9 during one year of left ventricular reverse remodelling in patients operated for severe aortic stenosis. *Eur J Heart Fail* 10:1201–1207.

Bonaca MP, Morrow DA, Braunwald E, Cannon CP, Jiang S, Breher S, Sabatine MS, Kempf T, Wallentin L, Wollert KC. (2011). Growth differentiation factor-15 and risk of recurrent events in patients stabilized after acute coronary syndrome: observations from PROVE IT-TIMI 22. *Arterioscler Thromb Vasc Biol* 31:203–210.

Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, Zhang HP, Donnellan M, Mahler S, Pryor K, Walsh BJ, Nicholson RC, Fairlie WD, Por SB, Robbins JM, Breit SN. (1997). MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc Natl Acad Sci USA* 94:11514–11519.

Böttner M, Laaff M, Schechinger B, Rappold G, Unsicker K, Suter-Crazzolara C. (1999). Characterization of the rat, mouse, and human genes of growth/differentiation factor-15/macrophage inhibiting cytokine-1 (GDF-15/MIC-1). *Gene* 237:105–111.

Cekanova M, Lee SH, Donnell RL, Sukhthankar M, Eling TE, Fischer SM, Baek SJ. (2009). Nonsteroidal anti-inflammatory drug-activated gene-1 expression inhibits urethane-induced pulmonary tumorigenesis in transgenic mice. *Cancer Prev Res (Phila)* 2:450–458.

Cheung PK, Woolcock B, Adomat H, Sutcliffe M, Bainbridge TC, Jones EC, Webber D, Kinahan T, Sadar M, Gleave ME, Vielkind J. (2004). Protein profiling of microdissected prostate tissue links growth differentiation factor 15 to prostate carcinogenesis. *Cancer Res* 64:5929–5933.

Chen YL, Lin PC, Chen SP, Lin CC, Tsai NM, Cheng YL, Chang WL, Lin SZ, Harn HJ. (2007). Activation of nonsteroidal anti-inflammatory drug-activated gene-1 via extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase revealed a isochaihulactone-triggered apoptotic pathway in human lung cancer A549 cells. *J Pharmacol Exp Ther* 323:746–756.

de Jager SC, Bermúdez B, Bot I, Koenen RR, Bot M, Kavelaars A, de Waard V, Heijnen CJ, Muriana FJ, Weber C, van Berkel TJ,Kuiper J, Lee SJ, Abia R, Biessen EA. (2011). Growth differentiation factor 15 deficiency protects against atherosclerosis by attenuating CCR2-mediated macrophage chemotaxis. *J Exp Med*. Available from: http://jem.rupress.org/content/early/2011/01/12/jem.20100370.long.

Ding Q, Mracek T, Gonzalez-Muniesa P, Kos K, Wilding J, Trayhurn P, Bing C. (2009). Identification of macrophage inhibitory cytokine-1 in adipose tissue and its secretion as an adipokine by human adipocytes. *Endocrinology* 150:1688–1696.

Dostálová I, Roubícek T, Bártlová M, Mráz M, Lacinová Z, Haluzíková D, Kaválková P, Matoulek M, Kasalicky M, Haluzík M. (2009). Increased serum concentrations of macrophage inhibitory cytokine-1 in patients with obesity and type 2 diabetes mellitus: the influence of very low calorie diet. *Eur J Endocrinol* 161:397–404.

Eggers KM, Kempf T, Allhoff T, Lindahl B, Wallentin L, Wollert KC. (2008). Growth-differentiation factor-15 for early risk stratification in patients with acute chest pain. *Eur Heart J* 29:2327–2335.

Eggers KM, Kempf T, Lagerqvist B, Lindahl B, Olofsson S, Jantzen F, Peter T, Allhoff T, Siegbahn A, Venge P, Wollert KC, Wallentin L. (2010). Growth-differentiation factor-15 for long-term risk prediction in patients stabilized after an episode of non-ST-segment-elevation acute coronary syndrome. *Circ Cardiovasc Genet* 3:88–96.

Ferrari N, Pfeffer U, Dell'Eva R, Ambrosini C, Noonan DM, Albini A. (2005). The transforming growth factor-beta family members bone morphogenetic protein-2 and macrophage inhibitory cytokine-1 as mediators of the antiangiogenic activity of *N*-(4-hydroxyphenyl) retinamide. *Clin Cancer Res* 11:4610–4619.

Foley PW, Stegemann B, Ng K, Ramachandran S, Proudler A, Frenneaux MP, Ng LL, Leyva F. (2009). Growth differentiation factor-15 predicts mortality and morbidity after cardiac resynchronization therapy. *Eur Heart J* 30:2749–2757.

Heger J, Schiegnitz E, von Waldthausen D, Anwar MM, Piper HM, Euler G. (2010). Growth differentiation factor 15 acts anti-apoptotic and pro-hypertrophic in adult cardiomyocytes. *J Cell Physiol* 224:120–126.

Huang CY, Beer TM, Higano CS, True LD, Vessella R, Lange PH, Garzotto M, Nelson PS. (2007). Molecular alterations in prostate carcinomas that associate with *in vivo* exposure to chemotherapy: identification of a cytoprotective mechanism involving growth differentiation factor 15. *Clin Cancer Res* 13:5825–5833.

Huh SJ, Chung CY, Sharma A, Robertson GP. (2010). Macrophage inhibitory cytokine-1 regulates melanoma vascular development. *Am J Pathol* 176:2948–2957.

Jang TJ, Kang HJ, Kim JR, Yang CH. (2004). Non-steroidal anti-inflammatory drug activated gene (NAG-1) expression is closely related to death receptor-4 and -5 induction, which may explain sulindac sulfide induced gastric cancer cell apoptosis. *Carcinogenesis* 25:1853–1858.

Kadara H, Schroeder CP, Lotan D, Pisano C, Lotan R. (2006). Induction of GDF-15/NAG-1/MIC-1 in human lung carcinoma cells by retinoid-related molecules and assessment of its role in apoptosis. *Cancer Biol Ther* 5:518–522.

Karan D, Holzbeierlein J, Thrasher JB. (2009). Macrophage inhibitory cytokine-1: possible bridge molecule of inflammation and prostate cancer. *Cancer Res* 69:2–5.

Kempf T, Eden M, Strelau J, Naguib M, Willenbockel C, Tongers J, Heineke J, Kotlarz D, Xu J, Molkentin JD, Niessen HW, Drexler H, Wollert KC. (2006). The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. *Circ Res* 98:351–360.

Kempf T, von Haehling S, Peter T, Allhoff T, Cicoira M, Doehner W, Ponikowski P, Filippatos GS, Rozentryt P, Drexler H, Anker SD, Wollert KC. (2007a). Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J Am Coll Cardiol* 50:1054–1060.

Kempf T, Björklund E, Olofsson S, Lindahl B, Allhoff T, Peter T, Tongers J, Wollert KC, Wallentin L. (2007b). Growth-differentiation factor-15 improves risk stratification in ST-segment elevation myocardial infarction. *Eur Heart J* 28:2858–2865.

Kempf T, Horn-Wichmann R, Brabant G, Peter T, Allhoff T, Klein G, Drexler H, Johnston N, Wallentin L, Wollert KC. (2007c). Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay. *Clin Chem* 53:284–291.

Kempf T, Sinning JM, Quint A, Bickel C, Sinning C, Wild PS, Schnabel R, Lubos E, Rupprecht HJ, Münzel T, Drexler H, Blankenberg S, Wollert KC. (2009a). Growth-differentiation factor-15 for risk stratification in patients with stable and unstable coronary heart disease: results from the AtheroGene study. *Circ Cardiovasc Genet* 2:286–292.

Kempf T, Wollert KC. (2009b). Growth differentiation factor-15: a new biomarker in cardiovascular disease. *Herz* 34:594–599.

Khan SQ, Ng K, Dhillon O, Kelly D, Quinn P, Squire IB, Davies JE, Ng LL. (2009). Growth differentiation factor-15 as a prognostic marker in patients with acute myocardial infarction. *Eur Heart J* 30:1057–1065.

Kim KS, Baek SJ, Flake GP, Loftin CD, Calvo BF, Eling TE. (2002). Expression and regulation of nonsteroidal anti-inflammatory drug-activated gene (NAG-1) in human and mouse tissue. *Gastroenterology* 122:1388–1398.

Kim KK, Lee JJ, Yang Y, You KH, Lee JH. (2008a). Macrophage inhibitory cytokine-1 activates AKT and ERK-1/2 via the transactivation of ErbB2 in human breast and gastric cancer cells. *Carcinogenesis* 29:704–712.

Kim JH, Kim KY, Jeon JH, Lee SH, Hwang JE, Lee JH, Kim KK, Lim JS, Kim KI, Moon EY, Lee HG, Ryu JH, Yang Y. (2008b). Adipocyte culture medium stimulates production of macrophage inhibitory cytokine 1 in MDA-MB-231 cells. *Cancer Lett* 261:253–262.

Kim JH, Chang JH, Rhee KH, Yoon JH, Kwon SH, Song K, Lee KW, Cho CI, Jeon JH, Kim KS. (2008c). Cyclooxygenase inhibitors induce apoptosis in sinonasal cancer cells by increased expression of nonsteroidal anti-inflammatory drug-activated gene. *Int J Cancer* 122:1765–1773.

Kim MY, Moon JY, Hwang JW, Lee SY, Joo YM, Han SI, Park HG, Kang HS. (2008d). Implication of NAG-1 in synergistic induction of apoptosis by combined treatment of sodium salicylate and PI3K/MEK1/2 inhibitors in A549 human lung adenocarcinoma cells. *Biochem Pharmacol* 75:1751–1760.

Lankeit M, Kempf T, Dellas C, Cuny M, Tapken H, Peter T, Olschewski M, Konstantinides S, Wollert KC. (2008). Growth differentiation factor-15 for prognostic assessment of patients with acute pulmonary embolism. *Am J Respir Crit Care Med* 177:1018–1025.

Lee SH, Yamaguchi K, Kim JS, Eling TE, Safe S, Park Y, Baek SJ. (2006). Conjugated linoleic acid stimulates an anti-tumorigenic protein NAG-1 in an isomer specific manner. *Carcinogenesis* 27:972–981.

Lee SH, Krisanapun C, Baek SJ. (2010). NSAID-activated gene-1 as a molecular target for capsaicin-induced apoptosis through a novel molecular mechanism involving GSK3beta, C/EBPbeta and ATF3. *Carcinogenesis* 31:719–728.

Lim JH, Park JW, Min DS, Chang JS, Lee YH, Park YB, Choi KS, Kwon TK. (2007). NAG-1 up-regulation mediated by EGR-1 and p53 is critical for quercetin-induced apoptosis in HCT116 colon carcinoma cells. *Apoptosis* 12:411–421.

Lind L, Wallentin L, Kempf T, Tapken H, Quint A, Lindahl B, Olofsson S, Venge P, Larsson A, Hulthe J, Elmgren A, Wollert KC. (2009). Growth-differentiation factor-15 is an independent marker of cardiovascular dysfunction and disease in the elderly: results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. *Eur Heart J* 30:2346–2353.

Liu T, Bauskin AR, Zaunders J, Brown DA, Pankhurst S, Russell PJ, Breit SN, Panhurst S. (2003). Macrophage inhibitory cytokine 1 reduces cell adhesion and induces apoptosis in prostate cancer cells. *Cancer Res* 63:5034–5040.

Meadows CA, Risbano MG, Zhang L, Geraci MW, Tuder RM, Collier DH, Bull TM. (2010). Increased expression of growth differentiation factor-15 in scleroderma-associated pulmonary arterial hypertension. *Chest*. Available from: http://chestjournal.chestpubs.org/content/early/2010/09/07/chest.10-0302.long.

Nickel N, Kempf T, Tapken H, Tongers J, Laenger F, Lehmann U, Golpon H, Olsson K, Wilkins MR, Gibbs JS, Hoeper MM, Wollert KC. (2008). Growth differentiation factor-15 in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 178:534–541.

Pang RP, Zhou JG, Zeng ZR, Li XY, Chen W, Chen MH, Hu PJ. (2007). Celecoxib induces apoptosis in COX-2 deficient human gastric cancer cells through Akt/GSK3beta/NAG-1 pathway. *Cancer Lett* 251:268–277.

Park JY, Park KH, Bang S, Kim MH, Koh SS, Song SY. (2008). Expression of nonsteroidal anti-inflammatory drug-activated gene-1 (NAG-1) inversely correlates with tumor progression in gastric adenomas and carcinomas. *J Cancer Res Clin Oncol* 134:1029–1035.

Park YJ, Lee H, Lee JH. (2010). Macrophage inhibitory cytokine-1 transactivates ErbB family receptors via the activation of Src in SK-BR-3 human breast cancer cells. *bmb Rep* 43:91–96.

Piyanuch R, Sukhthankar M, Wandee G, Baek SJ. (2007). Berberine, a natural isoquinoline alkaloid, induces NAG-1 and ATF3

expression in human colorectal cancer cells. *Cancer Lett* 258:230–240.

Schlittenhardt D, Schober A, Strelau J, Bonaterra GA, Schmiedt W, Unsicker K, Metz J, Kinscherf R. (2004). Involvement of growth differentiation factor-15/macrophage inhibitory cytokine-1 (GDF-15/MIC-1) in oxLDL-induced apoptosis of human macrophages *in vitro* and in arteriosclerotic lesions. *Cell Tissue Res* 318:325–333.

Senapati S, Rachagani S, Chaudhary K, Johansson SL, Singh RK, Batra SK. (2010). Overexpression of macrophage inhibitory cytokine-1 induces metastasis of human prostate cancer cells through the FAK-RhoA signaling pathway. *Oncogene* 29:1293–1302.

Shim M, Eling TE. (2005). Protein kinase C-dependent regulation of NAG-1/placental bone morphogenic protein/MIC-1 expression in LNCaP prostate carcinoma cells. *J Biol Chem* 280:18636–18642.

Shim M, Eling TE. (2008). Vitamin E succinate induces NAG-1 expression in a p38 kinase-dependent mechanism. *Mol Cancer Ther* 7:961–971.

Stahrenberg R, Edelmann F, Mende M, Kockskämper A, Düngen HD, Lüers C, Binder L, Herrmann-Lingen C, Gelbrich G, Hasenfuss G, Pieske B, Wachter R. (2010). The novel biomarker growth differentiation factor 15 in heart failure with normal ejection fraction. *Eur J Heart Fail* 12:1309–1316.

Stejskal D, Karpísek M, Humenanská V, Lacnák B, Svesták M. (2009). Macrophage-inhibitory cytokine-1 (mic-1) in differential diagnosis of dyspnea–a pilot study. *Clin Biochem* 42:1347–1351.

Strelau J, Strzelczyk A, Rusu P, Bendner G, Wiese S, Diella F, Altick AL, von Bartheld CS, Klein R, Sendtner M, Unsicker K. (2009). Progressive postnatal motoneuron loss in mice lacking GDF-15. *J Neurosci* 29:13640–13648.

Subramaniam S, Strelau J, Unsicker K. (2003). Growth differentiation factor-15 prevents low potassium-induced cell death of cerebellar granule neurons by differential regulation of Akt and ERK pathways. *J Biol Chem* 278:8904–8912.

Sun T, Huang Y, Phillips MI, Luo X, Zhu J, Shi H, Li J. (2010). Growth differentiation factor 15 and coronary collateral formation. *Clin Cardiol* 33:E1–E5.

Ago T, Sadoshima J. (2006). GDF15, a cardioprotective TGF-β superfamily protein. *Circ Res* 98:294–297.

Wang F, Guo Y, Yu H, Zheng L, Mi L, Gao W. (2010). Growth differentiation factor 15 in different stages of heart failure: potential screening implications. *Biomarkers* 15:671–676.

Wang X, Yang X, Sun K, Chen J, Song X, Wang H, Liu Z, Wang C, Zhang C, Hui R. (2010). The haplotype of the growth-differentiation factor 15 gene is associated with left ventricular hypertrophy in human essential hypertension. *Clin Sci* 118:137–145.

Wang Y, Yu H, Xu M, Gao W. (2010). Expression and significance of growth differentiation factor-15 in myocardial fibroblast. *Chin J Med Guide* 12:633–635.

Wollert KC, Kempf T, Peter T, Olofsson S, James S, Johnston N, Lindahl B, Horn-Wichmann R, Brabant G, Simoons ML, Armstrong PW, Califf RM, Drexler H, Wallentin L. (2007a). Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome. *Circulation* 115:962–971.

Wollert KC, Kempf T, Lagerqvist B, Lindahl B, Olofsson S, Allhoff T, Peter T, Siegbahn A, Venge P, Drexler H, Wallentin L. (2007b). Growth differentiation factor 15 for risk stratification and selection of an invasive treatment strategy in non ST-elevation acute coronary syndrome. *Circulation* 116:1540–1548.

Xu J, Kimball TR, Lorenz JN, Brown DA, Bauskin AR, Klevitsky R, Hewett TE, Breit SN, Molkentin JD. (2006). GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. *Circ Res* 98:342–350.

Yamaguchi K, Lee SH, Eling TE, Baek SJ. (2004). Identification of nonsteroidal anti-inflammatory drug-activated gene (NAG-1) as a novel downstream target of phosphatidylinositol 3-kinase/AKT/GSK-3beta pathway. *j Biol Chem* 279:49617–49623.

Yang H, Filipovic Z, Brown D, Breit SN, Vassilev LT. (2003). Macrophage inhibitory cytokine-1: a novel biomarker for p53 pathway activation. *Mol Cancer Ther* 2:1023–1029.

Yang H, Choi HJ, Park SH, Kim JS, Moon Y. (2009). Macrophage inhibitory cytokine-1 (MIC-1) and subsequent urokinase-type plasminogen activator mediate cell death responses by ribotoxic anisomycin in HCT-116 colon cancer cells. *Biochem Pharmacol* 78:1205–1213.

Zimmers TA, Gutierrez JC, Koniaris LG. (2010). Loss of GDF-15 abolishes sulindac chemoprevention in the ApcMin/+ mouse model of intestinal cancer. *J Cancer Res Clin Oncol* 136:571–576.