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Review

Insights Into Mechanisms of GDF15 and Receptor GFRAL: Therapeutic Targets

Luc Rochette,^{1,*} Marianne Zeller,¹ Yves Cottin,^{1,2} and Catherine Vergely¹

Growth and differentiation factor 15 (GDF15) belongs to the transforming growth factor- β (TGF- β) superfamily proteins. GDF15 acts as an inflammatory marker, and it plays a role in pathogenesis of tumors, ischemic diseases, metabolic disorders, and neurodegenerative processes. GDF15 is not normally expressed in the tissue; it is prominently induced following ‘injury’. GDF15 functions are critical for the regulation of endothelial adaptations after vascular damage. Recently, four research groups simultaneously identified glial-derived neurotrophic factor (GDNF)-family receptor α -like (GFRAL) in the brain, an orphan receptor as the receptor for GDF15, signaling through the coreceptor RET. In this article, new aspects of the biology of GDF15 and receptor GFRAL, and their relationship with various pathologies, are commented on.

GDF15: A Member of the TGF- β Superfamily

GDF15 (see [Glossary](#)) also termed macrophage inhibitory cytokine 1 (MIC-1), belongs to the transforming growth factor- β (TGF- β) superfamily proteins. TGF-secreted factor superfamily consists of more than 30 members. They were originally identified as molecules important for regulating development, differentiation, and tissue repair in various organs. The TGF- β superfamily comprises a lot of ligands including TGF- β s, activins, bone morphogenetic proteins (BMP), and GDFs. GDFs belong to the activin/myostatin subclass. Several members of this subfamily have been described, and named GDF1 through to GDF15 [1]. TGF- β family proteins bind to distinct type I and type II serine/threonine kinase receptors. TGF- β signaling is known for its pleiotropic regulatory role in the inflammatory process. GDFs have been involved in many of the pathophysiological processes. Signaling induced by the TGF family ligands are necessary for multiple processes during vertebrate development, tissue homeostasis, and repair [2–4].

A receptor of the **GDNF-family receptor α -like (GFRAL)** has been identified as the receptor for GDF15. The GDNF family is one of the best characterized families of neurotrophic factors, which supports the survival of a variety of target neurons. Four ligands are identified in this family. They require a heterodimeric receptor complex, and the receptor complex is comprised of two molecules: a common transmembrane receptor tyrosine kinase, and a glycosylphosphatidylinositol (GPI)-anchored membrane protein GDNF family receptor α (GFR α). The GDNF family of ligands (GFLs) is comprised of four related factors: GDNF, neurturin, artemin, and persephin [5].

Synthesis, Secretion, and Distribution of GDF15

The human GDF15 locus was mapped by fluorescence *in situ* hybridization (FISH) to chromosome 19p12.1-13.1, and it was shown that the gene contained a single 1820 bp intron [6]. Studies suggest that genetic factors play an important role in determining GDF15 concentrations. It has been reported that specific variants near the GDF15 gene on chromosome 19p13.11 were strongly associated with GDF15 concentration [7]. Several polymorphisms in the GDF15 gene have been identified. GDF15 is synthesized as a precursor protein: proGDF15 that undergoes disulfide-linked dimerization (a 25-kDa disulfide-linked dimer) like TGF- β . The unprocessed translated form of GDF15 (pre-pro-GDF15) is

Highlights

Growth and differentiation factor 15 (GDF15) belongs to the TGF- β superfamily proteins. Glial-derived neurotrophic factor-family receptor α -like is an endogenous receptor for GDF15 detected selectively in the brain. GDF15 serum levels rise in response to cell stress. GDF15 is an established predictive biomarker of adverse cardiovascular events.

GDF15-induced weight loss and reduction in food intake are centrally mediated. In obesity, GDF15 increases lipolysis, oxidative metabolism, and thermogenesis. These actions are associated with reduction of inflammatory process and insulin resistance.

Experimental and clinical studies support the potential use of GDF15 as a novel therapeutic target in relation to: (i) preventing and treating obesity by modulating metabolic activity; (ii) promoting an adaptive angiogenesis; and (iii) promoting regenerative processes.

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308 amino acids (aa) long, including the signal sequence (29 aa), the propeptide (167 aa) and a mature protein (112 aa), which contains a cystine knot typical for the TGF- β family. GDF15 is produced as a \approx 40kDa propeptide form. The mature protein is secreted as a homodimer linked by disulfide bonds and is released from the propeptide following intracellular cleavage at RXXR furine-like cleavage site. Mature GDF15 is soluble and evaluated in blood.

The mature peptide is a dimer with a broad normal circulating range of \sim 0.15–1.15 ng ml $^{-1}$ in healthy humans. GDF15 concentrations increase with age, but do not vary by sex in healthy elderly individuals. GDF15 concentrations have been associated with cystatin C and C-reactive protein concentrations [8,9]. Circulating GDF15 increases during exercise and during recovery from exercise in humans. GDF15 is expressed in most tissues including skeletal muscle. It is possible that GDF15 is secreted from muscle in response to cellular stress or injury, to act locally in an autocrine or paracrine manner [10].

GDF15 is not normally expressed in the adult myocardium, although it is prominently induced following ‘injury’ or in failed hearts. Several basic research studies have identified proteins produced by the heart, referred to as **cardiokines**, which may function analogously to adipokines. They are deregulated during tissular stress and inflammation in signaling either locally or peripherally. In recent years, there has been an increased comprehension of endocrine effects mediated by these factors produced and secreted by the heart. Cardiac fibroblasts and cardiomyocytes were proposed as the principal sources of inflammatory signals in pathological conditions, contributing to the proinflammatory environment in the myocardium by the production of various cardiokines. Within the TGF- β superfamily is the newly identified cardiokine: GDF15 [11]. During myocardial injury, GDF15 is expressed in cardiomyocytes, adipocytes, macrophages, endothelial cells, and vascular smooth muscle cells. The regulation of GDF15 expression in the cardiovascular system has been extensively reviewed [12,13]. GDF15 is expressed in various tissues, but there are limited data on various organs such as eyes (Box 1).

In conclusion, GDF15 is weakly produced in most tissues under physiological conditions, but is strongly induced in response to inflammation and tissue injury. In humans with cardiovascular disease, GDF15 has been detected in atherosclerotic plaque macrophages and in the infarcted myocardium [14].

Box 1. GDF15 and Ocular Pathologies

GDF15 gene is expressed in various tissues, but there are limited data on the ocular expression. Recent studies demonstrated the presence of GDF15 in ocular tissue such as human retinal pigment epithelial cells and murine retina. Alterations in the expression of genes were studied in the rat retina after optic nerve injury. GDF15 mRNA and protein are, respectively, six- and threefold upregulated in the murine retina at 1 day after optic nerve crush. GDF15 protein may be related to the regulation of vitreoretinal inflammatory processes [78].

Interestingly, it has been recently reported that, in aqueous humor, GDF15 was elevated in patients with primary open-angle glaucoma, compared to control cataract patients without glaucoma. GDF15 expression in the retina is specifically increased after acute injury to retinal ganglion cell axons, and in a murine chronic glaucoma model [79]. It is suggested that measurement of GDF15 in aqueous humor might give quantitative information about glaucomatous neurodegeneration [80]. HIF is a major transcription factor, in the different ocular tissue; it modulates the expression of many oxygen sensitive genes including vascular endothelial growth factor but also the TGF- β superfamily protein including GDF15. HIF pathway plays vital role in neovascularization in retinal diseases. HIF pathway is intimately associated with the pathobiology of several oxygen-dependent retinal diseases such as diabetic retinopathy, and glaucoma. The expression of proangiogenic genes such as GDF15, is implicated under hypoxic conditions. In retinal pigment epithelial cells, the expression of proangiogenic genes including GDF15, is dependent on histone lysine demethylases (KDMs); KDMs underlining gene regulation. The expression profile of GDF15 gene in the various cell types of retina tissue is not clearly elucidated. The evaluation of molecular mechanisms related to the ocular expression of GDF15 in retinal diseases, may help in understanding the ocular pathologies and has the potential to open a new field for ocular therapeutics.

Glossary

BT13: a compound that selectively targets GDNF family ligands receptor RET; a new drug to treat neuropathic pain.

Cardiokines: such as GDF15, are proteins produced by the heart, which may function analogously to adipokines. They are deregulated during tissue stress and inflammation in signaling locally or peripherally.

Glial-derived neurotrophic factor (GDNF)-family receptor α -like

(GFRAL): the receptor for GDF15, signaling through the coreceptor RET. RET is an abbreviation for ‘rearranged during transfection’.

Growth and differentiation factor 15

(GDF15): also termed macrophage inhibitory cytokine 1 (MIC-1) belongs to the transforming growth factor- β (TGF- β) superfamily proteins.

Growth hormone (GH): a hormone secreted from the pituitary gland that signals to the liver to stimulate the production of insulin-like growth factor-I (IGF1) and IGF1 acid-labile subunit. Circulating GDF15 acts on the liver to inhibit the actions of GH.

XIB4035: a nonpeptidyl agonist of GDNF receptor α 1 (GFR α 1), is an active treatment for small-fiber neuropathy.

Another important area of the biological actions of GDF15 is the tumor genesis. Several studies showed that higher expression of GDF15 mRNA and protein was found in cancer biopsies. GDF15 is one of the major secreted proteins induced by the tumor suppressor protein p53, which acts as a growth inhibitory molecule in tumor cells. This effect is associated with the prosurvival protein activating transcription factor 3, which is negatively regulated by p53 protein expression [15]. Some cellular environmental conditions are able to promote tumor angiogenesis via the increased release of GDF15. The interactions between a developing tumor and its micro-environment are known to implicate a complex ‘crosstalk’ among the factors produced by the population of cells. GDF15 could affect tumorigenesis both positively and negatively [16].

Molecular Mechanisms Underlying GDF15 Actions

GDF15 as a Regulator in Mitochondria

Recent findings suggest that GDF15 might modulate mitochondrial functions through induction of mitochondria-related genes. In mice in which the heart, muscle, and brain adenine nucleotide translocator isoform 1 (ANT1) was inactivated, the muscle mitochondria produce excess reactive oxygen species and are partially uncoupled. Muscle transcriptome analysis revealed the induction of mitochondrial biogenesis, and increased expression of the genes encoding the myokines: FGF21 and GDF15 [17]. ANT1 is the most abundant protein in the inner mitochondrial membrane. It forms as a homodimer, a gated channel by which ADP is brought into, and ATP transported out of the mitochondrial matrix. Mice in which the ANT1 gene is deleted promote mitochondrial myopathy in relationship with the hyperproliferation of skeletal muscle mitochondria [18]. In this context, muscle mitochondrial stress is correlated with the induction of GDF15 [19].

Various clinical studies in relationship with the mitochondrial metabolism, show a positive correlation between GDF15 and obesity, or type 2 diabetes mellitus (T2DM). Patients with obesity and insulin resistance exhibit impaired mitochondrial function [20]. In these patients, expression and secretion of GDF15 in skeletal myocytes, were associated with altered mitochondrial oxidative phosphorylation function [21]. These findings suggest that GDF15 is a regulator of systemic energy homeostasis.

Intriguingly, plasma GDF15 was recently reported to be increased in mitochondrial disease (MD) patients, with a lower current velocity of growth than expected values, and organ failures as well as muscular dystrophies. Among the various mitochondrial disorders’ subtypes, GDF15 concentrations were tenfold higher than controls. GDF15 has also been reported to be a useful biomarker for monitoring the state of heart failure and renal failure [19]. The mechanism of elevation of GDF15 is not clear in MDs patients; however, in this context, GDF15 is a useful biomarker for muscle-related diseases.

Carbon tetrachloride (CCl₄) intoxication and alcohol overdose are examples of hepatic injury resulting from microsomal metabolism and mitochondrial dysfunctions. It has been evoked that the mitochondrial dysfunction induced by alcohol and CCl₄ promotes GDF15 production in hepatocytes. In the work, it is reported that recombinant GDF15 decreased the expression of proinflammatory cytokines, and prevented the activation of T cells in the livers of mice with CCl₄-induced liver fibrosis [22]. Finally, and interestingly, a new concept is that GDF15 is a marker for diagnosis of MDs.

GDF15 and TGF β Signaling via SMAD Protein Activation

TGF- β family proteins bind to distinct type I and type II serine/threonine kinase receptors [23]. The specificity of the intracellular signaling is mainly determined by type I receptors [activin receptor-like kinase (ALK)-1 to ALK-7]. Intracellular signaling mechanisms induced by the TGF- β

superfamily are divided into SMAD-dependent and -independent pathways. SMAD proteins are important in TGF- β signaling [24]. GDF15 activates type 1 receptor and phosphorylates SMAD2/3 and SMAD1/5/8, which translocate to the nucleus in the form of heteromeric complex with SMAD4. This statement is controversial as use of contaminated GDF15 has been a common problem, which has affected experiments in this field. Purified recombinant proteins for use in biomedical research are invaluable to investigate protein function [25,26].

In patients, GDF15 levels showed a significant positive correlation with liver stiffness. The GDF15 treatment resulted in enhanced expression of α -smooth muscle actin and collagen I, as well as phosphorylation of SMAD2 and SMAD3. GDF15 elevation might be an adaptation mechanism for liver injury implicating the SMAD signaling system [27,28].

Identification of Glial-Derived Neurotrophic Factor GFRAL as the Receptor for GDF15

Interestingly, four research groups from different pharmaceutical companies simultaneously identified GFRAL as the receptor for GDF15, signaling through the coreceptor RET. (RET is an abbreviation for 'rearranged during transfection'.) Alternatively, several laboratories have shown that both GDNF and GFRAL signal independently of RET [29–32].

GFLs, the GDNF family of ligands, act as biologically active homodimers that signal canonically through the transmembrane receptor RET. RET is present in at least three isoforms, and activation of RET takes a major place within lipid rafts. The stoichiometry of GFL:GDNF receptor α (GFR α):RET binding interaction, is postulated to be one ligand homodimer to two GFR α molecules to two RET receptors, forming a hetero-hexameric complex [5] (Figure 1).

Expression of GFRAL gene mRNA was analyzed in various embryonic and adult mouse tissues. Both isoforms of GRAL mRNA were detected in the central nervous system (CNS) of adult mouse. By contrast, there was no detectable GRAL mRNA in peripheral organs examined, such as the heart, liver, spleen, lung, kidney, placenta, skeletal muscle, and small intestine. Among the various regions in the CNS, GFRAL mRNAs were relatively more abundant in some parts of the brain, such as the substantia nigra, the hippocampus, and the area postrema [33]. GFRAL is an orphan receptor, it is a transmembrane protein with a short cytoplasmic domain (Figure 1). The X-ray crystal structure of GDF15 in complex with the extracellular domain of GFRAL was demonstrated. GDF15 activates GFRAL-expressing neurons localized exclusively in the area postrema, and nucleus tractus solitarius of the mouse brainstem [32] (Figure 2).

GDF15–GFRAL signaling controls body weight. In normal conditions, the regulation of energy balance by the brain requires precise information regarding the influx of energy. Unlike gut-derived and adipose-tissue-derived hormones that are regulated by changes in nutrient intake and metabolic flux, GDF15 levels increase in response to tissue damage [34]. Recombinant GDF15 induces weight loss, in mice fed a high-fat diet and in nonhuman primates with spontaneous obesity [30]. In this study, germline *Gfral* gene deleted mice (*Gfral*^{–/–}) lost the anorexic and metabolic effects caused by recombinant GDF15. In addition, diet-induced obesity and insulin resistance are exacerbated in GFRAL-deficient mice, suggesting a homeostatic role for this receptor in metabolism [30].

Role of GDF15 in the Regulation of Body Growth and Hypertrophic Response

Heart disease is connected with GDF15 synthesis and secretion by cardiomyocytes. Recently, GDF15 was identified as a cardiac hormone that regulates body growth. **Growth hormone (GH)** secreted from the pituitary gland, signals to the liver to stimulate the production of insulin-like growth factor-I (IGF1) and IGF1 acid-labile subunit, via the JAK2-STAT5 pathway [35]. GH achieves its effects by influencing gene expression profiles, IGF1 being a key transcriptional

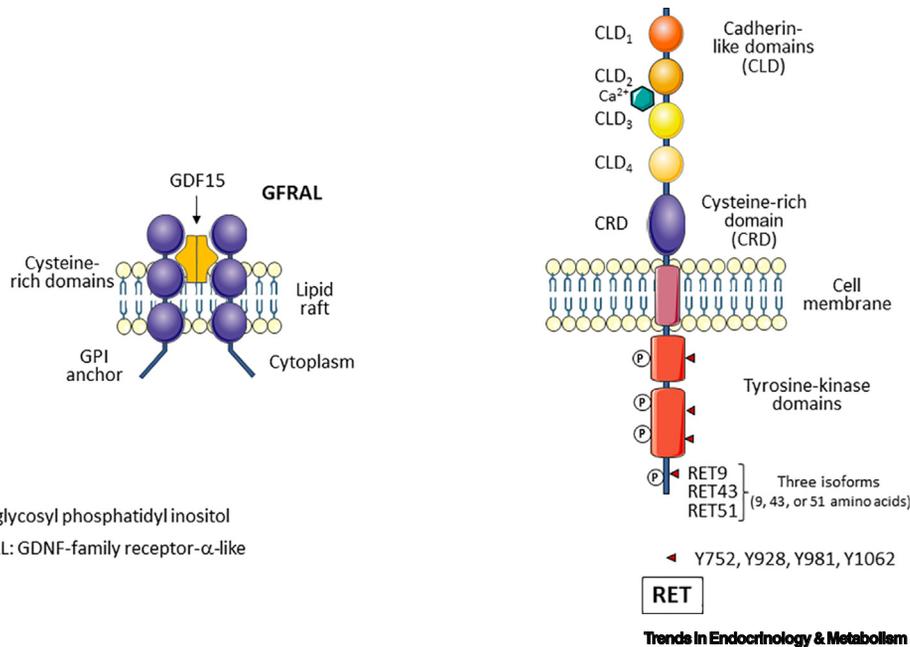


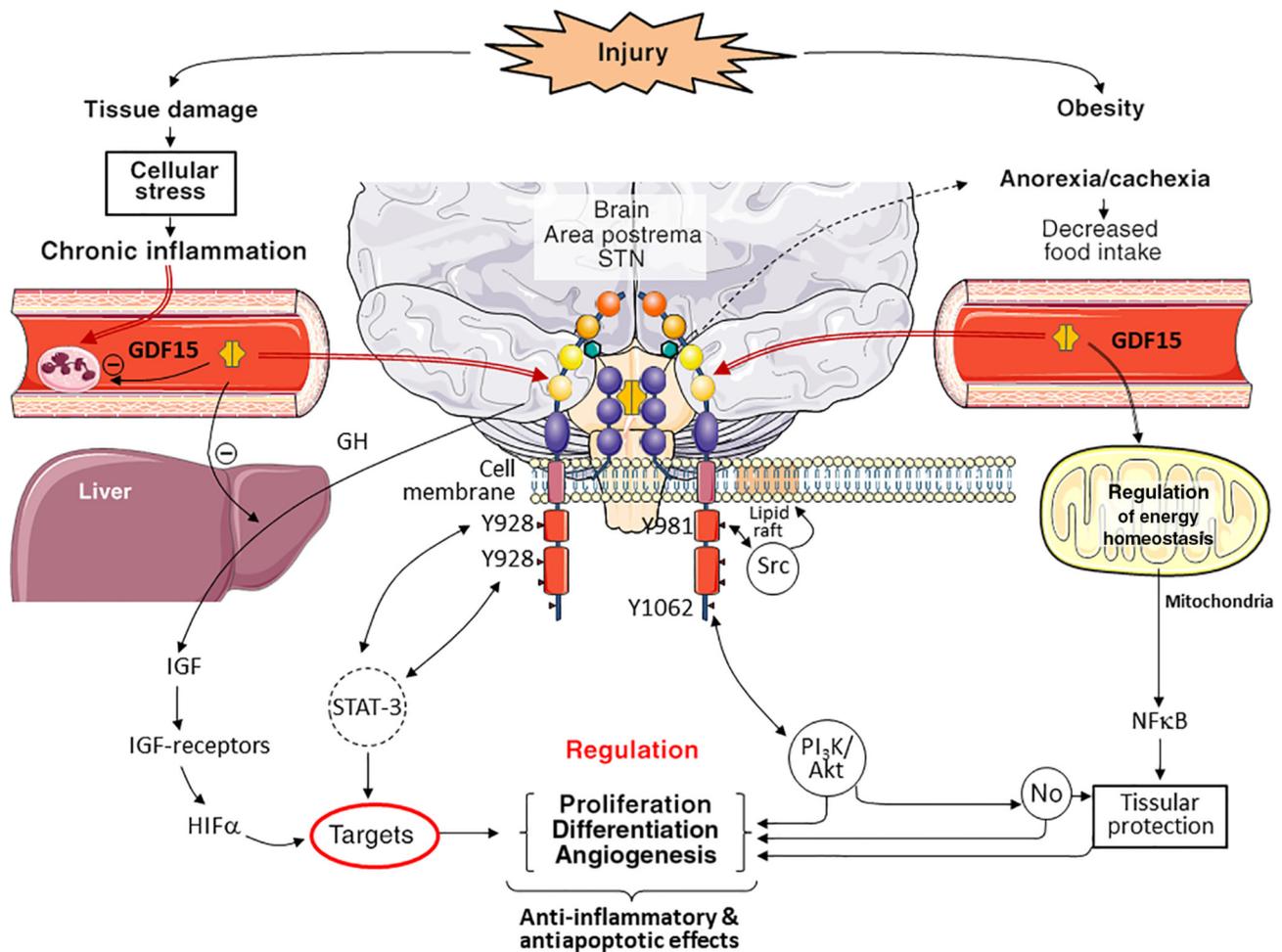
Figure 1. Schematic Structure of RET Protein and GDF15-GFRAL Complex. RET (rearranged during transfection) is a transmembrane tyrosine kinase receptor. The extracellular portion contains four cadherin-like domains (CLD 1,2, 3, and 4), a calcium-binding site, and a cysteine rich domain (CRD). Binding of Ca^{2+} ions to the extracellular CLD of RET is required for its activation. A single pass transmembrane exists. The intracellular portion contains a typical tyrosine-kinase domain. RET is subject to alternative splicing, that results in three alternate tails of 9, 43, and 51 amino acids, RET9, RET43, and RET51, respectively. GFRAL proteins have three cysteine-rich domains, and are attached to the plasma membrane by a glycosyl phosphatidyl inositol (GPI) anchor distributed within lipid rafts. The dimeric protein, GDF15, binds to GFRAL. The complex triggers the dimerization and phosphorylation of RET. The GFL-GFR complex brings together two RET molecules, thereby triggering autophosphorylation and intracellular signaling. The dimerization of RET activates its intracellular kinase domain, to initiate multiple signaling pathway through neurons in the brainstem. Abbreviations: GFRAL, GDNF-family receptor α -like; GDF15, growth differentiation factor 15; GDNF, Glial-derived neurotrophic factor.

target of GH signaling in the liver. Circulating GDF15 levels act on the liver to inhibit the actions of GH (Figure 2). Recent results demonstrated that plasma GDF15 is increased in children with concomitant heart disease and failure to thrive. These demonstrate that GDF15 mediates the communication between heart and liver during cardiac pathogenesis. It is an important advance in this area, in relation to clinical situations such as pediatric heart disease, however how GDF15 blocks GH signaling remains unresolved [36].

GDF15 is upregulated by various cardiovascular events associated with the oxidative stress such as coronary heart diseases, heart failure, and atherosclerosis. GDF15 is also induced during cardiac hypertrophy in a pressure-overload murine model, and its cardiac-specific overexpression protects the heart from hypertrophic responses [27]. Chronic heart failure patients had increased GDF15 concentrations that were closely related to disease severity. Increased cardiac GDF15 concentrations have been observed in mice with heart failure [27].

Role of GDF15 on Endothelium Functions and Vascular Homeostasis

As indicated before, vascular injury implies a degenerative inflammatory process, where different risk factors such as diabetes, hypertension, and dyslipidemia, are implicated; oxidative stress being an aspect in producing the endothelial dysfunction. The decreased synthesis, release and/or activity of endothelium-derived nitric oxide (NO) is the earliest and one of the most important events that characterizes endothelial dysfunction.



Trends in Endocrinology & Metabolism

Figure 2. GDF15 as a Signal in the Organism. Tissue damage, cellular stress, and/or chronic inflammation, induce the secretion and the release of GDF15 which appears in the blood to target specific receptors. GDF15 binds to GFRAL in the brainstem area postrema and solitary tract nucleus (STN), which induces activation and phosphorylation of RET dimer. GDF15-GFRAL-RET complex mediates regulation of food intake in anorexia-cachexia syndromes. RET is a kinase, which is capable of directly phosphorylating multiple downstream targets. Y1062 is a binding site for proteins. Phosphorylation of Y1062 results in activation of PI3K/AKT, nuclear factor κB (NFκB) pathways. GDF15 regulates signaling pathways for cardiovascular protection through activation of the phosphoinositide 3-kinase (PI3K)/Akt/nitric oxide (NO) axis. This axis signaling is essential in regulating cellular controls on proliferation and differentiation. The PI3K pathway plays an important role in regulating angiogenesis. The transcription factor STAT-3 is activated through phosphorylation of Y752 and Y928, and is essential for proliferation and differentiation. The STAT3 signaling pathway is also engaged to control angiogenesis. Angiogenic and neurotrophic factors such as hypoxia-inducible factor-1α (HIF-1α), insulin-like growth factor-1 (IGF1), and indirectly GH, are involved in angiogenesis. IGF1 signaling acts as an important modulator of cellular functions through its receptor, IGF1-R. GH secreted from the pituitary signals to the liver stimulates the production of IGF1. Circulating GDF15 acts on the liver to inhibit the actions of GH. Abbreviations: GFRAL, GDNF-family receptor α-like; GDF15, growth differentiation factor 15; GDNF, Glial-derived neurotrophic factor; GH, growth hormone; RET, rearranged during transfection.

GDF15 is able to modulate vascular contraction and relaxation responses in an endothelium-dependent fashion that involves the NO pathway, enhancing the basal release of NO [37]. Despite the pronounced effects of GDF15 on endothelial function, genetic deletion of GDF15 was not associated with significant hemodynamic effects in GDF15 knockout (KO) mice. The absence of a clear vascular phenotype of GDF15 KO mice may be explained by the statement that GDF15 expression is low in healthy animals and is prominently induced following ‘injury’.

There are several reports showing that GDF15 functions are essential for the regulation of endothelial functions after vascular damage. However, in this context, it is important to report that GDF15 is in relationship with a lot of endogenous pathways; the endothelium being endowed with a variety of constitutive and inducible mechanisms that act to reduce injury and accelerate repair [38]. Vascularization is vital to tissue regeneration. Cancer cells stimulate angiogenesis to supply oxygen and nutrients. However, unlike in healthy tissue, blood vessels in tumors are excessive in number. GDF15 is highly associated with malignant human cancers, and has been suggested to be involved in tumor angiogenesis. GDF15 secreted from cancer cells stimulates endothelial cell proliferation, by enhancing AP-1- and E2F-dependent expression of G(1)cyclins, via PI3K/Akt signaling pathway, increasing angiogenesis [39].

In terms of the relationship between cancer, angiogenesis, and chemotherapy, it has been demonstrated that GDF15 was produced in tissue exposed to chemotherapeutic agents evoking the 'Janus face' of GDF15 [40]. In human microvascular endothelial cells (HMEC-1) exposed to bleomycin or cisplatin, GDF15 levels increased. Elevated levels of GDF15 have been associated with increased risk of diseases. Several studies showed that GDF15 is a biomarker of chemotherapy-induced endothelial damage [41]. Similar to other angiogenic factors, GDF15 induced the proangiogenic effects. GDF15 promoted angiogenesis in hypoxic human umbilical vein endothelial cells. Hypoxia-inducible factor-1 α (HIF-1 α) activation and vascular endothelial growth factor signaling might be important mediators responsible for GDF15-induced angiogenic response [42].

As reported, GDF15 production may rise in response to tissue injury. GDF15 is the first anti-inflammatory cytokine to be shown to directly interfere with chemokine-triggered inside-out signaling, leading to leukocyte integrin activation. GDF15 is an inhibitor of polymorphonuclear leukocytes recruitment, by direct interference with chemokine signaling and integrin activation into inflamed tissue [43]. The receptor responsible for this GDF15-triggered anti-inflammatory mechanism on myeloid cells is identified. In an inflamed tissue, GDF15 reduces chemokine-triggered leukocyte integrin activation and neutrophil recruitment, by interacting with a receptor pair consisting of ALK-5 and TGF- β receptor 2. The same effects were observed for TGF- β 1 [44]. TGF- β signaling is well known for its pleiotropic regulatory role in the inflammatory process. Furthermore, depending on the pathophysiological context, TGF- β signaling can initiate pro- and anti-inflammatory effects, by modulating gene expression.

GDF15 as a Metabolic Regulator

GDF15 has been described in various diseases including acute injury, inflammation, and cancer. GDF15 plays pivotal role in the development and progression of diseases in relation to its function as a metabolic regulator. Studies suggest that GDF15 possesses a role in several biological processes, including energy homeostasis.

GDF15 released from macrophages, liver, and white adipose tissue may act as a metabolic regulator. GDF15 acts as an adipokine, such as adiponectin and leptin [45]. Increased GDF15 levels are associated with metabolic diseases including obesity, insulin resistance, and diabetes. A significant GDF15-mRNA expression is reported in adipose depots in diabetic patients, suggesting a possible paracrine role of GDF15 in adipose tissue metabolism. Serum GDF15 levels were increased in obese and type 2 diabetic patients, and correlated with body mass index [46]. Accumulating evidence has shown that GDF15 could associate with the development and prognosis of T2DM, but there is no evidence considering the predictive role of elevated GDF15 in T2DM subjects. Is GDF15 emerging as a critical prognostic marker in diabetics without remaining cardiovascular disease? It is still not evident.

GDF15 decreases food intake, body weight, adiposity, and improves glucose tolerance in normal and obesogenic diets [47]. Mice with tumors overexpressing GDF15 ate less and lost a substantial amount of adipose mass; in return, injection of recombinant GDF15 reproduced the weight loss. This effect was specific: a single injection of a monoclonal antibody to human GDF15 rapidly reversed the weight loss without having any effect on tumor size. The discovery of the GFRAL receptors for the protein GDF15 in the brain suggests that it regulates the energy balance and food uptake through this new pathway. Findings suggest that the central effect of GDF15 is directed at the hypothalamic arcuate nucleus; the major center for appetite control [48]. Direct administration of small amounts of GDF15 to the intracerebroventricular space of mice resulted in decreased food intake, which suggests that the brain is a site of action of GDF15 [49]. According to the results of recent studies, GDF15 appears to be an endocrine signal, that can be produced by any cell type in response to activation of the cellular integrated stress response. It is suggested that 'nutritional stress' induced by sustained overnutrition leads to increased circulating GDF15 levels, inducing an aversive endocrine signal to the brain [50]. Recently, experiments in rodents and shrews studied the impact of GDF15 on both emetic behaviors and energy balance. The results suggest that exogenous delivery of GDF15 produces either emesis or emetic-like behavior, prior to the induction of anorexia in shrews and rats, respectively. GDF15-GFRAL signaling causes emesis and nausea that appear before the onset of anorexia [51].

In the context of the potential functions of GDF15 as metabolic regulator, it is important to evoke the intricate role of GDF15 in the control of erythropoiesis. Complex interactions were demonstrated between erythropoiesis, iron metabolism, hepcidin, and erythroid factors such as GDF15 [52,53]. Interestingly, it has been reported that hepcidin expression can be suppressed by GDF15, thereby an increase in iron availability for hemoglobin synthesis can occur. Hepcidin regulates iron homeostasis and is stimulated by iron and inflammation, but it is suppressed by hypoxia and erythropoiesis. Erythropoiesis inhibits hepcidin synthesis, thereby promoting iron absorption and increasing the levels of circulating iron available [54]. GDF15, by affecting iron status, might be involved in the pathogenesis of anemia in patients with cardiovascular pathology. Finally, according to recent investigations in hematology and immunology, as a hypoxia effector molecule, GDF15 can be significantly upregulated by anemia and hypoxia, which as a signal molecule is associated with erythropoiesis and iron regulation [55].

In the metabolic context of the homeostasis, GDF15 play a role of modulator in relationship with the adaptation of tissue or organs submitted to a stress or injury. It is not easy to understand the direct association between metabolic stress connecting lipid metabolism and obesity, and the vascular dysregulation associated with diseases like cancer and blinding eye disease. In this context, the role of GDF15 is evoked through nonsteroidal anti-inflammatory drug activated gene-1 (NAG-1) activity. NAG-1 is also known as GDF15. Interestingly, various studies have suggested that peroxisome proliferator-activated receptors (PPARs), which are lipid-activated transcription factors playing a key role in the regulation of lipid metabolism, are factors involved in several physiological processes including modulation of cellular differentiation, development, and tumorigenesis. Studies have reported evidence for antitumorigenic activity of PPAR γ ligands. Among the PPAR γ ligands, the antitumorigenic activity of troglitazone (TGZ) is established. TGZ results in the increased expression of the NAG-1 [56].

Involvement of GDF15 in Pathologic Conditions: Perspectives for Clinical Applications

GDF15-Atherosclerosis and Cardiovascular Diseases

Development of atherosclerotic plaques is driven by endothelial dysfunction, oxidized low-density lipoprotein deposition in the subendothelial space, in association with recruitment of inflammatory

monocytes to the arterial vessel wall. GDF15 is highly expressed in atherosclerotic plaque. It has been shown that GDF15 inhibits proliferation of endothelial cells. *In vitro* and *in vivo*, GDF15 appears to protect against tissue injury by anti-inflammatory, and antiapoptotic properties; however, inconsistent reports show that the deficiency of GDF15 is beneficial against atherosclerosis [57]. Several studies have investigated associations between GDF15 gene polymorphisms and atherosclerosis-related disease, such as coronary disorders, but the results were inconsistent [58]. It is not precisely clear what the role of GDF15 is in the development of atherosclerosis, however, several lines of evidence have shown the cardioprotective effects of GDF15 in several experimental and clinical studies. GDF15 has been shown to have a local cardioprotective role presumably due to its autocrine/paracrine function. GDF15 expression is highly induced in cardiomyocytes after ischemia/reperfusion (I/R). Increased expression of GDF15 was observed in the heart within hours after myocardial infarction. Cardiomyocytes in the infarct border zone are the main source of GDF15. Circulated levels of GDF15 are increased in patients who are admitted to the hospital with an acute coronary syndrome. Patients with elevated levels of GDF15 (>1800 ng/L) had a high risk for mortality within 1 year [59,60]. In a rat ischemia model following different durations of reperfusion, the mRNA and protein expression levels of GDF15 were increased during the onset and development of no reflow and peaked at 24 h of reperfusion. GDF15 was negatively correlated with the activity of myeloperoxidase (MPO). MPO was selected as a representative leukocyte-generating oxidant system, accompanying ventricular remodeling and infarct size after coronary occlusion. MPO being an indicator of neutrophil infiltration; a possible explanation is that GDF15 protects the myocardium from no reflow by inhibiting the inflammatory like response. This response was characterized, as we previously reported, by neutrophil infiltration and trans-endothelial migration [43,61].

In the field of the cardiac protection, GDF15 shows antiapoptotic effect against I/R and reduced the size of myocardial infarction [62]. In a recent study, using GDF15 transgenic mice *in vivo* and using GDF15 expression adenovirus *in vitro*, it is reported that GDF15 induced a protective effect on cold I/R in heart transplantation. GDF15 exerts the protective effect through interaction with NF κ B signaling and Foxo3a; data indicates that GDF15 protects heart grafts from I/R injury through regulation of Foxo3a signaling [63]. A link exists between Foxo3 and NF κ B signaling, both of which play important role in antiapoptosis and oxidative stress. A crosstalk exists between Foxo3 and NF κ B signaling, and Foxo3 acts as antagonist of NF κ B signaling [64].

GDF15 has perhaps with other signaling molecules a regulatory role in tissue injury and regeneration. Interesting GDF15 and BMP-2 show similarities in their primary structure. Belonging to the TGF- β family, BMPs participate in organ regeneration through autocrine and paracrine actions. BMP-2 activates ALK-2/3/6 and phosphorylates SMAD1/5. Similarly, GDF15 activates type I receptors and SMAD1/5 [65]. Therefore, further experimental studies will be necessary to establish the relationship between GDF15 and BMP-2 in tissue regeneration. Taken together, available data demonstrate that the tissue protection is always accompanied by reduced recruitment of inflammatory cells and endogenous factors into the infarction. Several molecules such as GDF15 play a pivotal role in transendothelial leukocyte migration, in infarcted tissue. How GDF15 is involved in neutrophil infiltration and transendothelial migration (TEM) remains unresolved. The functional and metabolic links between GDF15 and endothelial cell remain to be firmly established by future studies.

GDF15 and Brain Stroke

The epidemiological investigations show that GDF15 is closely related to degenerative diseases (Box 2) and stroke. Several neurotrophic factors, such as GDF15, have been tested with regard to their neuroprotective and neurorestorative properties in animal models of neurodegenerative

Box 2. GDF15 Aging, Cognitive Decline, and Neurodegenerative Diseases

A relationship is envisaged between GDF15, aging, cognitive decline, and neurodegenerative diseases. As previously reported, several epidemiological studies have shown the association of GDF15 with aging, the GDF15 concentrations, and their changes over time are predictors of all-cause mortality. Several studies have found that inflammatory markers are elevated in the elderly, and are associated with cognitive decline and senescence. Senescence may be induced by various stimuli and cellular senescence is a complex stress response, with an irreversible state of cell function associated with a modification of expression of senescence regulators including GDF15 [81,82].

Cellular senescence was reversed by the downregulation of GDF15; GDF15 overexpression inducing growth arrest and cellular senescence [83]. Since the discovery of the TGF- β family, its biological activity has been a focal topic in the broad fields of cell proliferation and survival in relationship with senescence process. One of the well-studied functions of TGF- β is its cytostatic effect. The effect of TGF- β on apoptosis is highly dependent on the cell type and context. In addition to TGF- β itself, other TGF- β family members also play functional roles in various forms of cellular senescence. Among the TGF β family member proteins, pharmacology of GDF15 and GDF11 is raising exciting prospects for the development of antiaging therapeutics and senescence protection. According to the recent results concerning the cognitive aspect of GDF15, anti-inflammatory interventions using GDF15 may be a rational therapeutic strategy for cognitive impairment. GDF15 may represent responses toward an inflammatory state in the neuronal diseases [4,84]. Senescence is associated with complex mechanisms in both cellular and innate immunity; it is not easy to define the molecular mechanisms underlying GDF15 or GDF11 action in the onset of cognitive deficiency.

disease. GDF15 is ubiquitously expressed in rat and mouse brain. Murine GDF15 is found in the CNS; its site of highest expression being the choroid plexus and dopaminergic neurons. Following an ischemic injury induced by occlusion of the middle cerebral artery; GDF15 is significantly upregulated in the lesioned area: the hippocampus and parietal cortex, at 3 h and 24 h after lesion. GDF15 has been shown to be a potent neurotrophic factor for midbrain dopaminergic neurons both *in vitro* and *in vivo*. Recent results indicate the importance of GDF15 in promoting survival of dopaminergic neurons and regulating the inflammatory response post-lesion induced by application of the neurotoxin 6-hydroxydopamine [66]. The relationship between cardiac and neurological ischemic events has been studied. The aim of the study was to evaluate how prior cerebrovascular lesions affect myocardial function and signaling *in vivo* and *ex vivo* and how they influence cardiac vulnerability to I/R injury. Cerebral embolization was performed in adult Wistar male rats through the injection of microspheres into the left or right internal carotid artery. The stroke model produced large cerebral infarcts with severe neurological deficit. Following stroke, circulating levels of catecholamines and GDF15 increased. Stroke not only impairs cardiac contractility but also worsens myocardial vulnerability to ischemia [67]. The exact role of GDF15 after stroke may rely on different pleiotropic modes of action. In this field, a lot of studies investigated different biomarkers to predict functional outcome after stroke. Data corroborate that GDF15 is a potential biomarker in patients with stroke. In a clinical study performed in our team, data show that GDF15 plasma concentration at admission is independently associated with 3-month mortality in ischemic stroke patients treated with acute revascularization therapy [68].

The aim of a recent study was to evaluate the relationship between GDF15 gene polymorphisms, the serum GDF15 levels, and ischemic stroke susceptibility in patients. The distribution of rs1804826G/T polymorphism was significantly different between two groups. The data suggests that the GDF15 gene may play a role in the development of ischemic stroke. It is speculated that the rs1804826G/T polymorphism, contributes to variant molecule consequences, and may exert a direct impact on the 'dynamic' of mRNA, thereby affecting the GDF15 production [58].

GDF15 as a Biomarker

A biomarker is defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention'. Biomarkers are by definition objective, quantifiable characteristics of

biological processes, but to identify biomarkers requires the determination of their relevance and validity [69]. Among the more validated and currently in use biomarkers, inflammation related markers are prominent. These markers include GDF15. Associations of GDF15 with vascular and cardiac pathologies have been reported. These associations persist after adjustment for conventional cardiovascular risk factors. As we reported previously, GDF15 has been recognized as a consistent biomarker of mortality and cardiovascular events in patients with acute coronary syndrome (ACS) or coronary artery disease. Increased concentrations of GDF15 were observed in patients at increased risk for adverse left ventricular remodeling after ACS [12]. In fully-adjusted models, GDF15 predicted heart failure death [70]. It has been demonstrated that during cardiac surgery associated with cardiopulmonary bypass, GDF15 levels increased substantially and were associated with markers of cardiac injury and renal dysfunction [71]. This was the first report of the time course of GDF15 during cardiac surgery. Our study demonstrated that plasma GDF15 levels in humans increased during cardiac surgery associated with cardiopulmonary bypass and an acute inflammatory response. In patients with no history of atrial fibrillation, a low plasma level of GDF15 before coronary artery bypass graft surgery was a strong independent predictor of postoperative atrial fibrillation. Moreover, preoperative plasma GDF15 levels added an incremental predictive value to classic risk factors of postoperative atrial fibrillation. GDF15 may be of value to improve preoperative risk stratification among candidates for surgery [72]. Findings also suggested that the secreted protein, follistatin-like-1, could be an upstream inducer of GDF15 production and an independent prognostic biomarker in ACSs [73].

Finally, the use of biomarkers for disease prediction and prognosis has demonstrated promise for identifying groups at higher risk who may benefit from more intensive prevention and treatment. In this context, circulating levels GDF15 reflect acute and chronic cellular stressors; GDF15 appears as a highly emerging biomarker [74].

Concluding Remarks

The expression of GDF15 is upregulated in many different pathological or metabolic conditions, including inflammation, metabolic diseases, cardiovascular diseases, and tumor genesis. However, the exact biological functions of GDF15 are still poorly understood, and its function often exhibits differing and sometimes opposing roles (see [Outstanding Questions](#)). Consequently, GDF15 exhibits an intricate pattern of beneficial and harmful functions. GDF15 expression is low in healthy conditions, and is notably induced in response to cellular stressor following 'injury'. GDF15 functions are essential on regulation of endothelial adaptations after vascular damage.

GDF15 emerges as a relevant contributor in the energy-homeostasis field, and it appears as a biomarker of various cardiovascular and metabolic diseases. By modifying the amount of GDF15-GFRAL-RET signaling, a new class of GDF15/GFRAL/RET-based drugs is emerging. A screening identified a GDNF family ligand mimetic named **BT13**, as a compound that selectively targeted GDNF family ligands receptor RET to activate downstream signaling cascades. This GDNF mimetic small molecule, BT13, selectively activates RET. It offers opportunities for developing novel disease-modifying medications to treat neuropathic pain [75]. More recently, **XIB4035**, a nonpeptidyl agonist of GFR α 1, is an active treatment for small-fiber neuropathy. Initial stages of small-fiber neuropathy frequently involve nerve terminal degeneration before sensory neuron death. XIB4035 functions as a positive modulator of GDNF-family ligands signaling by prolonging ligand-induced RET receptor activation [76]. To investigate the role of GDF15 in metabolism and produce a potential therapeutic modality to treat obesity, recombinant (r) GDF15 proteins and antibodies were tested in a variety of disease models. (r)GDF15 and half-life extended variants of GDF15, were synthesized and tested in obese mice, rats, and monkeys. These molecules demonstrated durable efficacy in lowering body weight [77]. Taken together,

Outstanding Questions

GFRAL were identified as the receptor for GDF15, signaling through the coreceptor RET. Why is GFRAL expression restricted to the brainstem region? What neuronal circuits are activated, in relation to weight loss under stressed conditions?

Studies have identified proteins produced by the heart, referred to as cardiokines, which may function analogously to adipokines. What is the role of heart as an endocrine organ, and the specific function of the newly identified cardiokine: GDF15?

Serum GDF15 levels were increased in obese and type 2 diabetic patients and correlated with body mass index. What are the molecular mechanisms implicated in these clinical conditions?

Plasma GDF15 is increased in children with concomitant heart disease and failure to thrive; the mechanisms involved in such clinical situations remain to be determined.

GDF15 was identified as a cardiac hormone that regulates body growth. Circulating GDF15 acts on the liver to inhibit the actions of GH. How does GDF15 block GH signaling?

GDF15 exhibits an intricate pattern of beneficial and harmful functions. Is GDF15 emerging as a critical prognostic marker in cardiovascular disease? Is GDF15 a prognostic predictor of all-cause mortality in human disorders?

the overall results support potential use of GDF15 as a novel therapeutic target in: (i) preventing and treating obesity by modulating metabolic activity; (ii) promoting an adaptive angiogenesis; and (iii) promoting regenerative processes.

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Disclaimer Statement

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