Published in final edited form as:

Pharmacol Ther. 2017 July ; 175: 28–34. doi:10.1016/j.pharmthera.2017.02.032.

The Versatility and Paradox of GDF 11

A Jamaiyar^{1,2}, W Wan^{1,3}, DM Janota¹, MK Enrick¹, WM Chilian¹, and L Yin¹

¹Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH, USA

²School of Biomedical Sciences, Kent State University, Kent, OH, USA

³Department of Cardiology, Renmin Hospital of Wuhan University, Hubei, China

Abstract

In addition to its roles in embryonic development, Growth and Differentiation Factor 11 (GDF 11) has recently drawn much interest about its roles in other processes, such as aging. GDF 11 has been shown to play pivotal roles in the rescue of the proliferative and regenerative capabilities of skeletal muscle, neural stem cells and cardiomyocytes. We would be remiss not to point that some controversy exists regarding the role of GDF 11 in biological processes and whether it will serve as a therapeutic agent. The latest studies have shown that the level of circulating GDF 11 correlates with the outcomes of patients with cardiovascular diseases, cancer and uremia. Based on these studies, GDF 11 is a promising candidate to serve as a novel biomarker of diseases. This brief review gives a detailed and concise view of the regulation and functions of GDF 11 and its roles in development, neurogenesis and erythropoiesis as well as the prospect of using this protein as an indicator of cardiac health and aging.

Keywords

Growth differentiation factor 11; BMP signaling; Aging; Development; Biomarker

1. Introduction:

Bone Morphogenetic Protein 11 (BMP 11) or Growth/Differentiation Factor 11 (GDF 11) is a BMP/Transforming Growth Factor β (TGF β) family member protein that is globally secreted in several species including humans, mice, rats, etc. It has been hypothesized that GDF 11 is important in anterior/posterior axial patterning during embryonic development (McPherron, Lawler, & Lee, 1999). In humans, GDF 11 is encoded by its gene located on the long arm of chromosome 12 (band 13.2). It was first discovered in mice via in situ

Corresponding Author: Yin L, Department of Integrative Medical Sciences, Northeast Ohio Medical University, 4209 State Route 44, Rootstown, OH, USA 44272, Tel: 3303256521; lyin@neomed.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

¹¹Conflict of Interest Statement:

The authors declare that there are no conflicts of interest.

hybridization of sections and whole-mount embryos (Nakashima, Toyono, Akamine, & Joyner, 1999). At day 8.5 post coitus (dpc), GDF 11 is most highly expressed in the tail bud region but its expression spread to other parts of the embryo at 10.5 dpc. Protein BLAST analysis reveals that human and mouse GDF 11 proteins share 99.5% sequence homology.

The nascent GDF 11 peptide in mice is processed by having its N-terminal prodomain cleaved off and its C-terminal domain activated. The C-terminal domain has been found to form a noncovalent latent complex with its cleaved prodomain (Ge, Hopkins, Ho, & Greenspan, 2005). GDF 11 can bind type I TGF β superfamily receptors, such as Activin receptors, Activin receptor-like kinase 4 (ALK4), ALK5 and ALK7 (Andersson, Reissmann, & Ibanez, 2006). Most commonly, it signals through ALK4 and ALK5 receptors. Alk5 mutant embryos showed abnormalities in anterior/posterior patterning in vertebral, kidney and palate development in an Activin Receptor Type II B (Acvr2b)-null background, which are similar to the defects found in GDF 11 global knockout mice (Andersson, et al., 2006). Therefore, the TGF β receptor ALK5 is a vital component of GDF 11 signaling during the process of embryogenesis.

In humans, GDF 11 is expressed in nearly all major organs and tissues (Uhlen et al., 2015). The highest levels of Gdf 11 transcripts have been reported in the hippocampus region of the brain while the liver seems to have the lowest expression of the same. Spleen and myocardium expressed similar levels of the transcripts of Gdf 11. Within the spleen, most (~70%) of the Gdf 11 transcripts are expressed in lymphocytes and macrophages while the rest are expressed by other cells like endothelial cells and fibroblasts. Among all the cell types in the human heart, almost half of cells expressing GDF11 were fibroblasts. Endothelial cells were the second largest group of cells expressing GDF 11. Myocytes accounted for only 15-20% of the cells with detectable GDF 11 expression (Uhlen et al., 2015).

With regard to cell signaling, GDF 11 acts via pathways similar to those of other TGF β superfamily member proteins. It binds to two Activin Type IIR (ActRIIA, ActRIIB) and three Type IR (ALK4, ALK5 and ALK7) receptors. The Type II receptors phosphorylate the intracellular kinase domain of the Type I receptors. Next, SMAD2 and SMAD3 are phosphorylated. SMAD 2/3 recruit SMAD 4 and localize to the nucleus where they activate transcription of target genes such as homeobox genes. However, this is not the only signaling pathway through which GDF 11 acts. Mitogen Activated Protein Kinase Kinase Kinase 7 (MAP3K7)/MAP3K7IP1 (Tak1/Tab1) can also mediate the signal by activating MAPK14 (p38MAPK), as well as Phosphoinositide 3 Kinase (PI3K), RAS, MAPK1 (ERK) and MAPK8 (van Wijk, Moorman, & van den Hoff, 2007). Since GDF 11 signaling regulates crucial cell proliferation and differentiation responses, it is under a high level of extracellular and intracellular regulation.

Extracellular molecules like follistatin, decorin, chordin, noggin and follistatin-Like 3 (Fstl) directly interact with BMPs and prevent them from binding to their receptor (Figure 1). This regulation is further extended on to the cell membrane where a co-receptor, BMP and Activin Membrane-Bound Inhibitor (BAMBI) binds members of the TGF β superfamily. Since BAMBI lacks an intracellular domain to transduce the signal, the bound ligand is not

functional. Lastly, inside the cell, SMAD 6 and SMAD Specific E3 Ubiquitin Protein Ligase (SMURF) act to inhibit SMAD signaling of GDF 11 (van Wijk, et al., 2007).

Gdf 11 gene was thought to code for a single protein, but a recent study showed that a novel transcript of Gdf11, GDF 11 Ex1, that is void of exon 1, is expressed in mouse muscle (Jeanplong, et al., 2014). The GDF 11 Ex1 transcript was found in skeletal muscle, heart, brain and kidney. Between three to six weeks of age, this non-canonical transcript was highly expressed in skeletal muscle. The levels of GDF 11 Ex1 declined quickly after this period. There were also gender-based differences in the expression level of this transcript. Through in silico analysis of the GDF-11 Ex1 RNA, a secondary structure was predicted. This structure could, hypothetically, act as a protein scaffold to recruit other proteins or as a platform upon which signaling events occur. It could well be that this alternate transcript regulates expression of the classical Gdf 11 mRNA transcript.

Apart from the controversial role of GDF 11 in aging, the primary and best-studied function of GDF 11 is to regulate anterior/posterior axial patterning (McPherron, et al., 1999). Development of bones along the longitudinal axis is under a tightly orchestrated regulatory mechanism. Deletion of GDF 11 causes anteriorly directed homeotic transformation in the axial skeletal patterning of the thoracic and lumbar vertebrae. Later, posterior displacement of hind limbs occurs. This results in a mouse with an elongated body axis (Gad & Tam, 1999; Li, Kawasumi, Zhao, Moisyadi, & Yang, 2010). Tissue-specific overexpression of GDF 11 (BMP 11) propeptide in the bone leads to increased embryonic bone deposition. Such mice exhibit increased bone mineral content and bone density (Li, et al., 2011). Myostatin (GDF 8), a similar protein from the TGF β superfamily, plays a major role in inhibition of myogenesis, muscle cell growth and differentiation. Myostatin-null mice, cattle and dogs exhibit extreme muscle hypertrophy (Grobet et al., 1997). The proteins of GDF 11 and myostatin are 89% identical in humans by BLAST comparison. The controversy of GDF 11 in aging is related to myostatin, which will be discussed later in this review.

2. Regulation of GDF 11:

The mRNA of GDF 11 is initially translated to a precursor protein that undergoes a proteolytic cleavage to generate the C-terminal peptide or mature GDF 11, and the N-terminal peptide named GDF 11 propeptide. The propeptide can antagonize GDF 11 activity in vitro. GDF 11 and myostatin bind to Gdf Associated Serum Protein 1 (GASP 1) and GDF-Associated Serum Protein 2 (GASP 2). These two proteins can inhibit the expression of both GDF 11 and myostatin by blocking the initial signaling step of the ligand binding to its type II Receptor (Lee & Lee, 2013). Moreover, overexpression of GASP 1 in mice results in increased muscle growth, strongly suggesting that it acts upstream to inhibit myostatin activity. This has clinical implications for therapeutic approaches to several diseases such as muscular dystrophy and sarcopenia in the elderly (Zhang, et al., 2004).

The promoter region of the Gdf11 gene has been shown to be activated by trichostatin A (TSA), an antibiotic that inhibits enzymes called histone deacetylases (HDACs) (Zhang, et al., 2004). Deacetylation of histone proteins leads to DNA being coiled around the histone octamer more tightly, making that particular DNA sequence difficult to transcribe.

Acetylation has the opposite effect, allowing the transcription machinery access to the promoter present in that stretch of DNA. TSA-mediated inhibition of HDACs leads to hyperacetylation of a lysine residue in the H3 histone associated with the promoter region of the Gdf 11 gene. This causes cells to cease proliferation (Zhang, et al., 2004). Extracellular binding proteins like follistatin and follistatin-Like 3 (FSTL 3) bind to GDF 11 and inhibit its activity (Schneyer, et al., 2008). As part of its post-translational modification, the nascent GDF 11 polypeptide is acted upon by the proprotein convertase subtilisin/kexin 5 (PCSK5). PCSK5 cleaves the prodomain region and activates GDF 11 mature protein (Essalmani, et al., 2008). PCSK5 mutations cause abnormal expression of Hlxb9 and Hox genes in tissues, which in turn leads to anorectal malformations (Tsuda, et al., 2011). Deleting PCSK5 from mouse embryos causes skewed anteroposterior patterning with additional vertebrae and no tail. These embryos also exhibited kidney agenesis. The phenotypes of GDF 11-associated defects suggest the interaction of PCSK5 and GDF 11 as part of GDF 11's regulation. In humans, mutations in PCSK5 can cause caudal regression syndrome with defects similar to those described above (Szumska et al., 2008).

3. GDF 11 in Embryonic Development:

Gdf 11 is one of the significant genes that controls skeletal formation. Knockout of GDF 11 function causes abnormal patterning of the anterior/posterior axial skeleton. Transgenic mice that overexpressed the propeptide cDNA in skeletal tissue formed extra ribs on the seventh cervical vertebra (C7) as a result of transformation of the C7 vertebra into a thoracic vertebra (Li, et al., 2010). GDF 11 signaling is also important in trunk-to-tail transition (Jurberg, Aires, Varela-Lasheras, Novoa, & Mallo, 2013). During embryonic development, the main vertebrate body is formed by the addition of new tissue arising from progenitor cells at the posterior end. The elongation of the longitudinal axis takes place so that internal organs develop at their respective locations. Any disruption of this spatial correlation may lead to displacement of internal organs or anomalous trunk lengths. Trunk-to-tail transition is significantly delayed in the absence of GDF 11 signaling. On the other hand, the premature activation leads to formation of the mice with shortened trunks with the urogenital opening close to the forelimbs (Jurberg, et al., 2013).

The SMAD pathway has been shown to be involved in GDF 11 signaling (Lu, et al., 2016). One study showed that GDF 11 activated transcription of Hoxd11 genes (Gaunt, George, & Paul, 2013). When tail bud fragments from Hoxd11 transgenic mice with lacZ reporter were cultured in the presence of GDF 11, the Hoxd11/lacZ expression was activated. In vitro, when HepG2 cells were transfected with the Hoxd11/lacZ DNA, GDF 11 acted consistently to stimulate their expression. The activation of Hoxd11/lacZ expression occurred through a Hoxd11 enhancer region with a highly conserved SMAD3/4 binding element. This finding is consistent with other studies in which GDF 11 signals through the SMAD pathway (Oh, et al., 2002). Furthermore, the ability of GDF 11 to stimulate the expression of Hoxd11/lacZ is abolished by an inhibitor of SMAD3 called SIS3, lending more weight to the findings (Gaunt, et al., 2013).

While GDF 11 is required for the appropriate implementation of the vertebrate axial patterning plan (Andersson, et al., 2006; Gad & Tam, 1999; Oh, et al., 2002), it has also

been implicated in the formation and normal development of organs. As mentioned earlier, mutations in the GDF 11 regulator, PCSK5, can lead to kidney agenesis. It has also been reported that kidney-specific deletion of GDF 11 causes a wide range of defects including failure to develop kidneys in the mutant mice (Esquela & Lee, 2003). Deficiency of Gdf 11 might cause a failure of the formation of the ureteric bud, the initial structure that later gives rise to the kidneys. Ureteric bud development is directed by the glial cell line-derived neurotrophic factor (GDNF). Gdf 11-null embryos were found to be dysfunctional in terms of GDNF production in the metanephric mesenchymal layer. However, when exogenous GDNF was added to urogenital tracts from Gdf 11-null embryos, the ureteric bud was formed proximal to the Wolffian duct. Therefore, GDF 11 seems to control the expression of Gdnf in the metanephric mesenchyme, which leads to the development of the ureteric bud and kidney organogenesis (Esquela & Lee, 2003).

4. GDF 11 in Neurogenesis:

GDF 11 plays a pivotal role in the developing spinal cord. Neural progenitor cells give rise to both neurons and glial cells during neurogenesis, but the regulatory mechanism of this sequential generation of neuronal and glial cells has not been completely understood at the molecular level. In the development of spinal cord, GDF 11 is expressed for a brief period of time and plays a role in formation of neurons and glia. Gdf 11-null embryos exhibit a slower rate of neurogenesis in the spinal cord compared to wild type embryos (Shi & Liu, 2011). Once the peak expression window of GDF 11 has passed, the progenitor cells proliferated faster and gliogenesis becomes slower in the spinal cord in Gdf 11-null embryos. Moreover, these changes in progenitor properties can be induced in vitro by the addition of recombinant GDF 11. In addition, GDF 11 was shown to facilitate the temporal progression of neurogenesis by acting as a positive feedback signal on the progenitor cells to promote cell cycle exit and decrease proliferation ability, thus changing their differentiation potential. Hence, it is likely that GDF 11 produced by nascent neurons during spinal cord development regulates the rate of neurogenesis.

In the olfactory epithelium (OE) of mice, however, GDF 11 plays a very different role – negative feedback control of OE neurogenesis. Newly-formed neurons produce a signal to inhibit further neurogenesis from neuronal progenitor cells, and this inhibitory feedback signal has been found to be GDF 11 (Wu, et al., 2003). GDF 11 and its receptors are both expressed in neurons and neural progenitor cells. GDF 11 produced by neurons induces p27 (Kip1) in progenitor cells, leading to their cell cycle arrest. Another study from the same group found that activin collaborated with GDF 11 in feedback control of neuroepithelial stem cell proliferation and fate (Gokoffski, et al., 2011). Interestingly, FOXG1, a winged-helix transcription factor, is expressed in developing OE at the same time as GDF 11. FOXG1 promotes the development of anterior neural structures. To balance its action and to promote neurogenesis at the anterior end of the embryo, FOXG1 inhibits SMAD transcriptional complexes, a known component of the GDF 11 signaling pathway (Kawauchi, et al., 2009). Foxg1-null embryos exhibit retarded development of the OE and the right and left hemispheres of the brain. Mutation in even a single copy of Gdf 11 rescues the defects in Foxg1-null embryos. The balancing act of FOXG1 is highlighted by the fact that its deletion in the OE causes failure of expression of another GDF 11 inhibiting

molecule, follistatin (Fst) as well as increased expression of GDF 11. This study suggests that the effect of FOXP1 on GDF 11-mediated negative feedback of neurogenesis could be both direct and indirect. However, in the cerebral hemispheres of Foxg1-null embryos, mutations in GDF 11 do not rescue the developmental defects observed in the olfactory epithelium. GDF 11 did not express at high levels within these structures either. It is worth noting that there are different mechanisms of regulation of neurogenesis in different parts of the developing nervous system.

5. GDF 11 in Retinal Development:

The role of GDF 11 in the retinal development is different from its function in other tissues such as olfactory epithelium. In the developing retina, GDF 11 is expressed in multipotent progenitor cells from E12.5 onwards to at least one day after birth. It negatively regulates the number of neurons in the retina and controls the time period when multipotent progenitor cells are competent to give rise to various cell types like retinal ganglion cells (RGCs) and some inhibitory neurons called amacrine cells. Further study showed that instead of affecting proliferation of progenitors, GDF 11 controls the duration of expression of Math5 (Kim, et al., 2005). For progenitor cells to give rise to RGCs, the expression of the gene Math5 is a crucial requirement. In Gdf 11-null embryos, Math5 expression extends beyond its natural timespan and lasts up to E18, though it did not express at that stage of development in wild types. Furthermore, experiments in explants of the retina with exogenous GDF 11 showed that Math5 expression was rapidly down regulated in the presence of GDF 11. Similar results were obtained in follistatin-null retinas, as follistatin is a natural antagonist of GDF 11. This indicates a negative relationship between GDF 11 and Math5.

6. GDF 11 in Pancreatic Development:

The role of GDF 11 in pancreatic development is complex. Dichmann et al. studied the function of GDF 11 in pancreatic development and found that it is expressed in the embryonic pancreas epithelium before the secondary transition but decreases rapidly after this phase (Dichmann, Yassin, & Serup, 2006). They also found that wild type embryos had pancreases twice as big as Gdf 11-deficient embryos at E18. The exocrine compartment was found to have atrophied, while no change in size was observed for other compartments. However, endocrine precursor cells that were positive for Neurogenin 3 (NGN3) were found to be considerably more abundant in these mutant embryos than in their wild type counterparts. This strongly indicated that GDF 11 was involved in regulating the number of islet progenitor cells in the developing pancreas in mice. Maturation of these cells is also controlled by GDF 11. In the embryos, GDF 11 is expressed in the pancreatic epithelium at the same time that Neurogenin 3 (NGN3) positive cells are being formed. Knocking out GDF 11 results in an increase in the number of NGN3+ cells (Harmon, et al., 2004). This shows that proliferation of islet progenitor cells that express NGN3 is negatively regulated by GDF 11 (Figure 2). However, hyperplasia of NGN3+ cells does not result in more beta cells. On the contrary, Gdf 11-null embryos exhibit lower beta cell numbers than wild types, indicating that GDF 11 plays an important role in beta cell maturation. SMAD2 deficient mice also exhibit a similar number of islet progenitor cells and beta cell reduction. SMAD2 is also activated by the TGF β pathway; hence, both GDF 11 and SMAD2 are involved in

islet cell proliferation and differentiation into beta cells. In addition to the already established Notch pathway, GDF 11 and SMAD2 are thus also involved in the development of pancreas during embryogenesis.

7. GDF 11 as a Therapeutic Target in Abnormal Erythropoiesis

Erythropoiesis is the process of generation of new erythrocytes or red blood cells. In humans, the life span of red blood cells is 120 days. Erythrocyte progenitor cells proliferate and differentiate to give rise to mature erythrocytes. Initially, the first phase of colony-forming unit-erythroid (CFU-E) differentiation relies on erythropoietin (EPO) (Hattangadi, Wong, Zhang, Flygare, & Lodish, 2011). However, during the later stages, erythropoiesis becomes EPO independent. While EPO is a known and widely-used therapeutic for chronic anemia, it is unable to rescue erythropoiesis in a small subset of EPO independent anemic disorders. GDF 11 was found to negatively affect erythrocyte maturation by Suragani et al. when they studied the regulation of erythropoiesis using the ligand-trapping fusion protein ACE-536 (Suragani, et al., 2014). This fusion protein ACE-536 consisted, in part, of an Activin Receptor Type II B (ActRIIB) extracellular domain with decreased affinity for activin binding. ACE-536 also bound GDF 11 and subsequently inhibited SMAD2/3 signaling in erythrocyte progenitors. GDF 11 inhibited erythroid maturation in mice in vivo and ex vivo (Figure 3). Treatment with RAP-536 (a mouse version of ACE-536) significantly increased the number of mature erythrocytes and rescued anemia in murine models of myelodysplastic syndrome. As erythrocyte progenitors matured, GDF 11 and ActRIIB expression decreased in these cells. This points to the inhibitory effect of GDF 11 on erythroid differentiation in the later stages. ACE-536 is currently being tested in phase III clinical trials as a novel therapy under the name of Lustatercept by Acceleron Pharmaceuticals (Arlet, et al., 2016). In a mouse model of β -thalassemia intermedia, it was noted that splenic erythroblasts expressed high levels of GDF 11. When GDF 11 was inactivated, the cells showed decreased oxidative stress and matured into erythrocytes more frequently (Dussiot et al., 2014). The higher than normal expression of GDF 11 in the erythroblasts was found to be dependent on Reactive Oxygen Species (ROS). These findings strongly suggest that GDF 11 is involved in an autocrine amplification loop in β -thalassemia.

8. Role of GDF 11 in Aging:

Recently, GDF 11 has been implicated in playing a role in the reversal of age-related decline of skeletal muscle (Sinha et al., 2014), olfaction, neural stem cell proliferation (Katsimpardi, et al., 2014) and cardiac hypertrophy (Loffredo et al., 2013). It is also reported that the level of GDF 11 in the blood of mice declines with age (Poggioli et al., 2016). In a mouse model of heterochronic parabiosis, GDF 11 was determined to be the circulating factor in the blood of younger mice that reversed age-related cardiac hypertrophy in older mice (Loffredo et al., 2013). Daily injections of recombinant GDF 11 into old mice also recapitulated the effects of parabiosis between the young and old mice and reversed age-related hypertrophy in old mice. GDF 11 also restored skeletal muscle stem cell function in aged mice and enhanced muscle repair after injury (Sinha et al., 2014). The increase in skeletal muscle regeneration also translated to improved physiological function in the mice. Katsimpardi et al reported

that GDF 11 can also stimulate vascular remodeling and increase neurogenesis in aging mice. Injecting older mice with GDF 11 increased the number of neural stem cells and renewed angiogenesis in the brain (Katsimpardi et al., 2014). Treatment with GDF 11 also rescued olfactory ability of these older mice and allowed them to smell odors that are usually only detected by younger mice. These results strongly suggested that GDF 11 plays a role in the age-related global decline of physiological function in mice and that increasing the blood level of circulating factor GDF 11 could rescue several of these cellular and physiological dysfunctions in aged mice.

However, shortly after the paper on GDF 11-mediated reversal of age-related cardiac hypertrophy was published, another study (Egerman, et al., 2015) from the Glass group reported that the antibody used in the aforementioned study also detected myostatin (also known as GDF 8), a protein that shares 89% sequence homology with GDF 11 (Dschieltzig, 2014) but has a very different biological function. Myostatin has been known to inhibit myogenesis and myostatin knockout animals (poultry, cattle and canines) exhibit extreme muscle hypertrophy (Grobet, et al., 1997). Further, the Glass group showed that levels of GDF 11 rise with age in mice and humans by an immunoassay specific to GDF 11 (Egerman, et al., 2015). Also, mice injected with GDF 11 were reported to have decreased muscle regeneration. Another study conducted by Houser et al. showed that GDF 11 not only failed to reduce neonatal rat ventricular myocyte hypertrophy, but induced the hypertrophy instead (Smith, et al., 2015). Later on, the immunoassay used by Glass and colleagues was found to be non-specific to GDF 11 (Poggioli, et al., 2016) because it also detected immunoglobulin, a circulating protein whose levels also decline with age. This was confirmed by performing the assay on immunoglobulin knockout mice. The knockout mice tested negative for GDF 11 as well as myostatin (GDF 8) in the blood (Poggioli et al., 2016). The different results obtained after GDF 11 treatment have also been attributed to lot-to-lot variability in the commercially available recombinant GDF 11 product. Another study recently showed that treatment of young mice as well as old mice with exogenous GDF 11 resulted in a reduction in cardiomyocyte cross sectional area within a short period of time (9 days) (Poggioli et al., 2016).

Another study investigated the causes of variability in GDF 11 levels in different strains of mice and found out that most (74.52%) of the variation is due to the genetic background or strain of mice being used (Zhou, et al., 2016). Further, GDF 11 was found to have a positive quadratic correlation with the median life-span of a mouse strain. When outliers were excluded from this analysis, this correlation became linear, implying that in middle-aged mice, higher GDF 11 levels were indicative of longer life-spans (Zhou et al., 2016). In the same study, young mice were shown to have higher circulating levels of GDF 11 compared to older mice, thus being in agreement with the initial findings reported by Loffredo and colleagues (Loffredo et al., 2013). In a recent report, an innovative LC-MS/MS assay was used to distinguish between GDF 11 and its closely related homolog, GDF 8 (myostatin) (Schafer et al., 2016). In the study, GDF 11 did not decline with age in healthy men. Instead, myostatin levels were found to be lower in older male adults compared to younger ones. Future studies will be needed to further clarify the physiological effects of GDF 11 in aging.

9. GDF 11 as a Novel Diagnostic Biomarker:

Since GDF 11 is a circulating protein in mammals, its potential role in aging has led to studies which utilized serum/plasma levels of the protein as a biomarker for cardiovascular disease and/or lifespan. The design of these studies merged a large sample size with efficient use of statistical analyses and databases to investigate whether GDF 11 levels correlate with indicators of health, aging and a spectrum of pathological heart conditions. Olson and colleagues reported that higher levels of GDF 11/8 in humans with stable ischemic heart disease (IHD) correlate with a reduced risk of negative cardiovascular outcomes such as hospitalization due to heart failure, stroke, myocardial infarction and death (Olson et al., 2015). Patients of severe aortic stenosis who had high GDF 11 levels were more likely to have pre-existing cardiac pathologies, diabetes and overall frailty (Schafer et al., 2016). Subjects of valve replacement surgery with higher GDF 11 levels were more like to be re-hospitalized and develop new cardiovascular conditions such as myocardial infarction, deep vein thrombosis, arrhythmia, etc. (Schafer et al., 2016), (Bueno et al., 2016). However, in a canine model of Chronic Mitral Valve Insufficiency (CMVI), serum GDF 11 levels did not vary at different stages of CMVI-associated heart failure (Ahn, Suh, Moon, & Hyun, 2016). There was not any significant correlation between serum GDF 11 levels and age of the subjects.

In oncology, circulating proteins can also serve as novel diagnostic markers for various types of cancer. In a study of tumor samples from 130 individuals suffering from colorectal cancer, GDF 11 mRNA expression was significantly higher in tumor tissue than in normal tissue for 50% (n=65) of the patients (Yokoe et al., 2007). Further, the high expression of GDF 11 mRNA in colorectal tumors was significantly associated with incidences of lymph node metastases and cancer-related death. The 5-year survival rate for the low GDF 11 expression group was 77% while the rate for the high GDF 11 expression group was 58%. The results were statistically significant and it suggested that patients with higher GDF 11 mRNA expression in their tumor tissue had a poorer prognosis.

GDF 11 levels were also found to be significantly higher in uremic patients on hemodialysis than those in age matched healthy controls. High serum GDF 11 also correlated inversely with hemoglobin levels in these patients (Yamagishi, Matsui, Kurokawa, & Fukami, 2016). Bueno et al. analyzed plasma, serum and platelet lysate from 23 healthy subjects and discovered that platelet lysate had the highest levels of GDF 11 protein. They also found that GDF 11 levels increased with age in serum but not in plasma (Bueno et al., 2016).

10. Conclusion:

From embryonic development to an indicator of aging or diseases, the role of GDF 11 varies throughout the lifespan of an organism. The orthologs of GDF 11 have been found throughout vertebrates and the conservation of GDF 11 signaling and actions are favored by evolutionary mechanisms (Funkenstein & Olekh, 2010). From the examples quoted earlier, it is quite clear that GDF 11 performs vastly different functions in different developmental niches. Whereas neurogenesis slows down in the absence of GDF 11 during development of the spinal cord, higher levels of GDF 11 cause lower numbers of mature erythrocytes to

differentiate from erythroid progenitors in β – thalassemia. Gdf 11-null mutation is shown to be embryonic lethal, emphasizing the importance of this protein for the overall development and functioning of organ systems. By all accounts it appears that GDF 11 is not only a circulating factor, but also a paracrine signal which modulates critical function in the target cells. Although the cell signaling mechanisms of GDF 11 are well known, its roles in aging-related dysfunctions have contradictory reports. One possible reason for the controversy is that these studies rely on serum or plasma levels of GDF 11. However, methods of processing blood samples might result in lysis of platelets that could cause an increase in GDF 11 levels in either plasma or serum, thereby skewing results. This could be key to elucidating the circulatory dynamics of the secreted GDF 11 protein. This discrepancy is further compounded by the fact that most GDF 11 antibodies cross-react with myostatin (GDF 8). Development of more sensitive and specific molecular assays, such as the LC-MS/MS assay (Schafer et al., 2016) might help to solve this dispute. A genetic mouse model of GDF 11 might also be a promising step towards ending this controversy. Tissue-specific knockout or temporal inducible knockout mouse models of GDF 11 could shine more light on the various roles of this very versatile protein.

Acknowledgements:

This work was supported by grants 14BGIA18770028 from American Heart Association and 1R15HL115540-01 from NIH.

Abbreviations:

ALK	Activin receptor-like kinase
BAMBI	BMP and activin membrane-bound inhibitor
dpc	Days post coitus
EPO	Erythropoietin
GASP	GDF-associated serum protein
GDF 11	Growth/differentiation factor 11
GDF 8	Growth/differentiation factor 8
Gdnf	Glial cell line-derived neurotrophic factor
HDAC	Histone deacetylase
NGN3	Neurogenin 3
OE	Olfactory epithelium
PCSK5	Proprotein convertase subtilisin/kexin 5
TGF β	Transforming growth factor beta
TSA	Trichostatin A

15. References:

- Ahn ST, Suh SI, Moon H, & Hyun C (2016). Evaluation of growth differentiation factor 11 (GDF11) levels in dogs with chronic mitral valve insufficiency. *Can J Vet Res*, 80, 90–92. [PubMed: 26733738]
- Andersson O, Reissmann E, & Ibanez CF (2006). Growth differentiation factor 11 signals through the transforming growth factor-beta receptor ALK5 to regionalize the anterior-posterior axis. *EMBO Rep*, 7, 831–837. [PubMed: 16845371]
- Arlet JB, Guillem F, Lamarque M, Dussiot M, Maciel T, Moura I, Hermine O, & Courtois G (2016). Protein-based therapeutic for anemia caused by dyserythropoiesis. *Expert Rev Proteomics*, 1–10.
- Bueno JL, Ynigo M, de Miguel C, Gonzalo-Daganzo RM, Richart A, Vilches C, Regidor C, Garcia-Marco JA, Flores-Ballester E, & Cabrera JR (2016). Growth differentiation factor 11 (GDF11) - a promising anti-ageing factor - is highly concentrated in platelets. *Vox Sang*.
- Dichmann DS, Yassin H, & Serup P (2006). Analysis of pancreatic endocrine development in GDF11-deficient mice. *Dev Dyn*, 235, 3016–3025. [PubMed: 16964608]
- Dschietzig TB (2014). Myostatin - From the Mighty Mouse to cardiovascular disease and cachexia. *Clin Chim Acta*, 433, 216–224. [PubMed: 24680839]
- Dussiot M, Maciel TT, Fricot A, Chartier C, Negre O, Veiga J, Grapton D, Paubelle E, Payen E, Beuzard Y, Leboulch P, Ribeil JA, Arlet JB, Cote F, Courtois G, Ginzburg YZ, Daniel TO, Chopra R, Sung V, Hermine O, & Moura IC (2014). An activin receptor IIA ligand trap corrects ineffective erythropoiesis in beta-thalassemia. *Nat Med*, 20, 398–407. [PubMed: 24658077]
- Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE, Mallozzi C, Jacobi C, Jennings LL, Clay I, Laurent G, Ma S, Brachet S, Lach-Trifilieff E, Shavlakadze T, Trendelenburg AU, Brack AS, & Glass DJ (2015). GDF11 Increases with Age and Inhibits Skeletal Muscle Regeneration. *Cell Metab*, 22, 164–174. [PubMed: 26001423]
- Esquela AF, & Lee SJ (2003). Regulation of metanephric kidney development by growth/differentiation factor 11. *Dev Biol*, 257, 356–370. [PubMed: 12729564]
- Essalmani R, Zaid A, Marcinkiewicz J, Chamberland A, Pasquato A, Seidah NG, & Prat A (2008). In vivo functions of the proprotein convertase PC5/6 during mouse development: Gdf11 is a likely substrate. *Proc Natl Acad Sci U S A*, 105, 5750–5755. [PubMed: 18378898]
- Funkenstein B, & Olekh E (2010). Growth/differentiation factor-11: an evolutionary conserved growth factor in vertebrates. *Dev Genes Evol*, 220, 129–137. [PubMed: 20694476]
- Gad JM, & Tam PP (1999). Axis development: the mouse becomes a dachshund. *Curr Biol*, 9, R783–786. [PubMed: 10531023]
- Gaunt SJ, George M, & Paul YL (2013). Direct activation of a mouse Hoxd11 axial expression enhancer by Gdf11/Smad signalling. *Dev Biol*, 383, 52–60. [PubMed: 24016758]
- Ge G, Hopkins DR, Ho WB, & Greenspan DS (2005). GDF11 forms a bone morphogenetic protein 1-activated latent complex that can modulate nerve growth factor-induced differentiation of PC12 cells. *Mol Cell Biol*, 25, 5846–5858. [PubMed: 15988002]
- Gokoffski KK, Wu HH, Beites CL, Kim J, Kim EJ, Matzuk MM, Johnson JE, Lander AD, & Calof AL (2011). Activin and GDF11 collaborate in feedback control of neuroepithelial stem cell proliferation and fate. *Development*, 138, 4131–4142. [PubMed: 21852401]
- Grobet L, Martin LJ, Poncelet D, Pirottin D, Brouwers B, Riquet J, Schoeberlein A, Dunner S, Menissier F, Massabanda J, Fries R, Hanset R, & Georges M (1997). A deletion in the bovine myostatin gene causes the double-muscling phenotype in cattle. *Nat Genet*, 17, 71–74. [PubMed: 9288100]
- Harmon EB, Apelqvist AA, Smart NG, Gu X, Osborne DH, & Kim SK (2004). GDF11 modulates NGN3+ islet progenitor cell number and promotes beta-cell differentiation in pancreas development. *Development*, 131, 6163–6174 [PubMed: 15548585]
- Hattangadi SM, Wong P, Zhang L, Flygare J, & Lodish HF (2011). From stem cell to red cell: regulation of erythropoiesis at multiple levels by multiple proteins, RNAs, and chromatin modifications. *Blood*, 118, 6258–6268. [PubMed: 21998215]

- Jeanplong F, Falconer SJ, Oldham JM, Maqbool NJ, Thomas M, Hennebry A, & McMahon CD (2014). Identification and expression of a novel transcript of the growth and differentiation factor-11 gene. *Mol Cell Biochem*, 390, 9–18. [PubMed: 24378996]
- Jurberg AD, Aires R, Varela-Lasheras I, Novoa A, & Mallo M (2013). Switching axial progenitors from producing trunk to tail tissues in vertebrate embryos. *Dev Cell*, 25, 451–462. [PubMed: 23763947]
- Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, Chen JW, Lee RT, Wagers AJ, & Rubin LL (2014). Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science*, 344, 630–634. [PubMed: 24797482]
- Kawauchi S, Kim J, Santos R, Wu HH, Lander AD, & Calof AL (2009). Foxg1 promotes olfactory neurogenesis by antagonizing Gdf11. *Development*, 136, 1453–1464. [PubMed: 19297409]
- Kim J, Wu HH, Lander AD, Lyons KM, Matzuk MM, & Calof AL (2005). GDF11 controls the timing of progenitor cell competence in developing retina. *Science*, 308, 1927–1930. [PubMed: 15976303]
- Lee YS, & Lee SJ (2013). Regulation of GDF-11 and myostatin activity by GASP-1 and GASP-2. *Proc Natl Acad Sci U S A*, 110, E3713–3722. [PubMed: 24019467]
- Li Z, Kawasumi M, Zhao B, Moisyadi S, & Yang J (2010). Transgenic over-expression of growth differentiation factor 11 propeptide in skeleton results in transformation of the seventh cervical vertebra into a thoracic vertebra. *Mol Reprod Dev*, 77, 990–997. [PubMed: 21049546]
- Li Z, Zeng F, Mitchell AD, Kim YS, Wu Z, & Yang J (2011). Transgenic overexpression of bone morphogenetic protein 11 propeptide in skeleton enhances bone formation. *Biochem Biophys Res Commun*, 416, 289–292. [PubMed: 22093826]
- Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, Sinha M, Dall'Osso C, Khong D, Shadrach JL, Miller CM, Singer BS, Stewart A, Psychogios N, Gerszten RE, Hartigan AJ, Kim MJ, Serwold T, Wagers AJ, & Lee RT (2013). Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell*, 153, 828–839. [PubMed: 23663781]
- Lu Q, Tu ML, Li CJ, Zhang L, Jiang TJ, Liu T, & Luo XH (2016). GDF11 Inhibits Bone Formation by Activating Smad2/3 in Bone Marrow Mesenchymal Stem Cells. *Calcif Tissue Int*, 99, 500–509. [PubMed: 27395058]
- McPherron AC, Lawler AM, & Lee SJ (1999). Regulation of anterior/posterior patterning of the axial skeleton by growth/differentiation factor 11. *Nat Genet*, 22, 260–264. [PubMed: 10391213]
- Monestier O, Brun C, Heu K, Passet B, Malhouroux M, Magnol L, Vilotte JL, & Blanquet V (2012). Ubiquitous Gasp1 overexpression in mice leads mainly to a hypermuscular phenotype. *BMC Genomics*, 13, 541. [PubMed: 23046573]
- Nakashima M, Toyono T, Akamine A, & Joyner A (1999). Expression of growth/differentiation factor 11, a new member of the BMP/TGFbeta superfamily during mouse embryogenesis. *Mech Dev*, 80, 185–189. [PubMed: 10072786]
- Oh SP, Yeo CY, Lee Y, Schrewe H, Whitman M, & Li E (2002). Activin type IIA and IIB receptors mediate Gdf11 signaling in axial vertebral patterning. *Genes Dev*, 16, 2749–2754. [PubMed: 12414726]
- Olson KA, Beatty AL, Heidecker B, Regan MC, Brody EN, Foreman T, Kato S, Mehler RE, Singer BS, Hveem K, Dalen H, Sterling DG, Lawn RM, Schiller NB, Williams SA, Whooley MA, & Ganz P (2015). Association of growth differentiation factor 11/8, putative anti-ageing factor, with cardiovascular outcomes and overall mortality in humans: analysis of the Heart and Soul and HUNT3 cohorts. *Eur Heart J*, 36, 3426–3434. [PubMed: 26294790]
- Poggioli T, Vujic A, Yang P, Macias-Trevino C, Uygur A, Loffredo FS, Pancoast JR, Cho M, Goldstein J, Tandias RM, Gonzalez E, Walker RG, Thompson TB, Wagers AJ, Fong YW, & Lee RT (2016). Circulating Growth Differentiation Factor 11/8 Levels Decline With Age. *Circ Res*, 118, 29–37. [PubMed: 26489925]
- Schafer MJ, Atkinson EJ, Vanderboom PM, Kotajarvi B, White TA, Moore MM, Bruce CJ, Greason KL, Suri RM, Khosla S, Miller JD, Bergen HR, 3rd, & LeBrasseur NK (2016). Quantification of GDF11 and Myostatin in Human Aging and Cardiovascular Disease. *Cell Metab*, 23, 1207–1215. [PubMed: 27304512]

- Schneyer AL, Sidis Y, Gulati A, Sun JL, Keutmann H, & Krasney PA (2008). Differential antagonism of activin, myostatin and growth and differentiation factor 11 by wild-type and mutant follistatin. *Endocrinology*, 149, 4589–4595. [PubMed: 18535106]
- Shi Y, & Liu JP (2011). Gdf11 facilitates temporal progression of neurogenesis in the developing spinal cord. *J Neurosci*, 31, 883–893. [PubMed: 21248112]
- Sinha M, Jang YC, Oh J, Khong D, Wu EY, Manohar R, Miller C, Regalado SG, Loffredo FS, Pancoast JR, Hirshman MF, Lebowitz J, Shadrach JL, Cerletti M, Kim MJ, Serwold T, Goodyear LJ, Rosner B, Lee RT, & Wagers AJ (2014). Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. *Science*, 344, 649–652. [PubMed: 24797481]
- Smith SC, Zhang X, Zhang X, Gross P, Starosta T, Mohsin S, Franti M, Gupta P, Hayes D, Myzithras M, Kahn J, Tanner J, Weldon SM, Khalil A, Guo X, Sabri A, Chen X, MacDonnell S, & Houser SR (2015). GDF11 does not rescue aging-related pathological hypertrophy. *Circ Res*, 117, 926–932. [PubMed: 26383970]
- Suragani RN, Cadena SM, Cawley SM, Sako D, Mitchell D, Li R, Davies MV, Alexander MJ, Devine M, Loveday KS, Underwood KW, Grinberg AV, Quisel JD, Chopra R, Pearsall RS, Seehra J, & Kumar R (2014). Transforming growth factor-beta superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. *Nat Med*, 20, 408–414. [PubMed: 24658078]
- Szumaska D, Pielek G, Essalmani R, Bilski M, Mesnard D, Kaur K, Franklyn A, El Omari K, Jefferis J, Benthall J, Taylor JM, Schneider JE, Arnold SJ, Johnson P, Tymowska-Lalanne Z, Stammers D, Clarke K, Neubauer S, Morris A, Brown SD, Shaw-Smith C, Cama A, Capra V, Ragoussis J, Constam D, Seidah NG, Prat A, & Bhattacharya S (2008). VACTERL/caudal regression/Currarino syndrome-like malformations in mice with mutation in the proprotein convertase Pcsk5. *Genes Dev*, 22, 1465–1477. [PubMed: 18519639]
- Tsuda T, Iwai N, Deguchi E, Kimura O, Ono S, Furukawa T, Sasaki Y, Fumino S, & Kubota Y (2011). PCSK5 and GDF11 expression in the hindgut region of mouse embryos with anorectal malformations. *Eur J Pediatr Surg*, 21, 238–241. [PubMed: 21480163]
- Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szgyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, & Ponten F (2015). Proteomics. Tissue-based map of the human proteome. *Science*, 347, 1260419. [PubMed: 25613900]
- van Wijk B, Moorman AF, & van den Hoff MJ (2007). Role of bone morphogenetic proteins in cardiac differentiation. *Cardiovasc Res*, 74, 244–255. [PubMed: 17187766]
- Wu HH, Ivkovic S, Murray RC, Jaramillo S, Lyons KM, Johnson JE, & Calof AL (2003). Autoregulation of neurogenesis by GDF11. *Neuron*, 37, 197–207. [PubMed: 12546816]
- Yamagishi S, Matsui T, Kurokawa Y, & Fukami K (2016). Serum Levels of Growth Differentiation Factor 11 Are Independently Associated with Low Hemoglobin Values in Hemodialysis Patients. *Biores Open Access*, 5, 155–158. [PubMed: 27298756]
- Yokoe T, Ohmachi T, Inoue H, Mimori K, Tanaka F, Kusunoki M, & Mori M (2007). Clinical significance of growth differentiation factor 11 in colorectal cancer. *Int J Oncol*, 31, 1097–1101. [PubMed: 17912435]
- Zhang X, Wharton W, Yuan Z, Tsai SC, Olashaw N, & Seto E (2004). Activation of the growth-differentiation factor 11 gene by the histone deacetylase (HDAC) inhibitor trichostatin A and repression by HDAC3. *Mol Cell Biol*, 24, 5106–5118. [PubMed: 15169878]
- Zhou Y, Jiang Z, Harris EC, Reeves J, Chen X, & Pazdro R (2016). Circulating Concentrations of Growth Differentiation Factor 11 Are Heritable and Correlate With Life Span. *J Gerontol A Biol Sci Med Sci*.

Figure 1 –.

A schematic of extracellular, membrane-bound, and intracellular regulation of GDF 11 and the various inhibitors of GDF 11. SMURF – SMAD Specific E3 Ubiquitin Protein Ligase.

Figure 2–.

A summary of the dynamic between GDF 11 expression and proliferation of NGN3+ cells in the developing pancreas. In the Gdf 11-null pancreas, there are fewer insulin-producing cells and glucagon-producing cells than were found in wild type pancreas.

Figure 3 –.

Negative regulation of late stage erythropoiesis by GDF 11. Erythropoiesis independent of erythropoietin and regulated by GDF 11 leads to accumulation of immature erythrocyte progenitors. Consequently, mature erythrocytes are less abundant, leading to the pathological conditions described. ProE -Proerythroblast; BasoE – Basophilic Erythroblast; PolyE – Polychromatic Erythroblast; OrthoE – Orthochromatic Erythroblast.