# Bioinformatics Network Analyses of Growth Differentiation Factor 11 Anti-aging Study

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# Abstract

Growth differentiation factor 11 (GDF11) has been implicated in rejuvenative functions in age-related diseases. The molecular mechanisms connecting GDF11 with these anti-aging phenomena (reverse age-related cardiac hypertrophy, vascular and neurogenic rejuvenation, et al.) are still unclear. In this study, we aim to uncover the molecular functions of GDF11 using bioinformatics and network-driven analyses at human gene and transcription levels using gene co-expression network analysis and transcription factor network analysis. Our data suggests that GDF11 is involved in variety of functions such as apoptosis, DNA repair and telomere maintenance and interaction with key transcription factors such as MYC proto-oncogene (MYC), specificity protein 1 (SP1) and ETS proto oncogene 2 (ETS2). Our findings shed lights on the functions of GDF11 and provide insights into the role of GDF11 in aging.


# Introduction

Aging is a worldwide health issue. According to a report from Department of Economic and Social Affairs, Population Division, United Nations, the number of people aged 65 years or over was 727 million worldwide in 2020, with global elderly population increasing and estimated to reach 1.5 billion by 2050 [1]. Aging is not only the change of appearance, wrinkles or grey hairs, but also hypofunction of organs and tissues. Cell senescence is considered the fundamental reason underlying age-related dysfunctions [2]. The goal of geriatric research is to find solutions to reverse cell senescence and furthermore to decrease age-related disorders.

In 2013 Loffredo FS et al. identified growth differentiation factor 11 (GDF11) as a key factor in the rejuvenation of age-related heart hypertrophy, making GDF11 a prime candidate for reversing age-related diseases [3]. Specifically, they found that administering GDF11 for four weeks led to the normalization of the enlarged cardiac myocytes, increased myocardial contractility and decreased heart failure index, indicating heart rejuvenation at both physiological and functional levels. Additional studies have supported these findings, showing evidence for the role of GDF11 in rejuvenating vascular, nervous, and skeletal systems [4-7]. GDF11 is a member of transforming growth factor (TGF) –β super family, known to play an important role in development by maintaining the balance between proliferation and differentiation. GDF11 can halt the cell cycle at G1 phrase through p21cip1/p27kip1, and induce differentiation of cells into maturation [8], thereby controlling the amount of mature cells. However, these processes fail to explain the role of GDF11 in rejuvenation. To date, the exact mechanisms through which GDF11 rejuvenates organs and reverses aging remain unclear. It is our goal to implement bioinformatic and network driven analyses approach to understand the role of GDF11 in aging and anti-aging in biological systems to better improve global age-related diseases issue. Bioinformatics analysis is a useful method to investigate biological molecules with unclear functionalities, providing the potential to reveal novel functions of some specific genes, and may also reveal potential related molecules.

In this study, we employ various bioinformatics methods to explore the mechanisms involved in GDF11-mediated rejuvenative functions. By applying a bioinformatics analysis approach, we aim to expand our understanding of GDF11 and the underlying mechanisms involved in rejuvenating physiological systems, as these discoveries can provide the direction of future medical research. The bioinformatics methods used in this study include gene co-expression network analysis and transcription factor network analysis. These powerful methodologies have the capacity to reveal the functions of GDF11.

# Results

### 4.1 Gene Co-expression Network Analysis

Using gene co-expression networks we found GDF11 to be part of 23 modules (see Experimental Procedures 2.1) from 11 different systems or organs (see **Supplementary Table S1 online**). We analyzed the biological pathways of each GDF11-containing module (see **Supplementary Table S2 online**) to inform on the general functions of GDF11, as summarized in **Table 1**.

The common functions of GDF11 mostly related to apoptosis, DNA repair, telomere maintenance, some key pathways (PI3K/AKT pathway, ERK/MAPK pathway, DAG/IP3/Ca2+ pathway, toll-like receptor pathway, GPCR pathway), transcription regulation, proliferation and metabolism. However, endoplasmic reticulum (ER) stress, collagen formation, axon guide, ion transport and circadian rhythm were found in some specific tissue types. From **Table 1** and **Supplementary Table S2**, we conclude the main age-related functions of GDF11 gene co-expression modules in each tissue and system.

#### 4.1.1 Apoptosis

Apoptosis is the most common function among systems from our data analyses, also playing a major role in aging and age-related diseases. We observed GDF11 in caspase cascade related apoptotic cleavage in visceral adipose, hippocampus, cerebellar hemisphere, left ventricle, small intestine terminal ileum, skeletal muscle, pituitary and prostate tissues. In addition, our results suggested GDF11 in intrinsic apoptosis as SMAD/CTCF/MYC signaling, c-Jun N-terminal kinases (JNK) and pro-apoptotic molecule Bcl-2-associated death promoter (BAD) signals death, and in extrinsic apoptosis through death receptor (DR) 3 and DR4/5. In summary, GDF 11 was identified as a highly related player in intrinsic and extrinsic caspase apoptosis pathways in aging (see **Figure 1**).

#### 4.1.2 DNA repair

DNA damage increases with aging and declining DNA repair, subsequently leading to cell senescence and even cell death, conversely. We observed GDF11 as potentially beneficial for DNA repair in many aspects, including single stranded DNA damage repair (global genomic-NER (GG-NER), base excision repair (BER) and DNA mismatch repair (MMR)), and double-strand breaks repair (homology directed repair (HDR) as well as nonhomologous end-joining (NHEJ)). In adipose we found the GDF11 module involved into both single and double stranded repair GG-NER, BER, MMR, and HDR. Double stranded breaks repair is also present in left ventricle, both HDR and NHEJ, and in pituitary, only BER. Moreover, GDF11 module has a function of DNA damage check point in the tibial nerve.

#### 4.1.3 Telomere maintenance

Telomere and telomerase are likely to play a role in circadian biological clocks in cellular replicative senescence. Previous studies up until now have focused primarily on telomere maintenance and slowing down the aging process [9]. From our results, the gene co-expression module of GDF11 is involved in telomeres extension in adipose, and has been associated to telomeres, telomerase, cellular aging and immortality in left ventricle.

Genetics co-expression network studies of GDF11 have also implicated GDF11 in relieving endoplasmic reticulum (ER) stress which caused by aging, in NCAM signaling pathway which could benefit cell survival and regeneration, Toll-like receptor pathway for necroptosis which could help to clear aging cells, PI3K/AKT signaling for proliferation and lastly, in MAPK/ERK signaling for proliferation and angiogenesis. Our findings involving apoptosis, DNA repair, telomere maintenance and the other GDF11 functions may help explain the role of GDF11 in reversing age-related diseases.

### 4.2 Transcription Factor Network Analysis

We used FANTOM5 [10], a comprehensive analysis of gene regulation in different cell types, to analyze the transcriptomic network around GDF11 in different adult cell types. We found GDF11 transcriptomic network in 76 cell types across 10 human systems (the nervous system, cardiovascular system, digestive system, respiratory system, urinary system, skeletal muscle system, endocrine system, immune system, female and male reproductive system). And we found an interesting phenomenon that among different cell types the transcription factors were similar (see **Supplementary Table S3 online**). As an example, we analyzed the largest transcriptomic network of GDF11 in the neural stem cell (see **Figure 2**). Yellow nodes represent the first layer neighbors and lavender nodes are the second layer neighbors of GDF11.

From our data, we concluded that the most common transcription factor neighbors of GDF11 are cAMP response element-binding protein 1 (CREB1) and nuclear transcription factor Y subunit alpha (NFYA), showing in 93.42% cell types (see **Supplementary Table S3 online**). This suggests that CREB1 and NFYA are highly conserved and related to the most common function of GDF11 across cell types and systems. CREB1 is connecting GDF11 with CTCF (a transcriptional repressor of MYC [11]), ETS Proto-Oncogene 2 (ETS2, reported as a regulator of telomerase [12]), SMAD4 (a member of GDF11 downstream pathway) and first layer SP/KLF family (a transcription factor family that maintains stem cell proliferation and differentiation [13]). NFYA is the post-transcriptional regulation factor and also connected with SP/KLF family. These findings emphasize the importance of GDF11 and related transcription factors in apoptosis, DNA repair, telomere maintenance, proliferation and differentiation, which are associated with longevity. Lastly, we observed forkhead box (FOXO) family, homeobox (HOX) family, regulatory factor X (RFX) family, E2F transcription factor family and some other longevity related factors in this transcriptomic network.

## Discussion

Previous studies showed that GDF11 may reverse age-related heart hypertrophy, improve functions in the nervous system, and increase angiogenesis in aging animals [3,4]. During the embryonic stage, GDF11 could inhibit proliferation and promote differentiation of differentiable cells [8]. However, the functions of GDF11 in adults, especially its roles in reversing age-related diseases, remain poorly understood. One potential mechanism could involve GDF11 interacting with FOXO family and inhibit hypertrophy [3]. Our network analyses also confirm the involvement of the FOXO family in GDF11 functions in our transcription network. Despite this possibility, the mechanism behind the connection between GDF11 and rejuvenation are limited. Through our integrative network analyses, we aim to expand our knowledge of GDF11 and its functions in adulthood.

Based on our bioinformatics analyses of gene co-expression networks and transcription factor networks, we found that GDF11 is involved in apoptosis, DNA repair, telomere maintenance, transcription regulation, cell survival, angiogenesis and regeneration. We also identified potential novel related transcription factors of GDF11, including MYC, SP family, CREB1 and ETS2. Below we discuss in detail the common and novel functions uncovered by our GDF11 network analyses.

Based on human genetic data analysis, apoptosis is the most common function of GDF11 among systems (adipose tissue, nervous system, cardiovascular system, digestive system, skeletal muscle tissue and endocrine system), and through SMAD/CTCF/MYC signaling and JNK/BAD route to promote apoptosis both in the intrinsic and extrinsic caspase apoptosis pathways (**Figure 1**). Apoptosis plays a significant role in longevity. In stable cells and continuously dividing cells, apoptosis could eliminate presumably dysfunctional cells. Reactivation of

apoptosis may be beneficial in clearing cancerous and senescent cells in aging. During the aging process, apoptosis activity decreases, resulting in a decrease of normal homeostatic cell turnover rate [14]. Several experiments have been carried out to verify the role of GDF11 in promoting apoptosis. Wang et al. also found GDF11 could induce apoptosis. They added different concentrations of GDF11 to the culture medium of C17.2 neural stem cells. After 72 hours of culture, compared with the control group, different concentrations of GDF11 treatment group (12.5 ng/ml, 25 ng/ml, 50 ng/ml, 100 ng/ml) showed neural stem cell differentiation, and the high concentration of GDF11 treatment showed obvious apoptosis (the apoptosis rates of the four groups of 12.5, 25, 50, 100ng/ml were 2.1%, 9.8%, 13.1%, 17.7%, respectively) [15]. Zhang et al. found that exogenous GDF11 can cause apoptosis in myocardial H9C2 cell line, whereas GDF11 knockdown reduces apoptosis [16]. Liu et al. also found that the PANC-1 cell line overexpressing GDF11 by lentivirus showed increased apoptosis when analyzed for the role of GDF11 in pancreatic cancer. At the same time, cells treated with RNAi reduced GDF11 showed decreased apoptosis [17]. Millet et al. believe that the TGFβ family promotes apoptosis by binding to downstream SMAD2/3 and inhibiting bcl-2, an anti-apoptotic protein [18]. And after silencing TGFβ downstream SAMD2/3, apoptosis of stem cell-like cell lines NRP-154 and NRP-152 was reduced [19].

Our results suggest that GDF11 could promote apoptosis both in the intrinsic and extrinsic caspase apoptosis pathways, and provide a few novel specific GDF11 downstream apoptosis pathways as GDF11/SMADs/CTCF/MYC pathway and GDF11/JNK/BAD pathway (**Figure 1**). The former perfect previously discovered GDF11/SMADs/bcl-2 intrinsic apoptosis signaling with adding intermediate process of CTCF/MYC/p53. The later give us a new research direction of GDF11 involved in apoptosis. The classical pathway of GDF11 is through SMADs which acts by regulating diverse biological effects by partnering with various transcription factors [20]. MYC inhibitor CTCF is one of the GDF11/SMADs downstream transcription factor [11] and also appearing in our GDF11 transcription network with MYC. MYC has been recently linked to longevity [21]. A possible explanation for this might be through apoptosis as low MYC could activate p53 and induce BCL2-mediated intrinsic apoptosis. Previous studies have concerned p53 is the directly downstream of MYC and low level overexpression of MYC could induce sustained apoptosis [22]. So the GDF11-induced apoptosis signaling through SMADs/CTCF/MYC/p53 which suggested from our results is logical. Besides the GDF11/SMADs/CTCF/MYC signaling, our results also indicate GDF11 involved in apoptosis through JNK/BAD pathway and DR. The other two processes are not as specific as GDF11/MYC pathway, more experiments are needed for confirmation. From our discussion we can see the advantage of bioinformatics analysis of pointing out unknown information for future research. And from the above analyses we believe GDF11 could promote apoptosis through intrinsic and extrinsic caspase apoptosis pathways, and benefit age-related diseases as a reasonable result.

From our results, GDF11 is also involved in DNA damage repair and DNA damage checkpoints. In human cells, DNA damage occurs every day due to internal and external environmental reasons such as UV or x-ray. Extensive and cumulative DNA damage will result in cell carcinogenesis, cell death or apoptosis. Along with ageing, the rate of DNA repair decreases and a large amount of DNA damage accumulates. In this case, age-related diseases are prone to occur, and sometimes even cause cancer [23]. Another evidence is that several premature senility syndromes have potential DNA repair deficiencies [24]. In this study, we saw GDF11 was involved in DNA repair in many tissue systems, such as adipose tissue, nervous system, cardiovascular system, and endocrine system. Among these systems, GDF11 is involved in DNA damage repair checkpoint and single- or double-strand break repair signaling, and suggests that these DNA repair mechanism is related to PI3K/AKT signaling and SP1, the transcription factor associated to GDF11 at transcriptional level. Many studies have shown that the PI3K/AKT signaling pathway is the direct pathway in response to DNA damage, and several PI3K/AKT signaling downstream proteins guide cell cycle

checkpoint activation, DNA repair, and activation of apoptosis after unsuccessful repair [25,26]. SP1 is a promoter-binding protein and is a downstream molecule of PI3K/AKT. Most genes have multiple SP1 sites in the proximal promoter region. Studies have shown that the consumption of SP1 makes the cells sensitive to DNA damage, decrease the repair rate, and increase the double-strand DNA damage frequency [26]. Beishline et al. found that SP1 appeared at DNA damage region 7.5 minutes after DNA damage and persisted at the DNA break site for at least 8 hours, and the consumption of SP1 inhibited the repair of DNA breaks [27]. From our results of gene and transcriptional analysis in this study, GDF11 may be involved in the detection and repair of DNA damage through the SMADs/SP1 signaling pathway and the PI3K/AKT signaling pathway. With the successful repair of DNA damage, the organism will maintain stable internal environment. Normal cell proliferation activity and cell function are conducive to the recovery of organ function in aging tissues.

Our results also link GDF11 with telomere function. Telomere is a region of repetitive nucleotide sequences at each end of a chromosome, which protects the end of the chromosome from deterioration or from fusion with neighboring chromosomes. During chromosome replication, the enzymes that duplicate DNA cannot continue their duplication all the way to the end of a chromosome, so in each replication the end of the chromosome is shortened, which has been associated with aging. The length of telomere has become a symbol of longevity and telomere shortening is associated with age-related diseases, prolonging telomere length has become the research direction of delaying aging. Our research suggested that GDF11 can up-regulate telomere by regulating telomerase reverse transcriptase (TERT), through transcription-related factors SP1, ETS2 and GDF11/SMAD/CTCF/MYC signaling pathways. Telomerase acts as reverse transcriptase in the elongation of telomeres. TERT is a catalytic subunit of the enzyme telomerase, which, together with the telomerase RNA component (TERC), comprises the most important unit of the telomerase complex. The TERT gene promoter region has all SP1, ETS2 and MYC binding sites, and the three could synergistically activate TERT transcription and direct telomere elongation [28]. Among them, SP1 is effective but not necessary. SP1 overexpression can activate TERT, but SP1 site mutation has little effect on TERT [29]. ETS2 is important for driving TERT gene expression, silencing of ETS2 results in a decrease in TERT gene expression [30]. MYC plays an important role in TERT expression and telomere elongation. MYC regulates TERT in dual-direction, and there is a feedforward regulation [31]. MYC can activate telomerase to extend the terminal telomere of the gene and return the cell to a sustained division state[32]. SP1, ETS2 and MYC all belong to GDF11 transcriptomic network from our results. And transcription of TERT can be inhibited by E2F transcription factor 1 (E2F 1)[33], which is also involved in the GDF11-related transcription factor regulatory network. These results strongly suggest that GDF11 may have bidirectional regulation of TERT and telomere length. It can promote telomere synthesis by up-regulating TERT through SP1, ETS2 and MYC. At the same time, TERT and telomere synthesis can be inhibited by MYC and E2F1, also TERT level can be precisely regulated by MYC feedforward reaction. These bidirectional regulation for telomerase is necessary. Because TERT overexpression will induce tumor-like hyperproliferation, which is unfavorable to tissue and organ stability. But with TERT transcription bidirectional regulation, telomere can be regulated by controlling level and prolong telomere length, slow down cell senescence, restore organ function, maintain body homeostasis and prolong life of organism to a certain extent. Recent study has shown that GDF11 has a positive effect on the maintenance of telomere length which supports the conclusions of this study, but that paper does not provide specific mechanism analysis [34]. In this study, bioinformatics analyses showed that GDF11 could through SMAD/CTCF/MYC signaling pathway, SP1 and ETS2 regulate TERT to maintain telomere length, which provides direction and target for subsequent experimental verification.

# Conclusions

From our bioinformatics network analyses, promoting apoptosis, repairing DNA damage, and maintaining telomere length could be concluded, which are closely related to aging. They are the main mechanism for promoting proliferation and maintaining normal physiological functions of aging organisms. They can explain the anti-aging effects of GDF11 in various tissues and organs researches recently. Results in the gene co-expression network and transcription factor network analyses results turned out to be complementary of each other. Taken together, these functions of GDF11 from our study could explain the anti-aging phenomenon, and provide related transcription factors, like MYC, SP1 and ETS2, in these processes.

In summary, our comprehensive network analyses of GDF11 provide in silico predictions of GDF11 functions, revealing both previously known (such as transcription regulation) and novel (such as regulation of apoptosis, DNA repair and telomerase) functions of GDF11, which may underlie its anti-aging effects. Although we used multiple credible databases and complementary methodologies to cross-validate our findings, the accuracy of our results rely the quality and comprehensiveness of the datasets involved and further experimental testing is warranted to validate the predictions of the network analyses. It is our goal that these findings contribute to the clarity the of GDF11 mechanisms in rejuvenation and identify the candidate biomarkers or therapeutic targets for the future research.

# Experimental Procedures

### 7.1 Identification of Functional Modules Involving GDF11

To explore the molecular functions of GDF11, we first extracted functional modules that contain GDF11, as determined by various approaches. A functional module is defined as a group of molecules which share similar functions or involved in a common biological process [35]. Exploring functional modules could expand the research power from a targeted molecule such as GDF11, to a variety of related molecules, and thus provide further understanding of the functional roles of GDF11 as well as uncovering correlated genes around a target gene. To this end, we used 1) tissue-specific gene co-expression networks analysis, 2) transcription factor networks analysis.

7.1.1 Gene co-expression networks

Gene co-expression networks are statistics based on networks that capture genes from different microarray experiments [35] and the co-expressed genes are functionally related. It is a practical method to uncover the gene whose functions are still unknown and provides insight in how the gene perform in some specific biological processes [36]. We used weighted gene co-expression network analysis (WGCNA) [37] to construct gene co-expression networks. It is a widely used machine algorithm for selecting modules and constructing gene co-expression networks. Numerous studies have demonstrated the effectiveness of WGCNA in building up the biological functional modules [38,39].

7.1.1.1 Code availability

The WGCNA R routine source is https://horvath.genetics.ucla.edu/html/CoexpressionNetwork/Rpackages/WGCNA/ Tutorials.

Using WGCNA, we constructed human co-expression networks of various tissues using gene expression data from the Genotype-Tissue Expression project (GTEx) [40]. GTEx is the most comprehensive tissue-specific transcriptomic and genetic dataset derived from human tissues, providing a platform to understand the relationship between

genetic variation and gene expression. GTEx consists of whole-genome sequence and RNA-sequencing data from nearly 1000 deceased adult donors and 53 tissue sites (e.g. brain, liver, etc.). We used the human gene co-expression networks built by WGCNA using GTEx RNA-sequencing data to extract the genetic co-expression modules that contain GDF11. The GTEx data we applied and downloaded is the gene-level transcripts per millions (TPMs) data as GTEx_Analysis _2016-01-15_v7_RNASeQCv1.1.8_gene_tpm.gct.gz from GTEx official website https://gtexportal.org/ho me/datasets. We did a few steps of preprocessing for normalizations as WGCNA website suggests (https://horvath.genetics.ucla.edu/html/CoexpressionNetwork/Rpackages/WGCNA/faq.html): log-transforming the data using log2(x+1) as variance-stabilizing transformation and removing features whose counts are consistently low (removing all features that have a count of less than 1 in more than 60% of the samples).

7.1.2 Transcription factor network

We used Functional ANnoTation Of the Mammalian genome (FANTOM) 5, a comprehensive analysis of gene regulation in different cell types to analyze the transcriptomic network of GDF11. FANTOM5 provides an overall map of gene activity across the human body and as well as a holistic view of the complex networks that regulate gene expression across the wide variety of cell types [10]. The FANTOM5 networks are provided as tab-separated text files with three columns (column 1: the transcription factor gene, column 2: the target gene, column 3: the edge weight) [41]. We searched GDF11 at the second column and got it's related transcription factors and their weight value at FANTOM5 official website http://fantom.gsc.riken. jp.

### 7.2 Annotate the Functions of the GDF11 Modules Using Functional Enrichment Analysis

After identifying the co-expressed genes and relevant transcription factors of GDF11 from each functional module, we used gene set enrichment analysis (GSEA) [42]. With GSEA (website http://software.broadinstitute.org/gsea/index.jsp), we annotated the enriched biological pathways shared by the genes in the same module, providing us with insight of GDF11 functions. The annotated pathways were collected from BioCarta, KEGG and Reactome database. Pathways reaching $P \leq 0.05$ and false discovery rate (FDR) $\leq 0.01$ were collected. Overlaps with shared gene numbers $\geq 3$ and fold enrichment at top 30% were considered as significant pathways [43].

### 7.3 Visualize analysis results

Subsequently, we used pathway builder tool (website http://www.proteinlounge.com/ PathwayBuilder.aspx) to present the molecular mechanisms and pathways of apoptosis [44]. Cytoscape [45] (website https://cytoscape.org) was used to further investigate molecular relationships within transcription factor modules and pinpoint key regulators.

## Declarations

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http://www.proteinlounge.com/ PathwayBuilder.aspx) and Cytoscape (website https://cytoscape.org). We would like to thank Ingrid Cely (University of California, Los Angeles, USA) for English language editing.

### Data Availability

All data generated during this study are included in this published article (and its Supplementary Information files). The datasets analysed during the current study are available in the Genotype-Tissue Expression project (GTEx) repository (https://gtexportal.org/ho me/datasets, reference number 8), and the Functional ANnoTation Of the Mammalian genome (FANTOM) 5 repository (http://fantom.gsc.riken. jp., reference number 12).

### Author Contributions

F.Z. performed the experiments, analyzed the data, prepared figures 1-2 and wrote the main manuscript text. X.Y. and Z.B. conceived the project and revised the manuscript.

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# Tables

**Table 1 Top Functions of GDF11-containing Genetic Modules in Human Co-expression Networks**

| Tissue | Top Functions |
|---|---|
| Adipose | apoptosis, DNA Repair, telomere maintenance, transcription regulation, proliferation |
| Nervous System | apoptosis, DNA damage checkpoint, axon guidance, insulin/IGF-1 pathway, PI3K/AKT pathway, NCAM signaling, expression, immune, metabolism |
| Cardiovascular System | apoptosis, DNA repair, telomere maintenance, GPCR pathway, expression, metabolism |
| Digestive System | apoptosis, biological oxidation, ER stress, ERK/MAPK pathway, DAG/IP3/Ca2+ pathway, expression, metabolism |
| Liver | NOTCH pathway, collagen formation, NCAM signaling, extracellular matrix |
| Lung | ion transport, metabolism |
| Kidney | extracellular matrix |
| Skeletal Muscle | apoptosis, ERK/MAPK pathway, toll-like receptor pathway, muscle contraction, circadian rhythm |
| Endocrine System | apoptosis, DNA repair, PIK3/AKT pathway, axon guide, metabolism |
| Female Reproduction System | translation regulation, expression |
| Male Reproduction System | apoptosis, IGF signaling, transcription regulation, proliferation, metabolism |

*Top pathways: The pathways were selected and summarized from significant pathways ($P \leq 0.05$, $FDR \leq 0.01$, shared gene numbers$\geq 3$ and fold enrichment at top 30%) and listed in no particular order. Detailed data was showed in Table S2.

# Figures

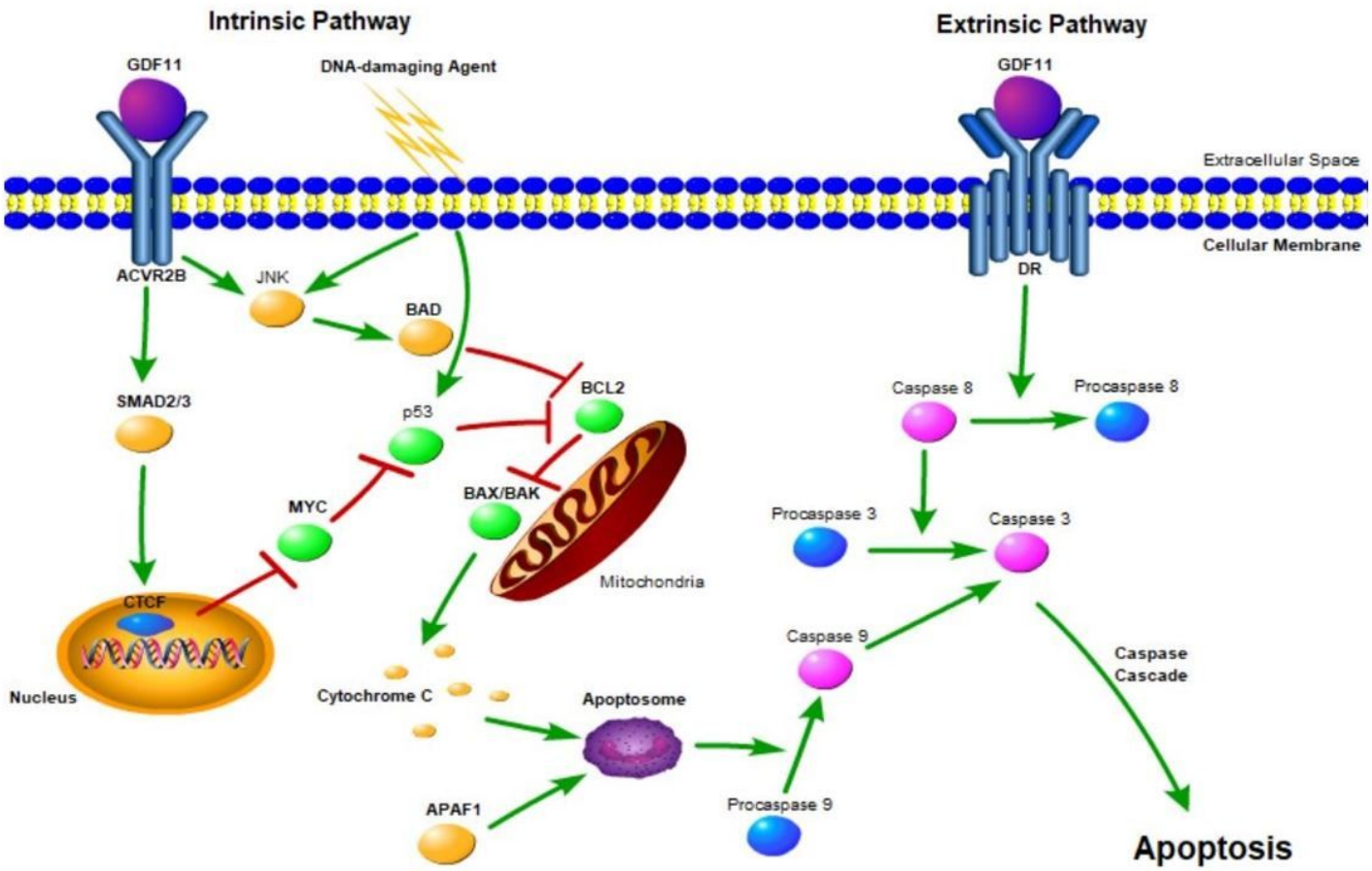


Figure 1

GDF11 Involved in Intrinsic and Extrinsic Apoptosis Pathway From our genetic co-expression network analysis results, GDF11 was involved in intrinsic and extrinsic apoptosis pathway. GDF11 could stimulate intrinsic apoptosis pathway through SMADs/CTCF/MYC/p53 pathway or JNK/BAD pathway and extrinsic apoptosis pathway through DR. The whole signaling pathways were suggested from our bioinformatics analysis. Some pathways are partly confirmed, but future experimental verification would be needed.

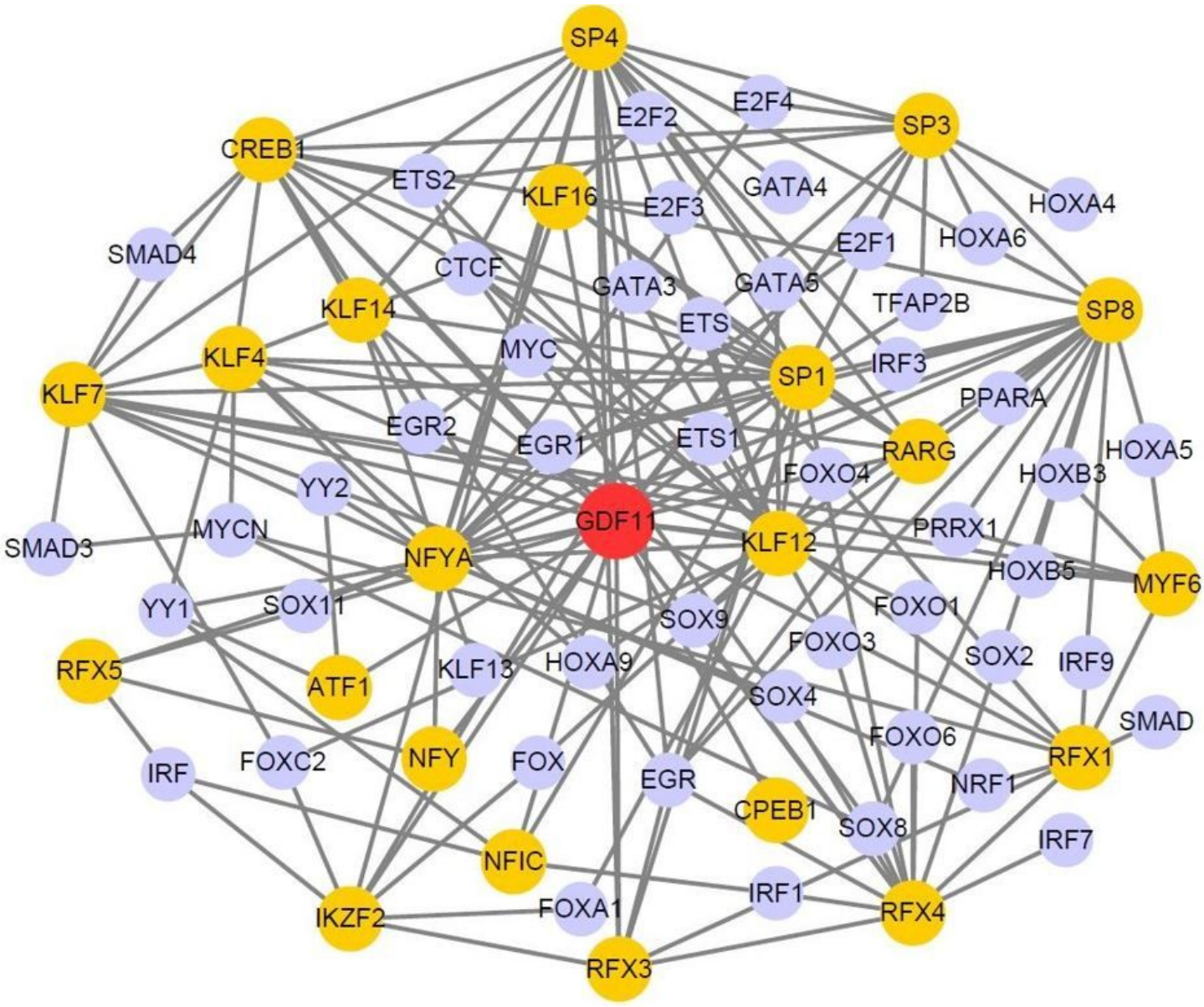


**Figure 2**

FANTOM5 Neural Stem Cell Transcription Factor Network of GDF11 Visualize GDF11 interactive transcription factors in neural stem cell. Yellow nodes represent the 1st layer interactive factor of GDF11 and lavender nodes represent the 2nd layer neighbor factors. The grey lines represent correlation of the transcription factors.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.pdf