RNA sequencing analysis of FGF2-responsive transcriptome in skin fibroblasts

Baojin Wu [1], **Xinjie Tang** [1], **Zhaoping Zhou** [1], **Honglin Ke** [2], **Shao Tang** [3], **Ronghu Ke** [Corresp. 1]

[1] Plastic, Huashan Hospital Affiliated to Fudan University, Shanghai, China

[2] Emergency, Huashan Hospital Affiliated to Fudan University, Shanghai, China

[3] Department of Statistics, Florida State University, CA, USA

Corresponding Author: Ronghu Ke
Email address: ronghuke@163.com

Objective: FGF2 plays important roles in the skin wounds. However, the mechanisms of FGF2 in wound repair have not been fully elucidated. This study aimed to comprehensively decipher the FGF2-responsive core genes and its regulatory networks in dermal fibroblasts. **Methods:** RNA-seq was performed to compare the transcriptional profiles between FGF2-treated skin fibroblasts and control. Subsequently, GO (Gene Ontology) analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis were used for the enrichment of DEGs (differentially expressed genes). Moreover, the co-expression networks between mRNAs and LncRNAs were constructed by Pearson correlation analysis and visualized by Cytoscape software. **Results:** A total of 1475 DEGs was identified. GO annotation showed these DGEs were mainly enriched in the ECM organization, cell adhesion and cell migration. KEGG pathway analysis demonstrated the FGF2-responsive DEGs were significantly involved in ECM-receptor interaction, PI3K-Akt signaling and Hippo pathway. Meanwhile, 213 differentially expressed LncRNAs were identified and three key LncRNAs (HOXA-AS2, H19, and SNHG8) were highlighted in FGF2-treated fibroblasts. **Conclusion:** The current study comprehensive analyzed the FGF2-responsive transcriptional profile and provide candidate mechanisms that may account for FGF2-mediated skin wounds.

Title page

Title: RNA sequencing analysis of FGF2-responsive transcriptome in skin fibroblasts

Authors: Baojin Wu[1#]; Xinjie Tang[1#]; Zhaoping Zhou[1]; Honglin Ke[2]; Shao Tang[3], Ronghu Ke[1§]

Affiliation: [1]Department of Plastic Surgery, [2]Department of Emergency, Huashan Hospital Affiliated to Fudan University, Shanghai, China. [3]Department of Statistics, Florida State University, FL, USA.

[#]Baojin Wu and Xinjie Tang are equal to this work.

[§]Correspondence to:

Ronghu Ke, MD

Department of Plastic Surgery, Huashan Hospital, Fudan University, Shanghai, China

No. 12, Wu Lu Mu Qi Road (M), Shanghai 200040, China.

Tel: +86(021) 5288 7832

Email: ronghuke@163.com

Abstract

Objective: FGF2 plays important roles in the skin wounds. However, the mechanisms of FGF2 in wound repair have not been fully elucidated. This study aimed to comprehensively decipher the FGF2-responsive core genes and its regulatory networks in dermal fibroblasts.

Methods: RNA-seq was performed to compare the transcriptional profiles between FGF2-treated skin fibroblasts and control. Subsequently, GO (Gene Ontology) analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis were used for the enrichment of DEGs (differentially expressed genes). Moreover, the co-expression networks between mRNAs and LncRNAs were constructed by Pearson correlation analysis and visualized by Cytoscape software.

Results: A total of 1475 DEGs was identified. GO annotation showed these DGEs were mainly enriched in the ECM organization, cell adhesion and cell migration. KEGG pathway analysis demonstrated the FGF2-responsive DEGs were significantly involved in ECM-receptor interaction, PI3K-Akt signaling and Hippo pathway. Meanwhile, 213 differentially expressed LncRNAs were identified and three key LncRNAs (HOXA-AS2, H19, and SNHG8) were highlighted in FGF2-treated fibroblasts.

Conclusion: The current study comprehensive analyzed the FGF2-responsive transcriptional profile and provide candidate mechanisms that may account for FGF2-mediated skin wounds.

Key word: FGF2, RNA-seq, fibroblast, wound healing

55

56

57 **INTRODUCTION**

58 Fibrotic diseases occur almost every organ including lung, liver, kidney, heart or skin.
59 Fibrosis is a fundamental wound healing process that allows the ordered replacement of dead or
60 damaged cells after injury. Despite numerous crucial differences among fibrotic pathologies of
61 various organs, the ubiquity of the fibrotic response reveals commonalities among these tissues
62 to the fibrotic program (Zeisberg & Kalluri 2013). One of these commonalities is the paradigm
63 of fibroblast activation, during which fibroblasts are responsible for deposition of extracellular
64 matrix proteins (Hinz 2016). Unfortunately, no curative treatments for fibroblast activation are
65 yet available, highlighting the critical need for a better fundamental understanding of molecular
66 mechanisms that may be therapeutically tractable.

67 Fibroblast growth factor 2 (FGF2), also known as basic fibroblast growth factor (bFGF), is
68 one of the family members of mammalian fibroblast growth factors. FGF2 has been shown to be
69 involved in mitogenesis, differentiation, proliferative lifespan, survival, oncogenesis, and stem
70 cell self-renewal (Akl et al. 2016). Exogenous FGF2 has shown to have both accelerative and
71 anti-fibrotic effects in various types of skin wounds including acute incision wounds, avulsions,
72 and burn wounds (Akita et al. 2008; Matsumine 2015; Ono et al. 2007). Moreover, FGF2
73 antagonized TGFβ1-induced differentiation of fibroblasts and thus affected fibrosis during
74 wound repair (Dolivo et al. 2017a). Importantly, FGF2 was observed to induce a shift in gene
75 expression to a more anti-fibrotic signature attenuated the expression of pro-fibrotic genes,
76 including collagen I, collagen III, α-SMA, and MMP-1(Dolivo et al. 2017b). In spite of the
77 extensive observations, the mechanisms by which FGF2 regulates the fibrotic response remain
78 incompletely understood.

Microarray and high-throughput sequencing technologies are powerful tools that can be used to investigate potential targets genes for diseases and underlying pathological mechanisms(Mery et al. 2019). In the present study, we performed RNA-seq to compare the expression profiles between FGF2-treated fibroblasts and control fibroblasts. Following the screening out the differentially expressed genes (DEGs), we identified the key genes and the signaling pathways triggered by FGF2 in skin fibroblasts by bioinformatics analysis. Thus, the study would provide a comprehensive understanding of the mechanisms regulated by FGF2 in skin fibroblasts, which may guide subsequent studies on skin wounds.

MATERIALS AND METHODS

Cell cultures and reagents

Human skin FBs were prepared as previously described (Xuan et al. 2014). The collection of skin tissues was approved by Huashan hospital of ethical review board (Ref: 2020-350). FBs were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (PAA Laboratories, Etobicoke, Ontario, Canada), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco BRL) at 37˚C in a humidified atmosphere containing 5% CO2. When confluent, the cells were trypsinized using a 0.25% trypsin/0.02% EDTA solution (Sigma, St Louis, MO, USA) and subcultured at a 1:3 ratio.

RNA isolation and RNA sequencing

After 48 hours of culture, total RNA was extracted from 7 samples (4 samples from FGF2-treated FBs, 3 samples from control FBs) using Trizol (Invitrogen, Carlsbad, CA). The libraries were constructed by RNA Library Prep Kit for Illumina (NEB, MA, and USA) according to the manufacturer's instructions. The library products were sequenced using Illumina HiSeqTM 2500 (Illumina, CA, USA). Index of the reference genome was built and paired-end clean reads were aligned to the reference genome using STAR. HTSeq v0.6.0 was used to count the reads numbers mapped to each gene. And then FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. Differentially expressed RNA between the two

groups was performed using the DESeq. 2R package (1.10.1). The resulting p values were adjusted using the Benjamini and Hochberg's approach. Genes with an adjusted $p < 0.05$ and $|\log2FC| > 1.0$ were assigned as differentially expressed genes (DEGs) or LncRNAs.

Annotation for DEGs

For the functional annotation, all the DEGs were analyzed by using the DAVID web tool (https://david.ncifcrf.gov/). GO Annotation for DEGs included three terms, molecular functions (MF), biological processes (BP), and cellular components (CC) of genomic products. As for pathway enrichment, DEGs was analyzed by Kyoto encyclopedia of genes and genomes (KEGG) pathway within the DAVID database (Version 6.7). $P< 0.05$ was considered as statistical significance.

LncRNA-mRNA co-expression network construction

To explore interactions among the differentially expressed mRNAs and LncRNAs, the co-expression networks between mRNAs and LncRNAs were constructed by Pearson correlation analysis. The lncRNA-mRNA networks were built according to the expression intensity in mRNAs and LncRNAs. The Pearson coefficients for each pair of mRNA-lncRNA were calculate and screened to obtain the significant correlation pairs with which to construct the networks (coefficient >0.95, $P<0.05$). The PPI network of co-expressed genes of the top 10 lncRNAs was constructed from the network analyst website (https://www.networkanalyst.ca/faces/home.xhtml) (Wang et al. 2020a).

RESULTS

Transcriptional profiles in FGF2-treated fibroblasts.

To determine the effect of FGF2 on the FBs, skin FBs were treated FBs with FGF2 at different doses and assessed the changes in morphology. After Day2 of FGF2 treatment, skin fibroblasts revealed a smaller size, less spindle-like shape in a dose-dependent manner (Fig. 1A), indicating fibroblasts activation was suppressed by FGF2. To understand differential expression profiles between FGF2-treated fibroblasts and control, RNA-seq was performed. Our results showed a

total of 1475 mRNAs were differentially expressed in FGF2-treated fibroblasts (Fig.1B, Table S1), among which 676 genes were up-regulated and 799 genes were down-regulated (Fig.1C). Meanwhile, there were 213 LncRNAs with a fold change > 2 (Fig.1B, Table S2), among which 80 LncRNAs were up-regulated and 133 were down-regulated (Fig.1C).

GO enrichment for DEGs.

To explore the functions of the DEGs, the 1475 DEGs were annotated by three GO categories (biological processes, molecular functions, and cellular components). For GO biological process, DEGs were involved in the extracellular matrix (ECM) organization, angiogenesis, cell adhesion, positive regulation of cell migration and collagen catabolic process (Fig.2). For molecular function, the DEGs were significantly enriched in regulation of calcium ion binding, growth factor activity, ECM structural constituent, heparin binding and frizzled binding (Fig.2). For cellular component annotation, the most significant terms were enriched in extracellular space, extracellular region, proteinaceous ECM, ECM and plasma membrane (Fig.2).

KEGG pathway analysis.

A KEGG pathway analysis was performed to identify the FGF2-associated pathways in FBs. The 1475 DEGs were mapped to 44 KEGG pathways, and 37 pathways were significantly enriched ($P \leq 0.05$) (Table S3). Several main pathways were represented, including ECM-receptor interaction, PI3K-Akt signaling pathway, hippo signaling pathway, complement and coagulation cascades, TGF-beta signaling pathway, MAPK signaling pathway, proteoglycans in cancer, protein digestion and focal adhesion (Fig.3). Using the STRING database, the 1475 DEGs were filtered into the PPI network.

lncRNA-mRNA co-expression network.

To explore interactions between the differentially expressed mRNAs and lncRNAs, pairs of mRNAs-lncRNAs were analyzed by the Pearson correlation analysis and were sorted by the coefficients, which represented the associations of interactions between mRNA and LncRNA in the networks (Table. S4). Subsequently, the PPI networks of 213 lncRNA were constructed from

the network analyst website. The results showed the top 10 significant LncRNAs included HOXA-AS2, LOC100130417, H19, LOC100507420 and SNHG8 (Fig. 4A). Based on the GO enrichment, ECM organization was significantly involved in the FGF2-mediated biological process. And the potential networks of mRNAs-lncRNAs in the ECM organization were constructed. The three key LncRNAs, including HOXA-AS2, H19 and SNHG8 were identified in the FGF2-mediated ECM organization. The three LncRNAs were associated with six DEGs, including LOX, TGFB1, ITGA10, COL4A1, COL3A1 and PDGFA. Among the 6 DEGs, only ITGA10 was upregulated and other 5 DEGs were downregulated (Fig. 4B).

DISCUSSION

Despite advancements in the understanding of the mechanism of skin wound repair, an effective method for accelerating the process remains to be identified. Fibroblasts play key roles in skin homeostasis. FGF2 contributes to the fibroblastic functions including cell migration, collagen synthesis and cell proliferation. Therefore, understanding the behavior of FGF2 in fibroblasts is essential to identify an effective way to improve wound healing. The present study performed RNA-seq to explore the DEGs of skin fibroblasts in response to FGF2. By bioinformatics analysis, we identified 1475 FGF2-responsive DEGs, including LOX, TGFB1, PDGFA, COL3A1, COL4A1 and ITGA10. Additionally, our results suggested HOXA-AS2, H19 and SNHG8 were involved in FGF2-mediated ECM organization. Thus, the study would provide a comprehensive understanding of the FGF2-responsive genes in fibroblasts, which may guide subsequent studies on wound healing.

Our first goal was to identify the important biological processes in human fibroblasts exposure to FGF2. Our GO analysis demonstrated that the FGF2-associated DEGs were mainly enriched in the ECM organization, cell adhesion and cell migration. This result accorded with the knowledge that FGF2 functioned as important regulator in cell behavior, growth and survival (Akl et al. 2016; Klagsbrun 1992; Przybylski 2009). Also, we further explored the effect of exogenous FGF2 on signal pathways in skin fibroblasts. KEGG enrichment results implied that

FGF2 was mainly involved in classical pathways including ECM-receptor interaction and PI3K-Akt signaling pathway. The result was accordance with our knowledge that FGF signaling is of critical importance during development and homeostasis. Cell interactions with the ECM are mediated by integrins and various signalling cascades are activated, which control cell adhesion, proliferation, morphogenesis, differentiation, and survival (DiPersio & Van De Water 2019). Apart from the two canonical pathways, our enrichment results indicated that FGF2 was significantly associated with Hippo signaling pathway. Yes-associated protein (YAP) and T-Box 5 (TBX5) are key regulators of the Hippo pathway. In line with our results, FGF2 was observed to promote the Hippo/YAP-signaling by inducing the nuclear-YAP expression during lens cell proliferation and differentiation, indicating FGF2 plays important roles in mediating Hippo suppression of YAP transcriptional activity(Dawes et al. 2018). Furthermore, FGFR1 and FGFR2 were showed to directly interact with YAP/TAZ at multiple tyrosine residues independent of upstream Hippo signaling (Azad et al. 2020). Thus, our study, together with previous observations, suggested that the pivotal role of the FGF/FGFR signaling in mediating Hippo signaling pathway.

In parallel, we deciphered the significant LncRNAs regulated by FGF2 in skin fibroblasts. Our study has identified 213 differentially expressed LncRNAs and highlighted the three key LncRNAs (HOXA-AS2, H19, and SNHG8) in FGF2-treated fibroblasts. HOXA-AS2 (HoxA cluster antisense RNA 2), located on chromosome 6, plays vital roles in the development of numerous cancers. HOXA-AS2 has been recently found to exhibit aberrant expression and promote cell migration and invasion abilities in a variety of malignancies, including non-small cell lung cancer, breast cancer, gastric cancer, gallbladder carcinoma, hepatocellular carcinoma and pancreatic cancer(Wang et al. 2018). But, whether HOXA-AS2 exhibits a certain function in fibroblasts remains elusive. Here, our result showed HOXA-AS2 was downregulated in FGF2-treated fibroblasts and was associated with the pathway of ECM organization. Based on the roles of ECM in skin wound, our study indicated HOXA-AS2 might involved in the FGF2-mediated ECM organization during wound healing. LncRNA H19 is an important imprinted gene locating

on human chromosome. H19 has been recently observed to be upregulated in keloid tissues and fibroblasts and silencing of H19 promoted cell viability, migration and invasion of the fibroblasts(Wang et al. 2020b). In the present study, H19 was downregulated in FGF2-treated fibroblasts, suggesting FGF2 attenuated the expression of H19. Conversely, Sun et al reported the H19 levels were remarkably increased in FGF2-treated human umbilical vein endothelial cells(Sun et al. 2019). Thus, our study and previous observation indicated the FGF2-mediated H19 expression appeared to exhibit a context-dependent pattern. SNHG8, an important member of SNHG, has been suggested to promote proliferation and invasion and act as an oncogene in colorectal cancer, gastric cancer and hepatocellular carcinoma. In spite of the significance of SNHG8 in cancer, the regulatory role of SNHG8 in fibroblasts as well as wound healing is rarely reported. Here, our study showed SNHG8 was downregulated in FGF2-treated fibroblasts. Taking into account the significance of FGF2 in wound healing, we postulated that the novel LncRNA provided candidate mechanisms that may account for FGF2-mediated wound healing.

CONCLUSIONS

In summary, the current study carried out RNA-seq to identify the crucial genes in FGF2-treated skin fibroblasts. Our results showed FGF2 was associated with ECM organization as well as other biological processes including cell adhesion and cell migration. Furthermore, our study identified the key genes (LOX, TGFB1, PDGFA, COL3A1, COL4A1 and ITGA10), with LOX being particularly prominent. Notably, our study highlighted the three key LncRNAs (HOXA-AS2, H19, and SNHG8) in FGF2-treated fibroblasts. Further studies are needed to delineate the mechanism that underlies the key LncRNAs in FGF2-mediated cellular functions. Therefore, the present study may provide new ideas and targets for the diagnosis and treatment of skin wound.

ACKNOWLEDGEMENTS

The authors gratefully thank Zi-Qi for his statistical assistance.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by Fund from National Natural Science Foundation of China (No.81401616) and Natural Science Foundation of Shanghai (14ZR1405100).

Declaration of interests

The authors declare that they have no competing interests

Authors' contributions

Baojin Wu , Xinjie Tang and Ronghu Ke conceived and designed the study. Xiejie Tang and Baojin Wu prepared the figures and tables. Zhaoping Zhou, Honglin Ke and Shao Tang analyzed the data. Ronghu Ke prepared the manuscript.

Availability of data and materials

Supporting data can be accessed if the corresponding author agrees.

References

Akita S, Akino K, Imaizumi T, and Hirano A. 2008. Basic fibroblast growth factor accelerates and improves second-degree burn wound healing. Wound Repair Regen 16:635-641. 10.1111/j.1524-475X.2008.00414.x

Akl MR, Nagpal P, Ayoub NM, Tai B, Prabhu SA, Capac CM, Gliksman M, Goy A, and Suh KS. 2016. Molecular and clinical significance of fibroblast growth factor 2 (FGF2 /bFGF) in malignancies of solid and hematological cancers for personalized therapies. Oncotarget 7:44735-44762. 10.18632/oncotarget.8203

Azad T, Nouri K, Janse van Rensburg HJ, Maritan SM, Wu L, Hao Y, Montminy T, Yu J, Khanal P, Mulligan LM, and Yang X. 2020. A gain-of-functional screen identifies the

Hippo pathway as a central mediator of receptor tyrosine kinases during tumorigenesis. Oncogene 39:334-355. 10.1038/s41388-019-0988-y

Dawes LJ, Shelley EJ, McAvoy JW, and Lovicu FJ. 2018. A role for Hippo/YAP-signaling in FGF-induced lens epithelial cell proliferation and fibre differentiation. Exp Eye Res 169:122-133. 10.1016/j.exer.2018.01.014

DiPersio CM, and Van De Water L. 2019. Integrin Regulation of CAF Differentiation and Function. Cancers (Basel) 11. 10.3390/cancers11050715

Dolivo DM, Larson SA, and Dominko T. 2017a. FGF2-mediated attenuation of myofibroblast activation is modulated by distinct MAPK signaling pathways in human dermal fibroblasts. J Dermatol Sci 88:339-348. 10.1016/j.jdermsci.2017.08.013

Dolivo DM, Larson SA, and Dominko T. 2017b. Fibroblast Growth Factor 2 as an Antifibrotic: Antagonism of Myofibroblast Differentiation and Suppression of Pro-Fibrotic Gene Expression. Cytokine Growth Factor Rev 38:49-58. 10.1016/j.cytogfr.2017.09.003

Hinz B. 2016. Myofibroblasts. Exp Eye Res 142:56-70. 10.1016/j.exer.2015.07.009

Klagsbrun M. 1992. Mediators of angiogenesis: the biological significance of basic fibroblast growth factor (bFGF)-heparin and heparan sulfate interactions. Semin Cancer Biol 3:81-87.

Matsumine H. 2015. Treatment of skin avulsion injuries with basic fibroblast growth factor. Plast Reconstr Surg Glob Open 3:e371. 10.1097/GOX.0000000000000341

Mery B, Vallard A, Rowinski E, and Magne N. 2019. High-throughput sequencing in clinical oncology: from past to present. Swiss Med Wkly 149:w20057. 10.4414/smw.2019.20057

Ono I, Akasaka Y, Kikuchi R, Sakemoto A, Kamiya T, Yamashita T, and Jimbow K. 2007. Basic fibroblast growth factor reduces scar formation in acute incisional wounds. Wound Repair Regen 15:617-623. 10.1111/j.1524-475X.2007.00293.x

Przybylski M. 2009. A review of the current research on the role of bFGF and VEGF in angiogenesis. J Wound Care 18:516-519. 10.12968/jowc.2009.18.12.45609

295 Sun B, Ding Y, Jin X, Xu S, and Zhang H. 2019. Long non-coding RNA H19 promotes corneal
296 neovascularization by targeting microRNA-29c. Biosci Rep 39. 10.1042/BSR20182394
297 Wang J, Su Z, Lu S, Fu W, Liu Z, Jiang X, and Tai S. 2018. LncRNA HOXA-AS2 and its
298 molecular mechanisms in human cancer. Clin Chim Acta 485:229-233.
299 10.1016/j.cca.2018.07.004
300 Wang J, Yu H, Yili A, Gao Y, Hao L, Aisa HA, and Liu S. 2020a. Identification of hub genes
301 and potential molecular mechanisms of chickpea isoflavones on MCF-7 breast cancer
302 cells by integrated bioinformatics analysis. Ann Transl Med 8:86.
303 10.21037/atm.2019.12.141
304 Wang Z, Feng C, Song K, Qi Z, Huang W, and Wang Y. 2020b. lncRNA-H19/miR-29a axis
305 affected the viability and apoptosis of keloid fibroblasts through acting upon COL1A1
306 signaling. J Cell Biochem. 10.1002/jcb.29649
307 Xuan YH, Huang BB, Tian HS, Chi LS, Duan YM, Wang X, Zhu ZX, Cai WH, Zhu YT, Wei
308 TM, Ye HB, Cong WT, and Jin LT. 2014. High-glucose inhibits human fibroblast cell
309 migration in wound healing via repression of bFGF-regulating JNK phosphorylation.
310 PLoS One 9:e108182. 10.1371/journal.pone.0108182
311 Zeisberg M, and Kalluri R. 2013. Cellular mechanisms of tissue fibrosis. 1. Common and organ-
312 specific mechanisms associated with tissue fibrosis. Am J Physiol Cell Physiol
313 304:C216-225. 10.1152/ajpcell.00328.2012
314
315

Figure 1

Figure 1. Transcriptional profiles in skin fibroblasts exposure to FGF2

(A) Representative morphology of skin fibroblasts treated with FGF2 at indicated dose. (B) volcano plot of mRNAs and LncRNAs expression profiles. (C) Summary of differently expressed mRNAs and LncRNAs (P<0.05 and fold change>2).

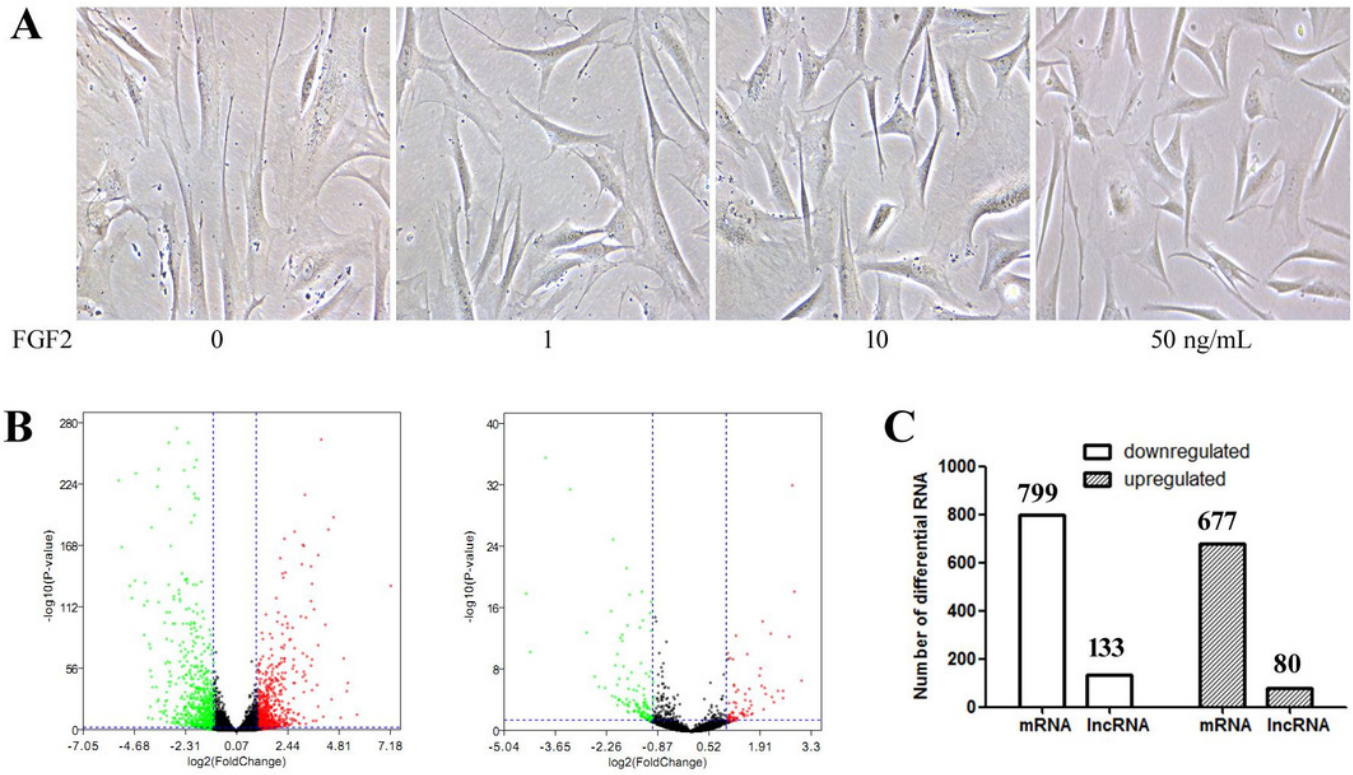


Figure 2

GO enrichment analysis of differently expressed mRNAs

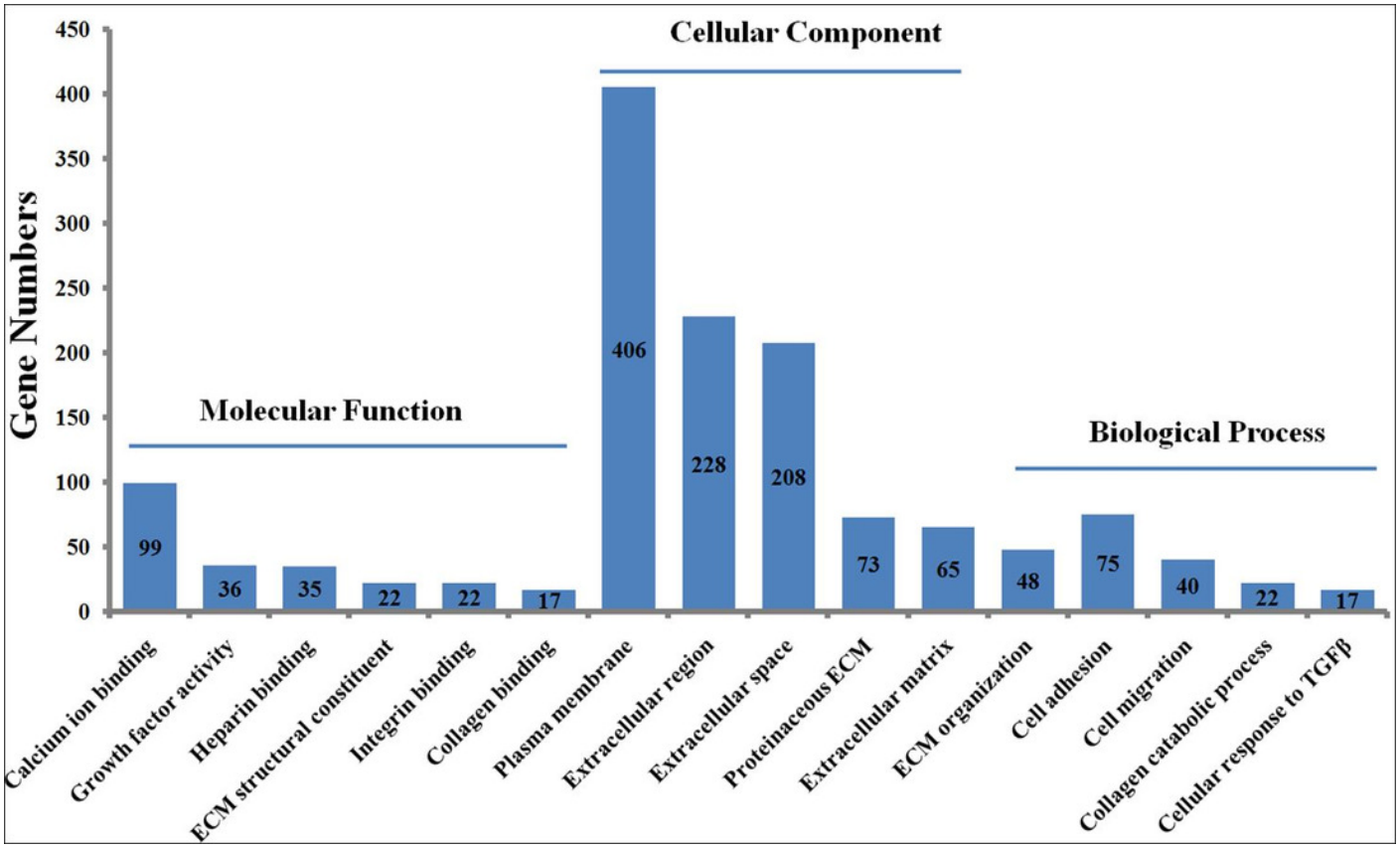


Figure 3

Figure 3. KEGG pathway analysis differently expressed genes

Figure 3. KEGG pathway analysis differently expressed genes.

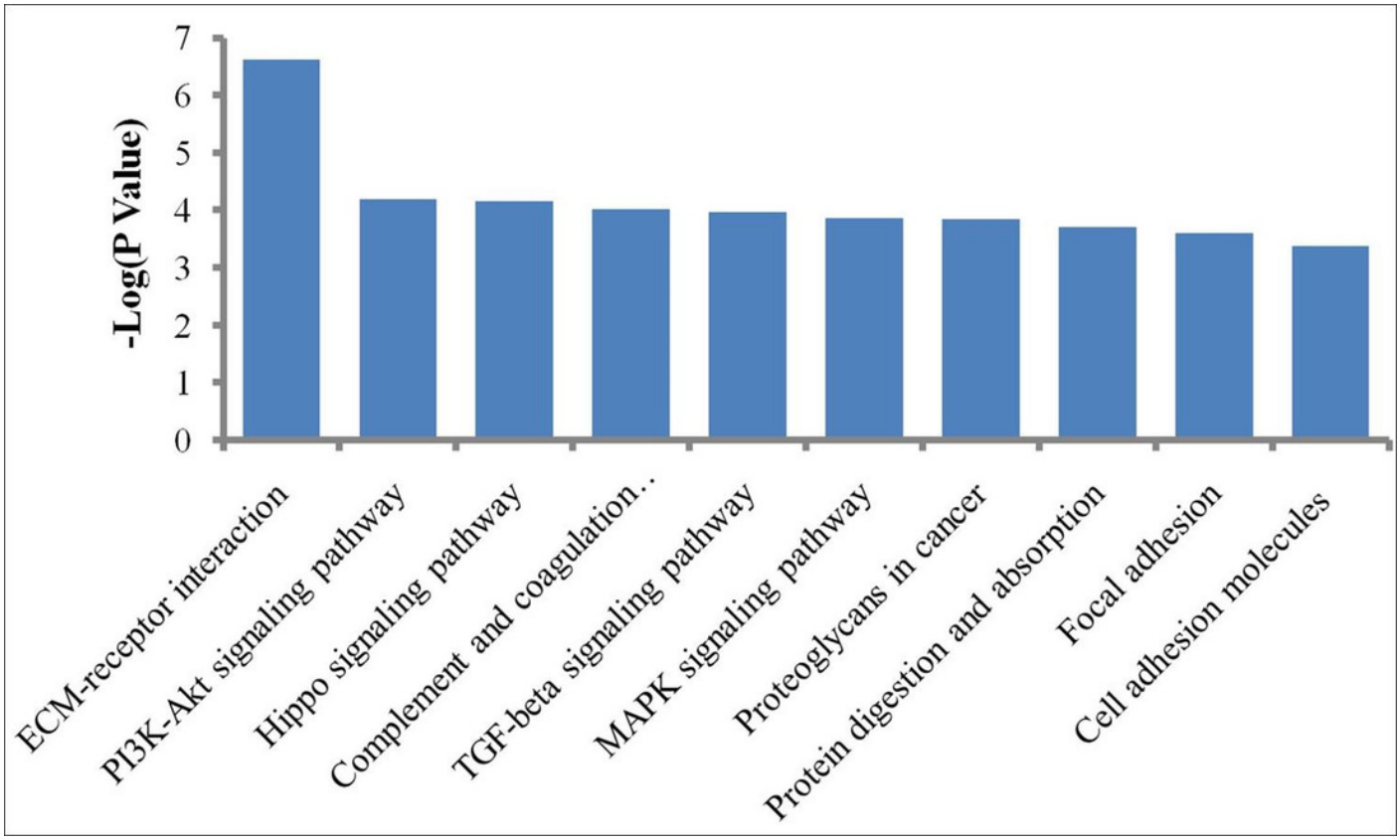
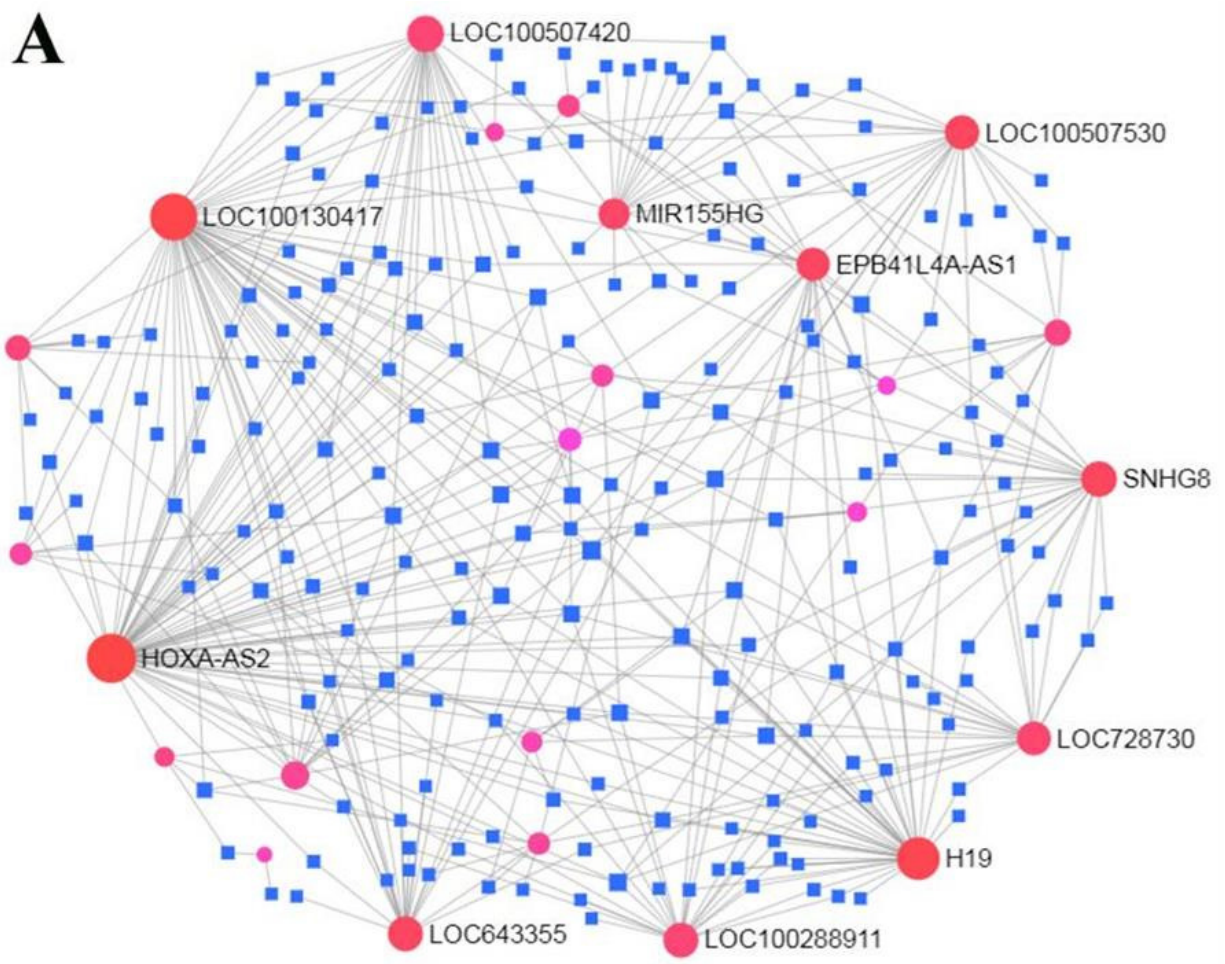
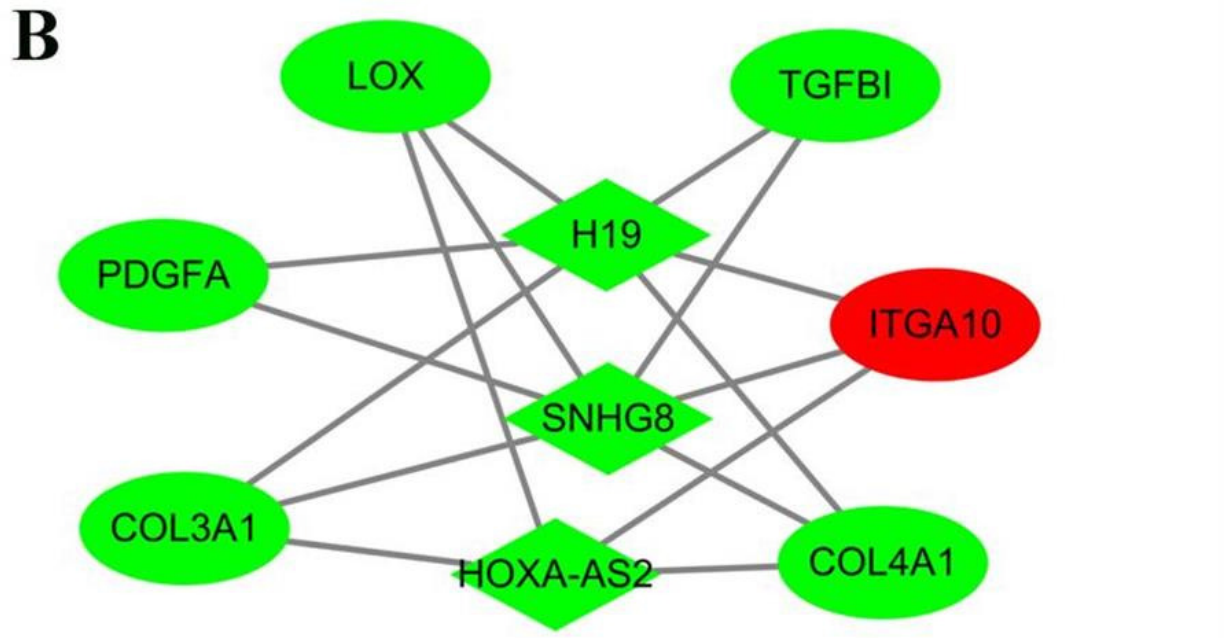


Figure 4

Figure 4. Networks of protein-protein interaction

(A). Networks of the differently expressed LncRNAs created by the network analyst website. (B). The co-expression network of the ECM-associated LncRNAs with their co-expression genes created by Pearson’s correlation. The diamond and ellipses nodes denote significant LncRNAs and mRNAs, respectively. The red and green nodes indicate upregulated and downregulated transcripts, respectively.


A
LOC100507420
LOC100507530
LOC100130417
MIR155HG
EPB41L4A-AS1
SNHG8
HOXA-AS2
LOC728730
H19
LOC643355
LOC100288911


B
LOX
TGFBI
H19
PDGFA
ITGA10
SNHG8
COL3A1
HOXA-AS2
COL4A1