Original Article

Lower expression of platelet derived growth factor is associated with better overall survival rate of patients with idiopathic nonspecific interstitial pneumonia

Xuyou Zhu[1*], Xia Fang[2*], Wei Chen[3], Fei Han[1], Ziling Huang[1], Benfang Luo[4], Pan Gu[1], Long Zhang[1], Weizhe Qiu[1], Yu Zeng[1], Weiwei Rui[1,5], Xianghua Yi[1]

[1]Department of Pathology, [2]Department of Hematology, [3]Department of Radiology, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China; [4]Department of Special Examination, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai 200433, China; [5]Department of Pathology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China

Contributions: (I) Conception and design: X Zhu, X Fang, W Rui, X Yi; (II) Administrative support: W Rui, X Yi; (III) Provision of study materials or patients: W Chen, F Han, Z Huang, B Luo; (IV) Collection and assembly of data: X Zhu, X Fang, P Gu, L Zhang, W Qiu; (V) Data analysis and interpretation: X Zhu, X Fang, Y Zeng, W Rui, X Yi; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*These authors contributed equally to this work.

Correspondence to: Xianghua Yi, MD. Department of Pathology, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China. Email: yixhxf@163.com; Weiwei Rui, MD. Department of Pathology, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China; Department of Pathology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China. Email: dilay_110@126.com.

Background: Idiopathic nonspecific interstitial pneumonia (INSIP) presents with varying degrees of interstitial inflammation and fibrosis exhibiting a uniform appearance. Lack of knowledge on the underlying mechanisms of INSIP has contributed to few effective treatment strategies. Our study is designed to explore aberrantly expressed cytokines involvement in INSIP development.

Methods: Oligo GEArray was employed to detect the expression of cytokines in INSIP patients, and idiopathic pulmonary fibrosis (IPF) was setup as isotype control. Real-time PCR and immunohistochemistry analysis were used to further confirm the expression of abnormally expressed cytokines. The correlationship between cytokines expression and overall survival rate of patients with IPF and INSIP were analyzed.

Results: From microarray detection, transforming growth factor-beta-1 (TGF-β1), fibroblast growth factor 10 (FGF10), and platelet derived growth factor (PDGF) were predominantly up-regulated in patients with INSIP. Real-time PCR and immunohistochemistry also showed these cytokines was abnormally expressed in INSIP. In addition to, the clinical relevance analysis demonstrated relatively lower expression of PDGF patients had longer overall survival rate than those with higher expression of PDGF.

Conclusions: Our study suggests that TGF-β1, FGF10, and PDGF are required for the pathogenesis of INSIP, and may therefore be ideal targets in INSIP treatment. Moreover, INSIP patients with lower expression of PDGF had better survival rate.

Keywords: Idiopathic nonspecific interstitial pneumonia (INSIP); idiopathic pulmonary fibrosis (IPF); transforming growth factor-beta-1 (TGF-β1); fibroblast growth factor 10 (FGF10); platelet derived growth factor (PDGF)

Submitted Jul 29, 2016. Accepted for publication Jan 09, 2017.
doi: 10.21037/jtd.2017.02.50
View this article at: http://dx.doi.org/10.21037/jtd.2017.02.50

Introduction

Idiopathic nonspecific interstitial pneumonia (INSIP) is generally characterized by hyperplastic type II pneumocytes with inflammatory cell infiltration, alveolar septum uniformly broadening with or without fibrosis, collagen deposition, occasional fibroblastic foci and honeycomb appearance of the lung (1,2) . INSIP is a major sub-type of idiopathic interstitial pneumonias (IIPs), which is a diverse group of lung disorders of unknown etiology characterized by various degrees of alveolar inflammation and remodeled alveolar structure that often result in pulmonary fibrosis (3,4). INSIP and idiopathic pulmonary fibrosis (IPF) share similar histomorphology, the latter also as the main sub-type of IIPs, and typically exhibits chronic fibrosis interstitial pneumonia, fibroblastic foci, and honeycomb changes (5), even though these two diseases were thought have etiological and inheritance heterogeneity. At present, many patients with IIPs respond to corticosteroid therapy to a certain degrees, but few achieve completely remission. Therefore, more effect needs to explore new potential strategy treatment for patients with IIPs, including INSIP and IPF.

Previous studies have suggested that many cytokines, including interleukins (ILs), transforming growth factor-beta (TGF-β), alpha-smooth muscle actin (α-SMA), and BMP-7 have pro-fibrogenic effects (6-9). Since patients with IPF frequently exhibit fibrotic lesion and have poor prognosis, many studies have focused on the role of these cytokines in IPF, and few investigations uncover the aberrantly expressed cytokines involved in the pathogenesis of patients with INSIP. In addition to, the clinical outcome of abnormally expressed cytokines in patients with INSIP is also still unclear.

In this study, we hypothesized that various cytokines were abnormally produced in the patients with INSIP. We determined the expression profile of cytokines in INSIP, including IPF by Oligo GEArray. Our initial results were validated by tissue-array with immunohistochemistry analysis and real-time PCR. Finally, the clinical outcome of related cytokines was further analyzed in 22 cases of INSIP and 25 cases of IPF. Our study aimed to identify the involvement of critical cytokines in the advancement of INSIP, and clarify whether these cytokines are related with the survival rate of patients with INSIP and IPF.

Methods

Human subjects

This study was approved by the ethics committee of Tongji Hospital [(Tong) No. 183 Ethics]. All samples were collected from patients with biopsy-confirmed INSIP and IPF from year 1999 to 2009, and all patients provided informed consent. Criteria for inclusion of subjects: (I) Biopsies for Oligo GEArray taken by video-assisted thoracoscope surgery or small incision lung biopsy. The control biopsy samples were collected from normal tissues of benign pulmonary tumors. Specimens used for tissue array and immunohistochemistry included 25 cases of IPF and 22 cases of INSIP (4 cases are cellular type, 18 cases are fibrosing type), which were collected at the time of diagnostic surgical lung biopsy through Tongji Hospital and Shanghai Pulmonary Hospital, affiliated with Tongji University School of Medicine; (II) the criteria of diagnosis referred to the American Thoracic Society (ATS)/European Respiratory Society (ERS) classification guidelines on IIP in 2002 (2), ATS/ERS views on INSIP Classification and Diagnostic Criteria in 2008 (10), Guidelines for Diagnosis and Management of IPF in 2011 (11) and Update of the International Multidisciplinary Classification of the Idiopathic Interstitial Pneumonias in 2013 (12); (III) the final diagnosis involved in multiple-disciplinary discussion is mutually made by pathologists, clinician and radiologists, and except other known causes of interstitial lung disease (ILD); (IV) each case had integrated clinical, radiologic, and pathologic data, including at least the follow-up data of more than 5 years. Besides, all patients received Glucocorticoids treatment, the Glucocorticoid use as following: (I) a large dosage: 100–200 mg/d methylprednisolone via intravenous injection, then 40 mg/d per os after 10 days, the dosage of methylprednisolone could be reduce until 4 weeks; (II) ordinary dosage: 0.5 mg/kg prednisone via per os, 0.25 mg/kg per os after 4 weeks, 0.125 mg/kg per os after 8 weeks or 0.25 mg/kg per os q.o.d (13). The clinical information of all patients included in the study is shown in *Table 1*.

Oligo GEArray

To monitor the expression profile of cytokines in patients with INSIP, including IPF, Oligo GEArray was employed. We extracted total RNA from human samples (3 cases of IPF and INSIP, and 1 case of normal control) that were grown on plastic plates, using the RNA Stat-60 reagent, and converted RNA into biotin-labeled cRNA target probes for microarray hybridization using the True Labeling-AMP linear RNA amplification kit. The cRNA targets (2 μg of cRNA) were next hybridized with oligonucleotide probes, representing different cytokines, printed on a nylon

Table 1 The clinical features of INSIP and IPF

Clinical features	Cases (%)	
	INSIP (n=22)	IPF (n=25)
Age (years)	50.86±9.8	58.16±10.7
Median age	49	58.5
Range	36–74	41–75
Gender		
Male	10 (45.5)	17 (73.3)
Female	12 (54.5)	8 (26.7)
Smoking history	9 (40.9)	10 (40.0)
Dust exposure	8 (36.4)	6 (24.0)
Symptoms		
Polypnea after activity	17 (77.2)	22 (88.0)
Velcro rale*	15 (68.2)	24 (96.0)
Acropachia	3 (13.6)	13 (52.0)
Average survival rate (months)	80.95±31.8	45.2±19.2

*, Inspiratory crackles. INSIP, idiopathic nonspecific interstitial pneumonia; IPF, idiopathic pulmonary fibrosis.

membrane. The resulting products on arrayed membranes were detected by a chemiluminescent detection kit, and analyzed by GEArray Analyzer data analysis software.

Tissue array design and immunohistochemistry (IHC)

Before constructing tissue array (TMA), typical lesions of 25 cases of IPF and 22 cases of INSIP were evaluated under light microscope. Then the TMA was performed using a core diameter of 2 mm by Shanghai Outdo Biotech laboratory. Each slide contained 77 lesion cores and 1 normal tissue core. IHC of paraffin-embedded sections was carried out using a standard streptavidin-biotinylated alkaline phosphatase (ABC-AP, DakoCytomation, Hamburg, Germany) method. The following antibodies were used: TGF-β1 (Santa Cruz, 1:100), fibroblast growth factor 10 (FGF10) (Santa Cruz, 1:100), platelet derived growth factor (PDGF) (Santa Cruz, 1:100). Under low-power magnification (100×), positive staining cells were screened and images of five representative fields were then captured under high-power magnification (400×) in Leica DMLA light microscope (Leica Microsystems, Wetzlar, Germany). The positive cell density of each core was counted by two independent investigators blind to clinical outcome and knowledge of the clinicopathological data. Data were expressed as mean value (± SE) of the triplicate cores taken from each patient.

Quantitative real-time reverse transcription polymerase chain reaction (QRT-PCR)

Total RNA was extracted from 50 mg biopsy using Trizol plus kit (TaKaRa, Japan). First-strand cDNA synthesis was done using Promega kit. Synthesized cDNA was used for QRT-PCR analysis using LightCycler (Roche, Switzerland) following the manufacturer's instructions. TGF-β1, FGF10, and PDGF primers were specifically designed by Biosune Bio-Technology Co., Ltd (China). β-actin was used as the internal control. The Nucleotide sequences for primers: *TGF-β1*: 5'-CGACTACTACGCCAAGGAG-3', 5'-GAGAGCAACACGGGTTCAG-3'; *FGF10*: 5'-AGAGCGACCCTCACATCAAG-3', 5'-TCGTTTCAGTGCCACATACC-3'; *PDGF*: 5'-CCTGCCCATTCGGAGGAAGAG-3', 5'-TTGGCCACCTTGACGCGCG-3'; β-*actin*: 5'-CCTGTACGCCAACACAGTG-3', 5'-ATACTCCTGCTTGCTGATCC-3'. Amplifications were carried out in the 20 μL reaction mixtures in the following conditions 95 ℃ for 2 min and followed by 40 cycles of 95 ℃ for 20 s, 55 ℃ for 30 s and 72 ℃ for 40 s, and then 72 ℃ for 5 min. The same experiment was repeated 3 times and similar results were obtained. The relative mRNA expression level was calculated and statistically analyzed using delta-delta-Ct method.

Microarray data analysis

Hierarchical clustering of 127 cytokines were performed using the software CLUSTER 3.0 (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm) and displayed by the software Java TreeView (http://www.yiiframework.com/forum/index.php?/topic/9180-the-tree-view/). For the network analysis, all genes were uploaded into the STRING 9.0 database (Search Tool for the Retrieval of Interacting Genes) to analyze the Protein-Protein interaction (PPI). Based on the neighborhood, gene fusions, co-occurrence, co-expression, experiments, and literature mining, STRING database provides information on both experimental and predicted interactions. In this study, we constructed the PPI network based on confidence score of 0.4, which implied that all possible interactions with low level of confidence were

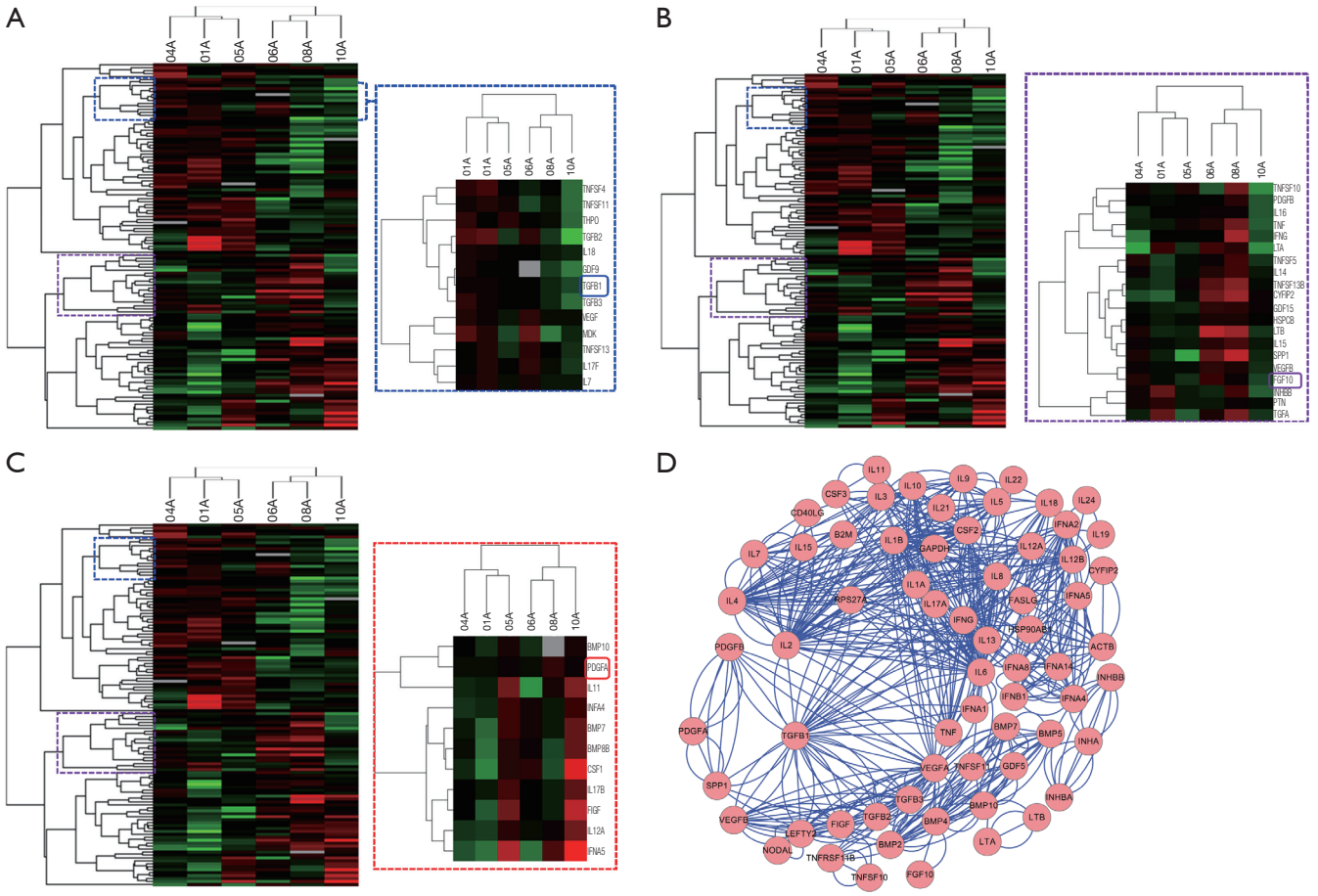

Figure 1 The hierarchical clustering and network analysis of cytokines microarray in IIPs patients. (A-C) The hierarchical clustering of 127 cytokines in patients were analyzed by cluster 3.0 and displayed by the software Java TreeView. Cytokines are color-coded according to their inheritance status (IPF: n=3, INSIP: n=3); (D) the network map of cytokines tightly involved in advancement of patients with IPF and INSIP. IIP, idiopathic interstitial pneumonia; IPF, idiopathic pulmonary fibrosis; INSIP, idiopathic nonspecific interstitial pneumonia.

extracted from the database and as many as possible were considered, and we used Cytoscape v2.8.3 software for visual analysis of the constructed networks.

Statistical analysis

All measurement data were expressed as mean ± SD, the difference among groups compared using ANOVA, enumeration data were analyzed by chi-square test. Kaplan-Meier method was employed to evaluate survival curve, and the log-rank test was used to compare survival time among groups. The test results were reported as 2-tailed P values, where $P<0.05$ was considered to be statistically significance.

Results

Identification of cytokines involved in the pathogenesis of INSIP

To identify cytokines involved in the pathogenesis of INSIP, with IPF as isotype control, Oligo GEArray that profiled 127 cytokines was employed. The analysis identified 109 cytokines as differentially expressed more than 2-fold with P value <0.05 between normal and lesion tissues. These included cytokines involved in regulation of cell cycle and proliferation, growth factor activity, and protein biosynthesis (*Table S1*). Specifically, TGF-β1, FGF10, and PDGF were dramatically up-regulated in patients with INSIP (*Figure 1A,B,C*), and were found closely related with

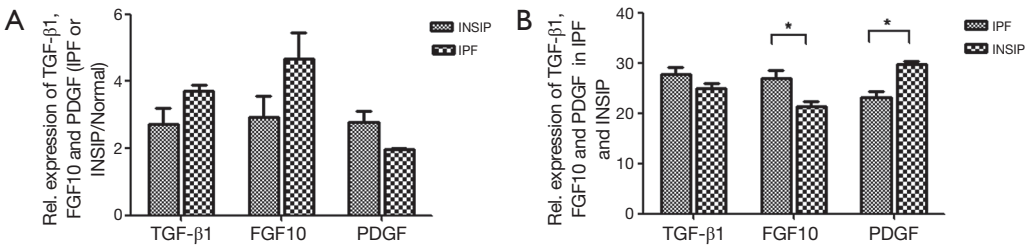

Figure 2 Relative expression of TGF-β1, FGF10, and PDGF in patients. (A) The quantification of microarray results, TGF-β1 and FGF10 were relatively higher in IPF, while PDGF was highly expressed in INSIP; (B) the expression of above genes in INSIP and IPF were confirmed by RT-PCR, and the experiment was independently repeated three times, *P<0.05. TGF-β1, transforming growth factor-beta-1; FGF10, fibroblast growth factor 10; PDGF, platelet derived growth factor; INSIP, idiopathic nonspecific interstitial pneumonia; IPF, idiopathic pulmonary fibrosis; RT-PCR, reverse transcription polymerase chain reaction.

pathogenesis of INSIP (*Figure 1D*). Similar phenotype was also found in patients with IPF. The quantitative analysis of these cytokines from GEArray is shown in *Figure 2A*. Interestingly, we found that the expression of TGF-β1 and FGF10 were higher in IPF than in INSIP, while PDGF was expressed at a higher level in patients with INSIP, though there have no statistical significance between INSIP and IPF groups.

TGF-β1, PDGF, and FGF10 expression were increased in INSIP patients

To confirm above results, we also performed RT-PCR to detect the expression of T*GF-β1*, *PDGF*, and *FGF10* in patients with INSIP, including IPF. Consistence with results of Oligo GEArray, these genes was obviously increased in patient samples regardless of INSIP and IPF comparing the normal tissue (P<0.05). Their expressions were also individually analyzed in both INSIP and IPF patients. As demonstrated in *Figure 2B*, PDGF was relatively overexpressed in INSIP, whereas TGF-β1 and FGF10 were highly expressed in IPF (P<0.05), indicating the potential existence of a predominant expression axis of these cytokines in different subtypes of IIPs. Taken together, these results suggested that TGF-β1, FGF10, and PDGF were abnormally expressed in IIPs disease.

The expression and location of TGF-β1, PDGF, and FGF10 in INSIP by IHC detection

Since we observed dramatically increased expression of TGF-β1, PDGF, and FGF10 in both INSIP and IPF at the transcriptional level, we analyzed 22 cases of INSIP and 25 cases of IPF to determine the expression of these cytokines by immunohistochemical analysis. Comparing with normal lung tissues, TGF-β1, PDGF, and FGF10 were highly expressed in various type of cells in both INSIP and IPF (*Figure 3A-I*), including bronchial epithelial cells, alveolar epithelial cells, macrophage in alveolus and its mesenchyme, vascular endothelial cells, fibroblast (FB) and smooth muscle cells. Importantly, we observed that PDGF was more strongly expressed in patients with INSIP than patients with IPF (*Figure 3C,F*). Of note, the alveolar macrophages (AM) showed stronger expression of these cytokines than other type of cells. Consistently, through quantification of these cytokines, TGF-β1 and FGF10 were highly expressed in IPF than in INSIP, while PDGF was strongly expressed in INSIP (*Table 2*), which was consistent with the results of GEArray and RT-PCR. Based on these results, we hypothesized that there was likely a priority of the effect of TGF-β1, PDGF, and FGF10 in INSIP and IPF, and PDGF may be the predominant disease-promoting factor in INSIP, while TGF-β1 and FGF10 may be more important for progression of IPF, although they were all highly expressed in both IPF and INSIP.

The relationship between PDGF and overall survival rate of patients with INSIP

Due to the relatively high expression of PDGF in patients with INSIP, we wonder whether its expression may be associated with the survival rate of patients. Additionally, the relationship between TGF-β1 and FGF10 expression with the patients' survival rate were also studied. As mentioned in previously, each case had integrated clinical follow-up data of more than five years. Interestingly, we found that patients with above average PDGF expression survived better than those with PDGF expression below the

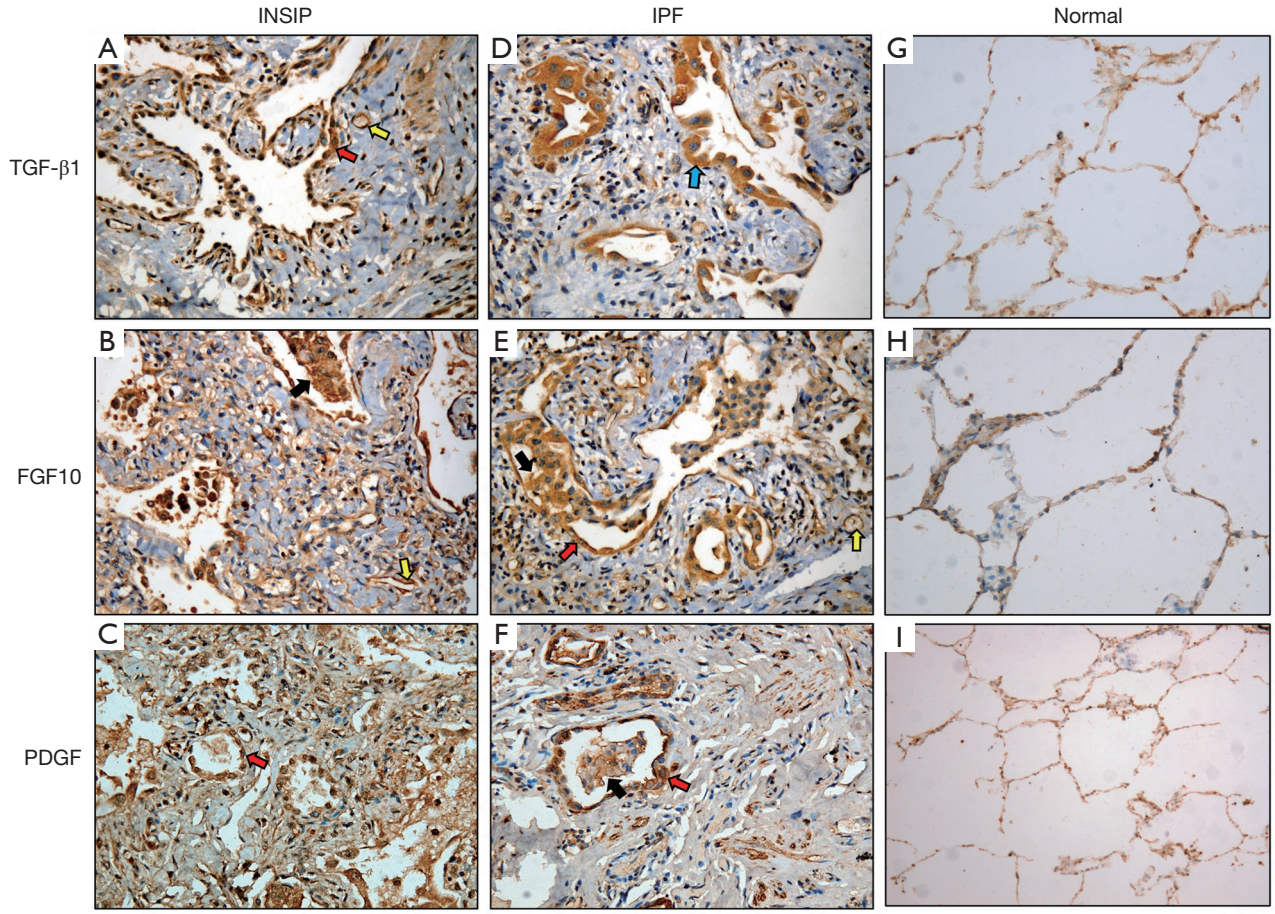

Figure 3 The expression of TGF-β1, FGF10, and PDGF in INSIP and IPF by immunohistochemistry detection. (A-C) TGF-β1, FGF10, and PDGF were strongly expressed in alveolar epithelial cells (red arrow), vascular endothelial cells (yellow arrow), alveolar macrophages (black arrow) of patients with INSIP (Envison ×200); (D-F) above cytokines also highly expressed in various type of cells in patients with IPF, including bronchial epithelial cells (blue arrow), alveolar epithelial cells (red arrow), vascular endothelial cells (yellow arrow), alveolar macrophages (black arrow) (Envison ×200); (G-I) the expression of TGF-β1, FGF10 and PDGF in normal lung tissues (Envison ×200). TGF-β1, transforming growth factor-beta-1; FGF10, fibroblast growth factor 10; PDGF, platelet derived growth factor; INSIP, idiopathic nonspecific interstitial pneumonia; IPF, idiopathic pulmonary fibrosis.

average level. However its expression levels had no significant correlation with the survival rate of IPF patient (*Figure 4A,B*). Additionally, PDGF is not an independent prognostic factor of INSIP patients. Besides, our data also showed that TGF-β1 expression also had no significant correlation with patients' survival rate in INSIP or IPF as well as FGF10 correlation analysis (*Figure 4C,D,E,F*). Taken together, our data suggests there is significant correlation ship between PDGF expression and survival rate of patients with INSIP.

Discussion

Our results demonstrated that IIPs, including IPF and INSIP, are associated with many abnormally-expressed cytokines. Oligo GEArray identified several cytokines which appeared to be important in the pathogenesis and advancement of INSIP and IPF. In further analysis, we found that TGF-β1, FGF10, and PDGF were dominantly up-regulated in patients with INSIP, as well as IPF. These results were also confirmed by RT-PCR and IHC. Interestingly, TGF-β1 and FGF10 were preferentially increased in IPF than that in INSIP, while PDGF was increasingly expressed in INSIP, indicating there was likely a priority-effect of these cytokines in the progression of IPF and INSIP. Importantly, we found that the negative correlationship between PDGF expression and overall

Table 2 The expression and location of cytokines in cells of INSIP and IPF patients ($\bar{x}\pm s$)

Groups	Cases	TGF-β1	PDGF	FGF10
Bronchial epithelial cell				
INSIP group	22	$3.91\pm1.48^{*\Delta1}$	$5.41\pm1.65^{*\Delta3}$	$3.08\pm1.15^{*\Delta5}$
IPF group	25	$5.24\pm1.27^{\Delta2}$	$4.52\pm1.09^{\Delta4}$	$4.04\pm1.02^{\Delta6}$
Alveolar epithelial cell				
INSIP group	22	3.86±1.39*	5.32±1.67*	3.16±1.14*
IPF group	25	5.12±1.01	4.44±1.04	4.00±1.04
Alveolar macrophage				
INSIP group	22	4.32±1.13*	6.68±1.99*	4.52±1.12*
IPF group	25	5.16±1.49	5.20±1.73	5.22±1.00
Vascular endothelial cell				
INSIP group	22	3.14±0.89*	4.73±1.12*	2.20±1.44*
IPF group	25	4.44±1.23	4.04±0.68	3.00±1.21
Fibroblast				
INSIP group	22	2.68±0.89*	4.77±1.07*	2.12±1.83*
IPF group	25	3.32±1.03	3.96±0.54	3.30±1.02
Smooth muscle cell				
INSIP group	22	1.41±1.05	4.41±1.05*	1.04±0.61*
IPF group	25	1.88±1.33	3.80±0.41	2.30±0.82

*, P<0.05, compared with IPF; $^{\Delta1}$, P=0.000, r=0.782; $^{\Delta2}$, P=0.000, r=0.885; $^{\Delta3}$, P=0.000, r=0.967; $^{\Delta4}$, P=0.000, r=0.930; $^{\Delta5}$, P=0.000, r=0.939; $^{\Delta6}$, P=0.000, r=0.980, compared with alveolar epithelial cell in original group. INSIP, idiopathic nonspecific interstitial pneumonia; IPF, idiopathic pulmonary fibrosis.

survival rate of patients with INSIP.

Recently, high throughput technique was employed to screen potential therapeutic targets and biomarkers for IIPs. Kaminski *et al.* described global changes of gene expression in IPF by using the reductionist "cherry picking" and quantitative "systems" approach (6). Yang *et al.* evaluated transcriptional signatures between sporadic IIPs and familial IIPs, and CXCL12 was identified as a key regulator in the pathogenesis of the disease (8). Similarly, we screened the expression of 127 various cytokines in INSIP by Oligo GEArray, with IPF as isotype control. Expectedly, many cytokines were disorderly expressed in INSIP, including ILs, tumor necrosis factor, osteogenesis protein families and so forth. It is noteworthy that TGF-β1, FGF10, and PDGF were dominantly over-expressed in disease, suggesting they might be disease-drivers of INSIP and IPF. Several studies uncovered TGF-β1 as a well-known pro-fibrogenic factor (7,14-17), and pirfenidone or nintedanib could be used in IPF-involved dysfunction of TGF-β1 (18-21). But little is known about FGF10 and PDGF in the pathogenesis of pulmonary fibrosis and their therapeutic potential in INSIP and IPF, especially as studies have lacked human clinical relevance evidence that may identify the role of these cytokines in IIPs.

To clarify the clinical outcome of TGF-β1, FGF10, and PDGF in INSIP, we used tissue array and IHC to detect their expression in 22 cases of INSIP and 25 cases of IPF subjects. Similar to the report by Gu *et al.*, which identified TGF-β1 and FGF as highly expressed in these two diseases, and localized in alveolar epithelial cells, AM and bronchial epithelium (17), we found that TGF-β1 and FGF10 were strongly expressed in these cells in our subjects. Interestingly, all these cytokines were predominantly expressed in AM, indicating AM are a major resource for the production of

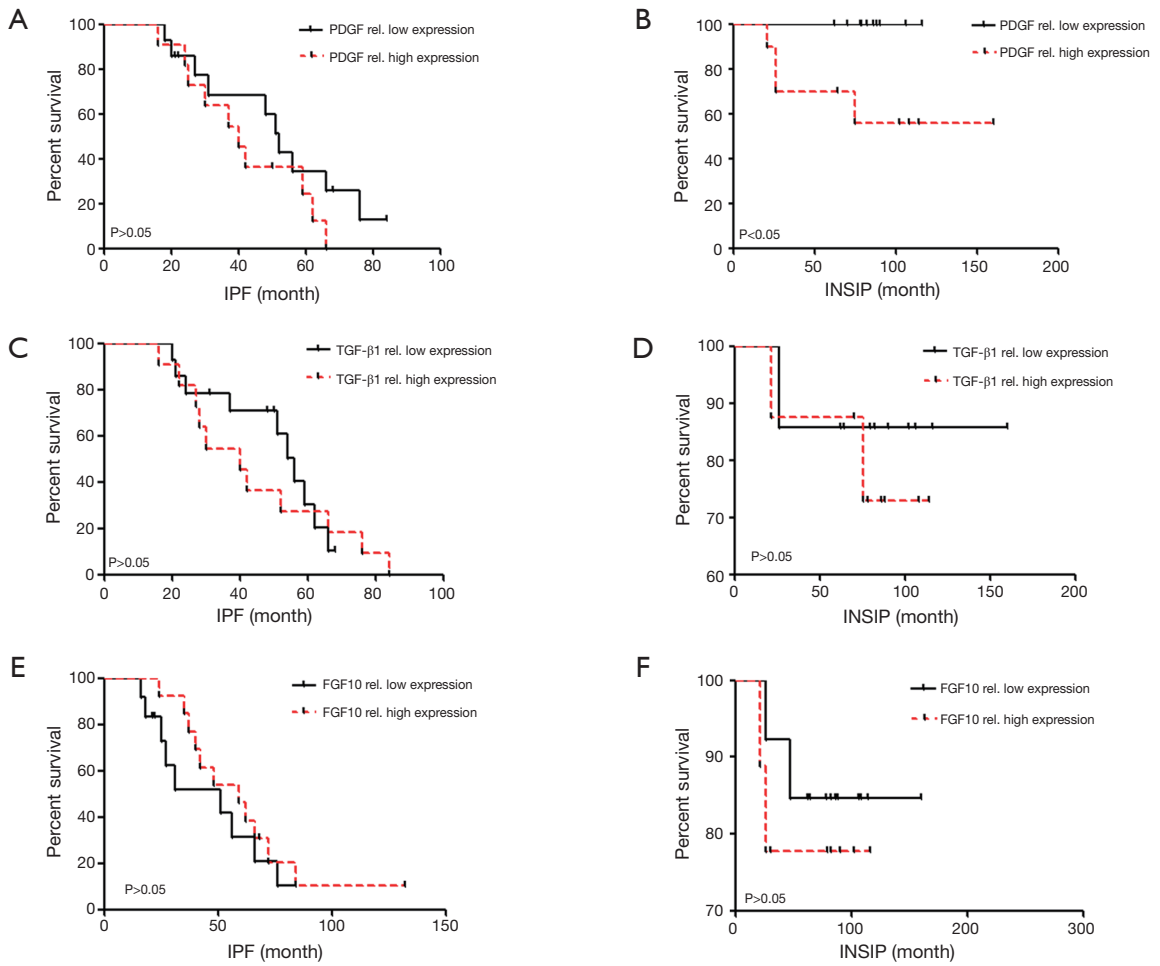

Figure 4 The correlation of TGF-β1, FGF10, and PDGF expression and the patients' survival rate. (A,B) Lower expression of PDGF was closely associated with better survival rate of INSIP rather than IPF patients (*P<0.05); (C-F) the relationship between differential expression level of TGF-β1, FGF10 and the survival time of IPF and INSIP patients, there no statistical significance among these groups. TGF-β1, transforming growth factor-beta-1; FGF10, fibroblast growth factor 10; PDGF, platelet derived growth factor; IPF, idiopathic pulmonary fibrosis; INSIP, idiopathic nonspecific interstitial pneumonia.

these cytokines. Lemaire *et al.* also found that AM isolated from lung fibrosis in rats induced by asbestos, releases a FGF which persisted over time (22). Importantly, we discovered that PDGF, which was more strongly expressed in INSIP. Intriguingly, some studies indicate that PDGF can promote the proliferation of fibroblasts (23). In bleomycin-induced mice, PDGF was significantly increased in murine pulmonary tissues (24), and target its expression could availably prevent the progress of fibrosis. In human studies, similar results show that imatinib, which specifically inhibits PDGF tyrosine kinase (25), could obviously improve the pulmonary function in IPF patients (26). Accordingly, our study also suggests that PDGF may a potential target for pulmonary fibrosis, especially for INSIP patients since its expression was negatively associated with the survival rate of patients with INSIP in our study. However, further analysis indicates that PDGF is not an independent prognostic factor for INSIP patients. This may due to the relatively small sample size. Thus the value of PDGF in the prognosis of INSIP need verified in more large samples in future studies.

In summary, our study investigated cytokine expression in INSIP and IPF subjects. To the best of our knowledge, most previous studies that have explored the potential

therapeutic target or mechanisms of interstitial pneumonia have used cells or animal models, but few studied at the human pathologic level, especially for Asian population. Through analyzing gene expression profiling, we found that the expression of TGF-β1, FGF10, and PDGF were all increased in patients. Importantly, our results suggests that a potential priority effect exists among these cytokines in INSIP and IPF, whereby PDGF may be more important in the pathogenesis of INSIP, whereas TGF-β1 and FGF10 may be more critical for the advancement of IPF. Importantly, our findings suggest that lower expression of PDGF is associated with better overall survival rate of patients with INSIP.

Acknowledgements

Funding: This work was supported by the Science and Technology Commission Foundation of Key Medical Research Foundation of Shanghai, China (034119868; 09411951600), Key Medical Research Foundation of the Health Bureau of Shanghai, China (20134034) and National Nature Science Foundation of China (81570053).
We sincerely thank Yudong Zhang, Jun Gu for their skillful technique support.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the ethics committee of Tongji Hospital [(Tong) No. 183 Ethics]. And all patients provided informed consent.

References

1. Daniil ZD, Gilchrist FC, Nicholson AG, et al. A histologic pattern of nonspecific interstitial pneumonia is associated with a better prognosis than usual interstitial pneumonia in patients with cryptogenic fibrosing alveolitis. Am J Respir Crit Care Med 1999;160:899-905.
2. American Thoracic Society.; European Respiratory Society. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med 2002;165:277-304.
3. Swigris JJ, Kuschner WG, Kelsey JL, et al. Idiopathic pulmonary fibrosis: challenges and opportunities for the clinician and investigator. Chest 2005;127:275-83.
4. Green FH. Overview of pulmonary fibrosis. Chest 2002;122:334S-339S.
5. Fang X, Luo B, Yi X, et al. Usual interstitial pneumonia coexisted with nonspecific interstitial pneumonia, What's the diagnosis? Diagn Pathol 2012;7:167.
6. Kaminski N, Rosas IO. Gene expression profiling as a window into idiopathic pulmonary fibrosis pathogenesis: can we identify the right target genes? Proc Am Thorac Soc 2006;3:339-44.
7. Murray LA, Chen Q, Kramer MS, et al. TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P. Int J Biochem Cell Biol 2011;43:154-62.
8. Yang IV, Burch LH, Steele MP, et al. Gene expression profiling of familial and sporadic interstitial pneumonia. Am J Respir Crit Care Med 2007;175:45-54.
9. Gu P, Luo B, Yi X, et al. The expressions and meanings of BMP-7 and TGF-β in idiopathic pulmonary fibrosis and idiopathic nonspecific interstitial pneumonia. Zhonghua Jie He He Hu Xi Za Zhi 2014;37:664-70.
10. Travis WD, Hunninghake G, King TE Jr, et al. Idiopathic nonspecific interstitial pneumonia: report of an American Thoracic Society project. Am J Respir Crit Care Med 2008;177:1338-47.
11. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788-824.
12. Travis WD, Costabel U, Hansell DM, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 2013;188:733-48.
13. Wu XY, Yi XH, Li HP, et al. A study on the efficacy of glucocorticoid therapy for idiopathic nonspecific interstitial pneumonia. Zhonghua Jie He He Hu Xi Za Zhi 2010;33:593-6.
14. Wei X, Xia Y, Li F, et al. Kindlin-2 mediates activation of TGF-β/Smad signaling and renal fibrosis. J Am Soc Nephrol 2013;24:1387-98.
15. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. Cell Mol Life Sci 2014;71:549-74.
16. Goodwin A, Jenkins G. Role of integrin-mediated TGFbeta activation in the pathogenesis of pulmonary

fibrosis. Biochem Soc Trans 2009;37:849-54.
17. Gu L, Xu WB, Liu HR, et al. Different cytokine profiles in usual interstitial pneumonia and nonspecific interstitial pneumonia. Zhonghua Jie He He Hu Xi Za Zhi 2003;26:350-3.
18. Akhurst RJ, Hata A. Targeting the TGFβ signalling pathway in disease. Nat Rev Drug Discov 2012;11:790-811.
19. Iyer SN, Gurujeyalakshmi G, Giri SN. Effects of pirfenidone on transforming growth factor-beta gene expression at the transcriptional level in bleomycin hamster model of lung fibrosis. J Pharmacol Exp Ther 1999;291:367-73.
20. Iyer SN, Gurujeyalakshmi G, Giri SN. Effects of pirfenidone on procollagen gene expression at the transcriptional level in bleomycin hamster model of lung fibrosis. J Pharmacol Exp Ther 1999;289:211-8.
21. Margaritopoulos G. Challenges in IPF diagnosis, current management and future perspectives: Patient case 2. Sarcoidosis Vasc Diffuse Lung Dis 2015;32 Suppl 1:38-9.
22. Lemaire I, Beaudoin H, Dubois C. Cytokine regulation of lung fibroblast proliferation. Pulmonary and systemic changes in asbestos-induced pulmonary fibrosis. Am Rev Respir Dis 1986;134:653-8.
23. Walsh J, Absher M, Kelley J. Variable expression of platelet-derived growth factor family proteins in acute lung injury. Am J Respir Cell Mol Biol 1993;9:637-44.
24. Maeda A, Hiyama K, Yamakido H, et al. Increased expression of platelet-derived growth factor A and insulin-like growth factor-I in BAL cells during the development of bleomycin-induced pulmonary fibrosis in mice. Chest 1996;109:780-6.
25. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 1996;2:561-6.
26. Distler JH, Manger B, Spriewald BM, et al. Treatment of pulmonary fibrosis for twenty weeks with imatinib mesylate in a patient with mixed connective tissue disease. Arthritis Rheum 2008;58:2538-42.

Cite this article as: Zhu X, Fang X, Chen W, Han F, Huang Z, Luo B, Gu P, Zhang L, Qiu W, Zeng Y, Rui W, Yi X. Lower expression of platelet derived growth factor is associated with better overall survival rate of patients with idiopathic nonspecific interstitial pneumonia. J Thorac Dis 2017;9(3):519-528. doi: 10.21037/jtd.2017.02.50

Supplementary

Table S1 The expressed profile of cytokines involved in the pathogenesis of patients with IPF and INSIP

Symbol	Description	GO term	1A: standard value/control standard value	4A: standard value/control standard value	6A: standard value/control standard value	5A: standard value/control standard value	8A: standard value/control standard value	10A: standard value/control standard value
RPS27A	Ribosomal protein S27a	Intracellular; protein biosynthesis; structural constituent of ribosome; ribosome	0.6984812	0.88565386	1.15274426	0.71415456	1.21272104	1.169643001
AREG	Amphiregulin (schwannoma-derived growth factor)	Integral to membrane; cell proliferation; extracellular space; growth factor activity; cell-cell signaling; cytokine activity	0.36046713	0.54934792	1.94266824	0.13030534	0.60418898	1.82350768
ARS	ARS component B	Extracellular; cytokine activity; biological process unknown	1.08435248	1.51541266	0.88175234	0	0.62953952	0.915287115
ATP6AP1	ATPase, H+ transporting, lysosomal accessory protein 1	ATP binding; hydrolase activity; transporter activity; hydrogen-transporting ATP synthase activity, rotational mechanism; hydrogen-transporting ATPase activity, rotational mechanism; ATP synthesis coupled proton transport; proton-transporting two-sector ATPase complex; proton transport	0.57892632	0.81518827	1.20603347	0.21065414	1.18184632	0.870057203
BMP1	Bone morphogene tic protein 1	Calcium ion binding; extracellular; proteolysis and peptidolysis; zinc ion binding; development; growth factor activity; cytokine activity; metallopeptidase activity; cartilage condensation; astacin activity; procollagen C-endopeptidase activity	0.44907116	1.53291465	2.36971787	1.49313203	1.77526707	1.941768055
BMP10	Bone morphogene tic protein 10	Extracellular; cell growth and/or maintenance; growth factor activity; cell-cell signaling; regulation of cell proliferation; cytokine activity; embryonic development; growth; cardioblast differentiation; embryonic heart tube development	0.88372109	1.25930558	0.8686157	1.55617516	0	1.551193053
BMP15	Bone morphogene tic protein 15	Extracellular; cell growth and/or maintenance; growth factor activity; cytokine activity; female gamete generation	1.63601749	1.70915606	1.30182138	1.87381476	0.65265595	2.545671034
BMP2	Bone morphogene tic protein 2	Extracellular; cell growth and/or maintenance; growth factor activity; skeletal development; cell-cell signaling; cytokine activity; growth	0.41148814	0.53024484	1.28156685	0.67825423	0.89965975	1.225958449
BMP3	Bone morphogene tic protein 3 (osteogenic)	Growth factor activity; skeletal development; cell-cell signaling; cytokine activity	0.4218214	0.80764214	0.67738821	0.58399011	0.8947683	0.926961445
BMP4	Bone morphogene tic protein 4	Mesoderm development; signal transducer activity; growth factor activity; skeletal development; cytokine activity; growth	0.68117675	0.94967571	1.35208101	0.40737043	0.37587385	1.032840747
BMP5	Bone morphogene tic protein 5	Cellular component unknown; growth factor activity; skeletal development; cytokine activity; growth	0.80459129	0.88061403	0.84268902	0.63724704	3.87536045	1.74188967
BMP6	Bone morphogene tic protein 6	Growth factor activity; skeletal development; cytokine activity; growth	0.4338411	0.45341569	1.28857593	0.4882698	1.85010961	1.305614283
BMP7	Bone morphogene tic protein 7 (osteogenic protein 1)	Growth factor activity; skeletal development; cytokine activity; growth	0.60980249	1.16194954	1.70558442	2.43253239	1.17500204	3.415602872
BMP8B	Bone morphogene tic protein 8b (osteogenic protein 2)	Extracellular; cell growth and/or maintenance; growth factor activity; skeletal development; cytokine activity; growth	0.44606252	1.00370687	1.26870185	1.57379029	0.63197258	2.800244206
CSF1	Colony stimulating factor 1 (macrophage)	Integral to membrane; cell proliferation; positive regulation of cell proliferation; cell differentiation; hematopoiesis; macrophage differentiation; macrophage colony stimulating factor receptor binding	0.39762743	0.85268102	1.69268968	1.62542045	0.62966387	7.966210029
CSF2	Colony stimulating factor 2 (granulocyte-macrophage)	Cell surface receptor linked signal transduction; development; extracellular space; cytokine activity; cellular defense response; granulocyte macrophage colony-stimulating factor receptor binding	0.86439825	2.1039088	1.56077784	2.30494703	3.91822998	4.675454723
CSF3	Colony stimulating factor 3 (granulocyte)	Extracellular; immune response; positive regulation of cell proliferation; cell surface receptor linked signal transduction; development; extracellular space; cell-cell signaling; cytokine activity; interleukin-6 receptor binding; cellular defense response; granulocyte colony-stimulating factor receptor binding	0.1869539	0.40499611	0.40866589	0.43818644	0.56068614	0.823405602
CYFIP2	Cytoplasmic FMR1 interacting protein 2	Extracellular; immune response; chemokine activity	0.21420331	0.25539393	1.30377059	0.44353184	1.90888968	0.527755359
EBAF	Endometrial bleeding associated factor (left-right determination, factor A; transforming growth factor beta superfamily)	Growth factor activity; cell-cell signaling; cytokine activity; transforming growth factor beta receptor signaling pathway; transforming growth factor beta receptor binding; cell growth; oocyte axis determination	1.21764497	0	1.15646718	2.06761498	0.81054292	0.924354838
FAM3B	Family with sequence similarity 3, member B	Extracellular; cytokine activity; insulin secretion	0.78531381	0.65905041	2.229818	1.28087945	4.95090772	4.701887783
FAM3C	Family with sequence similarity 3, member C	Extracellular; cytokine activity; biological process unknown	0.49964231	0.89692954	3.18000379	1.77520073	6.25169364	3.125564115
FGF10	Fibroblast growth factor 10	Signal transduction; nucleus; regulation of cell cycle; extracellular space; heparin binding; organogenesis; growth factor activity; cell-cell signaling; response to stress; regulation oftranscription; fibroblast growth factor receptor signaling pathway; cell surface; protein-nucleus import, translocation; fibro blast growth factor receptor binding; embryonic limb morphogenesis; positive regulation of receptor mediated endocytosis; positive regulation of urothelial cell proliferation; urothelial cellproliferation	3.19600254	4.89922147	5.88637612	2.51792617	4.16436653	2.088285455
FIGF	C-fos induced growth factor (vascular endothelial growth factor D)	Cell proliferation; regulation of cell cycle; positive regulation of cell proliferation; membrane; extracellular space; growth factor activity; platelet-derived growth factor receptor binding; angiogenesis	0.34008861	0.73039865	0.6914384	1.90344681	0.57572558	3.60552752
FLT3LG	Fms-related tyrosine kinase 3 ligand	Integral to membrane; signal transduction; positive regulation of cell proliferation; soluble fraction; cytokine activity	0.14434829	0.93506638	1.40548726	1.0582324	1.66671187	1.121110141
GDF1	Growth differentiation factor 1	Extracellular; cell growth and/or maintenance; growth factor activity; cell differentiation; cytokine activity	0.71304012	1.20313207	0.13961967	1.73922252	0.58195599	0.568924283
GDF10	Growth differentiation factor 10	Growth factor activity; skeletal development; cytokine activity; transforming growth factor beta receptor signaling pathway	0.19112295	0.4487812	0.51587711	0.59738119	1.10692823	1.029270193
GDF11	Growth differentiation factor 11	Cellular component unknown; mesoderm development; growth factor activity; skeletal development; neurogenesis; cytokine activity; growth	0.49771627	0.92771365	1.70170279	1.02288945	0.75853251	0.946061695
GDF15	Growth differentiation factor 15	Signal transduction; extracellular; growth factor activity; cell-cell signaling; cytokine activity; transforming growth factor beta receptor signaling pathway	1.16324212	1.25200137	2.17175906	1.03014713	2.08572453	1.630074383
GDF2	Growth differentiation factor 2	Extracellular; cell growth and/or maintenance; growth factor activity; cytokine activity; growth	0.67075334	1.78379253	1.15515494	1.24631474	0.9995993	1.320697065
GDF3	Growth differentiation factor 3	Extracellular; cell growth and/or maintenance; growth factor activity; cytokine activity	0.42614976	1.46617164	5.84659285	1.18540565	2.58311126	12.87213207
GDF5	Growth differentiation factor 5 (cartilage-derived morphogene tic protein-1)	Protein binding; growth factor activity; cell-cell signaling; cytokine activity; transforming growth factor beta receptor signaling pathway; growth	0.09844814	0.76816607	1.50746376	1.1620905	3.08452196	3.312303835
GDF8	Growth differentiation factor 8	Growth factor activity; muscle development; cytokine activity; transforming growth factor beta receptor signaling pathway; growth	0	0	0	0	0	0
GDF9	Growth differentiation factor 9	Extracellular; cell growth and/or maintenance; growth factor activity; cytokine activity; transforming growth factor beta receptor signaling pathway; female gamete generation	1.5551875	1.88818857	0	1.51274806	0.93594492	0.554337305
IFNA1	Interferon, alpha 1	–	1.54500454	1.16844903	0.59446116	1.71518551	1.27340387	0.919061148
IFNA13	Interferon, alpha 13	Extracellular; defense response; interferon-alpha/beta receptor binding	1.82956475	1.08339289	1.27299482	1.85809945	0.72289898	1.125587879
IFNA14	Interferon, alpha 14	Extracellular; defense response; hematopoietin/interferon-class (D200-domain) cytokine receptor binding	1.18464548	0.91799836	1.20976248	1.22168442	1.50826849	1.573881337
IFNA2	Interferon, alpha 2	Extracellular; induct ion of apoptosis; inflammatory response; cell surface receptor linked signal transduction; cell-cell signaling; defense response; interferon-alpha/beta receptor binding	1.88261574	1.7431851	1.38292725	1.92205808	0.96346873	1.423555669
IFNA4	Interferon, alpha 4	Extracellular; response to virus; cell-cell signaling; defense response; interferon-alpha/beta receptor binding	1.17361593	1.06252523	1.92194785	2.76220278	1.33646694	2.699104604
IFNA5	Interferon, alpha 5	Extracellular; defense response; hematopoietin/interferon-class (D200-domain) cytokine receptor binding	1.23818065	1.65148932	1.27750868	14.6657583	6.37817411	33.94323995
IFNA8	Interferon, alpha 8	Extracellular; defense response; hematopoietin/interferon-class (D200-domain) cytokine receptor binding	0.114951	0.58061597	0.74191419	0.50904098	0.76927753	0.482888981
IFNB1	Interferon, beta 1, fibroblast	Extracellular; negative regulation of cell proliferation; cell surface receptor linked signal transduction; response to virus; Caspase activation; B-cell proliferation; defense response; natural killer cell activation; positive regulation of innate immune response; interferon-alpha/beta receptor binding; anti-inflammatory response; negative regulation of virion penetration; regulation of MHC class I biosynthesis	1.77661581	0.70740493	1.18248114	1.72889506	1.12276541	0.984607959
IFNG	Interferon, gamma	Regulation of cell growth; extracellular; immune response; cell surface receptor linked signal transduction; cell motility; cell-cell signaling; cytokine activity; interferon-gamma receptor binding	1.7167365	0.67814498	1.88857383	1.93853225	7.61475888	0.973028806
IFNK	Interferon, kappa	Extracellular; negative regulation of cell proliferation; response to virus; defense response; regulation of transcription; cytokine and chemokine mediated signaling pathway; natural killer cell activation; cellular defense response (sensu vertebrata); positive regulation of innate immune response; Interferon-alpha/beta receptor binding	1.31719373	1.01248042	0.53252278	0.92103371	0.58158402	1.143468571
IFNW1	Interferon, omega 1	Extracellular; response to virus; cell cycle arrest; defense response; interferon-alpha/beta receptor binding	1.42434696	1.21945239	1.18832803	1.71327051	0.58954076	1.049929566
IK	IK cytokine, down-regulator of HLA II	Immune response; extracellular space; soluble fraction; cell-cell signaling; cytokine activity	1.64646668	1.8741568	1.06467638	1.10080838	0.38592034	0.599044565
IL10	Interleukin 10	Extracellular; Immune response; anti-apoptosis; cell-cell signaling; B-cell proliferation; cytokine activity; B-cell differentiation; T-helper 2 type immune response; regulation of isotype switching; interleukin-10 receptor binding; cytoplasmic sequestering of NF-kappaB; hematopoiesis; immune cell chemotaxis; negative regulation of MHC class ii biosynthesis; negative regulation of t-cell proliferation; negative regulation of interferon-alpha biosynthesis; negative regulation of interferon-gamma biosynthesis; negative regulation of nitric oxide biosynthesis	0.63884315	1.6675018	0.53983092	0.63422976	0.95138832	0.453962045
IL11	Interleukin 11	Extracellular; positive regulation of cell proliferation; cell-cell signaling; plateletactivation; cytokine activity; B-cell differentiation; interleukin-11 receptor binding; adipocyte differentiation; megakaryocyte differentiation	1.96323506	1.85343374	0.73993446	6.74102002	3.14383543	7.594803581
IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	–	1.24653313	1.34306261	1.84622945	2.99207674	2.21844084	5.456309813
IL12B	Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)	Signal transducer activity; membrane; extracellular space; antimicrobial humoral response (sensu vertebrata); positive regulation of interferon-gamma biosynthesis; cytokine activity; hematopoietin/interferon-class (D200-domain) cytokine receptor activity; interleukin-12 receptor binding; T-helper cell differentiation; interferon-gamma biosynthesis; natural killer cell activation; positive regulation of activated T-cell proliferation; regulation of cytokine biosynthesis	1.70500651	1.06834274	0.95909508	3.1452199	0.82850509	0.876244301
IL13	Interleukin 13	Cell proliferation; signal transduction; extracellular; immune response; inflammatory response; signal transducer activity; soluble fraction; cell motility; antimicrobial humoral response (sensu vertebrata); cell-cell signaling; chemokine activity; interleukin-13 receptor binding	1.21004911	1.62433632	0.72171694	0.75596692	0.66550881	0.642189933
IL14	Taxilin	Cell proliferation; extracellular; high molecular weight B-cell growth factor receptor binding	0.52526398	0.88536095	1.06673542	0.71710052	1.42119473	0.658870839
IL15	Interleukin 15	Golgi apparatus; cell proliferation; signal transduction; immune response; positive regulation of cell proliferation; membrane fraction; signal transducer activity; integral to plasmamembrane; extracellular space; endosome; cell-cell signaling; hematopoietin/interferon-class (D200-domain) cytokine receptor binding	0.70213539	0.82335532	1.10137384	0.6390786	1.47449891	0.625214574
IL16	Interleukin 16 (lymphocyte chemoattractant factor)	Immune response; protein binding; extracellular space; chemotaxis; cytokine activity	1.58139564	1.24719248	1.82767665	1.73220471	1.88149791	0.803103305
IL17	Interleukin 17 (cytotoxic T-lymphocyte-associated serine esterase 8)	Immune response; apoptosis; extracellular space; cell death; cell-cell signaling; cytokine activity; protein amino acid glycosylation	9.80067608	7.4405083	1.39902053	5.41101957	1.64419825	3.46121765
IL17B	Interleukin 17B	Immune response; signal transducer activity; cell-cell signaling; cytokine activity	1.15724256	1.33395919	1.42822417	2.55111625	1.08124331	3.214249272
IL17C	Interleukin 17C	Inflammatory response; cell surface receptor linked signal transduction; extracellular space; soluble fraction; cell-cell signaling; cytokine activity	1.73479304	3.92625774	1.46620866	1.39194436	0.58171142	1.654175954
IL17E	Interleukin 17E	Membrane; cytokine activity; interleukin-17E receptor binding; biological process unknown	0.82060846	0.76920313	1.22449616	1.68156722	0.20627228	0
IL17F	Interleukin 17F	Extracellular; cytokine activity; negative regulation of angiogenesis; cytokine biosynthesis; proteoglycan metabolism	1.76371006	1.32606734	1.93859107	1.24426497	1.16497143	0.810622138
IL18	Interleukin 18 (interferon-gamma-inducing factor)	Extracellular; immune response; induction of apoptosis via death domain receptors; signal transducer activity; interleukin-1 receptor binding; cell-cell signaling; angiogenesis; interferon-gamma biosynthesis; positive regulation of activated T-cell proliferation; T-helper 2 type immune response; chemokine biosynthesis; granulocyte macrophage colony-stimulating factor biosynthesis; Interleukin-13 biosynthesis; Interleukin-2 biosynthesis; regulation of cell adhesion; sleep	1.04899764	1.11062992	0.95779984	0.8448451	0.77969744	0.662700197
IL19	Interleukin 19	Signal transduction; extracellular; immune response; cytokine activity; actin binding	3.01819304	1.93573526	1.63277351	1.63144336	0.87518143	1.82184604
IL1A	Interleukin 1, alpha	Cell proliferation; immune response; cytoplasm; apoptosis; anti-apoptosis; negative regulation of cell proliferation; regulation of cell cycle; inflammatory response; signal transducer activity; extracellular space; interleukin-1 receptor binding; cell-cell signaling; chemotaxis	0.92241657	2.3689492	1.7958075	1.79342661	1.68340863	1.482172667
IL1B	Interleukin 1, beta	Cell proliferation; signal transduction; immune response; apoptosis; negative regulation of cell proliferation; regulation of cell cycle; inflammatory response; signal transducer activity; extracellular space; interleukin-1 receptor binding; antimicrobial humoral response (sensu vertebrata); cell-cell signaling	1.69998232	1.59534836	2.28855983	1.25859418	11.2163116	1.721077691
IL1F10	Interleukin 1 family, member 10 (theta)	Extracellular; immune response; interleukin-1 receptor antagonist activity	1.64501992	6.15108437	1.53726768	4.18960317	1.67940221	1.643218455
IL1F5	Interleukin 1 family, member 5 (delta)	Extracellular; immune response; interleukin-1 receptor antagonist activity	1.49668861	7.90464734	1.45959404	3.85410687	1.31685159	4.478736387
IL1F6	Interleukin 1 family, member 6	Extracellular; immune response; inflammatory response; interleukin-1 receptor binding	1.35784015	1.39803102	0.70685224	1.81789403	0.22921418	0.926796096
IL1F7	Interleukin 1 family, member 7 (zeta)	Extracellular; immune response; interleukin-1 receptor antagonist activity	1.52926815	1.70695734	1.27934853	1.20834604	0.31098844	1.094279548
IL1F8	Interleukin 1 family, member 8 (eta)	Extracellular; immune response; interleukin-1 receptor binding	7.63819507	3.66816028	1.75098682	2.81217976	0.68113273	4.543417181
IL1F9	Interleukin 1 family, member 9	Extracellular; immune response; cell-cell signaling; interleukin-1 receptor antagonist activity; response to pest, pathogen or parasite	10.6074733	0.91372598	2.00002979	4.22983771	1.52435628	1.226817076
IL2	Interleukin 2	–	7.68503718	0.77761967	1.10690276	1.77467021	0.83628448	1.282571949
IL20	Interleukin 20	Extracellular; immune response; interleukin-20 receptor binding; positive regulation of epidermal cell differentiation; positive regulation of keratinocyte differentiation; positive regulation of tyrosine phosphorylation of Stat3 protein; regulation of inflammatory response	8.13303416	0.60438263	0.66200638	2.12157085	1.04528241	1.454492023
IL21	Interleukin 21	Signal transduction; Nucleus; cytokine activity; lymph gland development	3.93130568	1.93819257	0.50450821	1.88498697	0.28569141	1.256471497
IL22	Interleukin 22	Extracellular; inflammatory response; acute-phase response; cell-cell signaling; interleukin-22 receptor binding	2.24882983	1.00427292	0.82387008	2.06062241	0.70705756	1.627601657
IL24	Interleukin 24	Extracellular; immune response; apoptosis; cytokine activity	0.91127363	0.8669872	0.94603635	1.89331206	0.60682735	1.33606596
IL26	Interleukin 26	Immune response; extracellular space; soluble fraction; antimicrobial humoral response (sensu vertebrata); cell-cell signaling; cytokine activity	1.59430708	1.88389603	0.75213864	1.71489213	0.50874937	0.402468096
IL3	Interleukin 3 (colony-stimulating factor, multiple)	Cell proliferation; extracellular; immune response; positive regulation of cell proliferation; cell-cell signaling; neurogenesis; cytokine activity; interleukin-3 receptor binding	1.31838154	1.96864535	1.67101865	1.69908821	0.59010334	2.797392551
IL4	Interleukin 4	Cell proliferation; extracellular space; chemotaxis; interleukin-4 receptor binding; B-cell differentiation; T-helper 2 type immune response; cellular defense response; cholesterol metabolism; connective tissue growth factor biosynthesis; regulation of isotype switching	9.63679381	1.16890173	0.81613764	3.7220775	2.20043825	1.565717883
IL5	Interleukin 5 (colony-stimulating factor, eosinophil)	Extracellular; immune response; inflammatory response; positive regulation of cell proliferation; cytokine activity; interleukin-5 receptor binding; hypersensitive response	7.93629877	1.49643167	1.30074841	3.63138859	1.43450147	1.559777562
IL6	Interleukin 6 (interferon, beta 2)	Humoral immune response; negative regulation of cell proliferation; positive regulation of cell proliferation; cell surface receptor linked signal transduction; extracellular space; acute-phase response; cell-cell signaling; cytokine activity; interleukin-6 receptor binding	3.4689024	1.01811895	3.96001262	1.05246174	2.00258984	4.185069361
IL7	Interleukin 7	Extracellular; humoral immune response; positive regulation of cell proliferation; organogenesis; cell-cell signaling; interleukin-7 receptor binding	2.27361139	1.96237312	2.19677239	1.5142724	1.44988391	1.418583441
IL8	Interleukin 8	Immune response; intracellular signaling cascade; negative regulation of cell proliferation; G-protein coupled receptor protein signaling pathway; cell cycle arrest; extracellular space; soluble fraction; cell motility; cell-cell signaling; chemokine activity; chemotaxis; angiogenesis; regulation of cell adhesion; neutrophil chemotaxis; calcium-mediated signaling; interleukin-8 receptor binding; induction of positive chemotaxis; neutrophil activation; regulation of retroviral genome replication	0.1335222	0.35942406	0.13590094	0.23689785	1.83093334	1.03244623
IL9	Interleukin 9	Extracellular; immune response; inflammatory response; positive regulation of cell proliferation; cell-cell signaling; interleukin-9 receptor binding	1.19731974	1.23996092	0.43462676	1.55514456	0.40694034	0.946526229
INHA	Inhibin, alpha	Extracellular; protein binding; cell growth and/or maintenance; negative regulation of cell cycle; induction of apoptosis; cell surface receptor linked signal transduction; cell cycle arrest; growth factor activity; hormone activity; skeletal development; cell-cell signaling; cell differentiation; neurogenesis; cytokine activity; negative regulation of interferon-gamma biosynthesis; activin inhibitor activity; erythrocyte differentiation; hemoglobin biosynthesis; negative regulation of B-cell differentiation; negative regulation of follicle-stimulating hormone secretion; negative regulation of macrophage differentiation; negative regulation of phosphorylation; ovarian follicle development; positive	1.32746285	1.2769295	0.88358932	1.04287598	0.67358137	0.730762637
INHBA	Inhibin, beta A (activin A, activin AB alpha polypeptide)	Extracellular; protein binding; cell growth and/or maintenance; negative regulation of cell cycle; induction of apoptosis; mesoderm development; cell surface receptor linked signal transduction; cell cycle arrest; growth factor activity; hormone activity; skeletal development; cell-cell signaling; cell differentiation; Neurogenesis; defense response; cytokine activity; transforming growth factor beta receptor binding; negative regulation of interferon-gamma biosynthesis; growth; activin inhibitor activity; erythrocyte differentiation; hemoglobin biosynthesis; negative regulation of b-cell differentiation; negative regulation of follicle-stimulating hormone secretion; negative	1.08073029	1.87227915	1.17236683	1.69650078	2.13238195	3.614676832
INHBB	Inhibin, beta B (activin AB beta polypeptide)	Extracellular; protein binding; cell growth and/or maintenance; growth factor activity; hormone activity; cell-cell signaling; cell differentiation; defense response; cytokine activity; protein homodimerization activity; growth; negative regulation of follicle-stimulating hormone secretion; ovarian follicle development; positive regulation of follicle-stimulating hormone secretion; response to external stimulus; host cell surface receptor binding; negative regulation of hepatocyte growth factor biosynthesis	3.59633698	1.68064664	1.09103593	1.25698631	2.60211969	0.677419361
LTA	Lymphotoxin alpha (TNF superfamily, member 1)	Signal transduction; immune response; induction of apoptosis; membrane; cell-cell signaling; tumor necrosis factor receptor binding	6.03872643	0.92303735	6.50682307	2.52759587	5.2510886	1.149333892
LTB	Lymphotoxin beta (TNF superfamily, member 3)	Integral to membrane; signal transduction; immune response; cell-cell signaling; tumor necrosis factor receptor binding	1.0086348	1.792797	6.96505111	1.09358159	5.41109041	0.882881616
MDK	Midkine (neurite growth-promoting factor 2)	Cell proliferation; signal transduction; regulation of cell cycle; extracellular space; heparin binding; growth factor activity; cell-cell signaling; cell differentiation; neurogenesis; cytokine activity	2.94380443	4.66579429	5.27988906	1.2295348	0.82412643	1.679094814
NODAL	Nodal homolog (mouse)	Development; growth factor activity; cytokine activity	4.9657434	1.81908917	1.40132893	1.7936727	0.72246576	1.594742743
PDGFA	Platelet-derived growth factor alpha polypeptide	Cell proliferation; regulation of cell cycle; cell surface receptor linked signal transduction; membrane; extracellular space; growth factor activity; cell-cell signaling; platelet-derived growth factor receptor binding	1.9297869	2.02531946	1.99717728	2.48247569	3.45445054	2.414740302
PDGFB	Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	Cell proliferation; extracellular; cell growth and/or maintenance; regulation of cell cycle; membrane; response to wounding; growth factor activity; platelet-derived growth factor receptor binding	0.83853903	0.97079553	0.95900676	0.85307547	1.31218767	0.441073007
PTN	Pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	Cell proliferation; regulation of cell cycle; positive regulation of cell proliferation; extracellular space; heparin binding; growth factor activity; neurogenesis; cytokine activity; protein phosphatase inhibitor activity; transmembrane receptor protein tyrosine phosphatase signaling pathway	1.5980138	1.24542208	0.87483804	0.91393046	1.16807059	0.985116858
SPP1	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein, early T-lymphocyte activation 1)	Protein binding; anti-apoptosis; cell-matrix adhesion; extracellular matrix; growth factor activity; cell-cell signaling; cytokine activity; immune cell chemotaxis; integrin binding; positive regulation of T-cell proliferation; T-helper 1 type immune response; ossification; negative regulation of bone mineralization; regulation of myeloid blood cell differentiation; induction of positive chemotaxis; extracellular matrix (sensu Metazoa)	1.87831749	3.00932904	5.74522182	0.62071966	12.4820223	1.80158236
TGFA	Transforming growth factor, alpha	Cell proliferation; protein binding; protein-tyrosine kinase activity; regulation of cell cycle; signal transducer activity; integral to plasma membrane; extracellular space; soluble fraction; growth factor activity; cell-cell signaling; epidermal growth factor receptor activating ligand activity	4.31496433	1.40547436	2.29988862	0.83922367	5.21695189	1.199012061
TGFB1	Transforming growth factor, beta 1 (Camurati-Engelmann disease)	Cell proliferation; anti-apoptosis; regulation of cell cycle; growth factor activity; cell-cell signaling; transforming growth factor beta receptor signaling pathway; transforming growth factor beta receptor binding; cell growth	3.87456127	3.87868445	3.38612691	3.62205385	2.5987023	1.925692836
TGFB2	Transforming growth factor, beta 2		6.74713211	5.89042355	4.58762198	1.92222283	1.89256555	0.568599573
TGFB3	Transforming growth factor, beta 3	Cell proliferation; signal transduction; regulation of cell cycle; organogenesis; growth factor activity; cell-cell signaling; transforming growth factor beta receptor binding; cell growth	5.09166362	6.78416947	4.71856209	4.15792631	3.33086612	1.843379256
THPO	Thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor)	Cell proliferation; extracellular; development; growth factor activity; hormone activity; cytokine activity;	1.96988678	2.80676172	1.73069457	2.75051882	1.63698552	0.789714774
TNF	Tumor necrosis factor (TNF superfamily, member 2)	Integral to membrane; signal transduction; immune response; regulation of transcription, DNA-dependent; apoptosis; anti-apoptosis; inflammatory response; response to virus; soluble fraction; cell-cell signaling; tumor necrosis factor receptor binding; leukocyte cell adhesion; necrosis	1.40274268	1.33969398	2.29927615	2.06736798	4.45361783	0.795004677
TNFRSF11 B	Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	Signal transduction; extracellular; protein binding; apoptosis; receptor activity; skeletal development; cytokine activity	2.43667195	1.07216513	0.51648813	1.81762159	1.09420177	0.76648775
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	Signal transduction; immune response; induction of apoptosis; apoptosis; positive regulation of I-kappaB kinase/NF-kappaB cascade; integral to plasma membrane; soluble fraction; cell-cell signaling; tumor necrosis factor receptor binding	0.29859957	0.40336945	0.17101673	0.47425768	0.94754173	0.107686114
TNFSF11	Tumor necrosis factor (ligand) superfamily, member 11	Extracellular; immune response; receptor activity; integral to plasma membrane; cell differentiation; tum or necrosis factor receptor binding; osteoclast differentiation	1.84514703	1.58894407	0.76369915	1.55912449	1.15241823	0.537289446
TNFSF12	Tumor necrosis factor (ligand) superfamily, member 12	Signal transduction; immune response; induction of apoptosis; apoptosis; integral to plasma membrane; angiogenesis; tumor necrosis factor receptor binding	0.57298826	1.18267977	1.8772737	0.35582279	2.28919263	1.530630561
TNFSF13	Tumor necrosis factor (ligand) superfamily, member 13	Signal transduction; immune response; positive regulation of cell proliferation; membrane; tumor necrosis factor receptor binding	1.32075171	0.99920833	1.40025956	0.69674831	0.98571721	0.678497647
TNFSF13B	Tumor necrosis factor (ligand) superfamily, member 13b	Cell proliferation; signal transduction; immune response; positive regulation of cell proliferation; integral to plasma membrane; soluble fraction; tumor necrosis factor receptor binding	0.33414425	0.40715534	1.1565197	0.57007853	1.72446091	0.49062492
TNFSF14	Tumor necrosis factor (ligand) superfamily, member 14	Integral to membrane; signal transduction; immune response; induction of apoptosis; tumor necrosis factor receptor binding; Caspase inhibitor activity; inhibition of Caspase activation; release of cytoplasmic sequestered NF-kappaB; T-cell proliferation; T-cell homeostasis	1.56294653	1.72750559	0.74842096	2.28521965	1.01825948	0.973083683
TNFSF15	Tumor necrosis factor (ligand) superfamily, member 15	Signal transduction; immune response; regulation of cell cycle; membrane; integral to plasma membrane; soluble fraction; tumor necrosis factor receptor binding	1.66997676	1.44107317	0.90215988	1.84584106	0.93673721	1.080413403
TNFSF18	Tumor necrosis factor (ligand) superfamily, member 18	Integral to membrane; signal transduction; immune response; anti-apoptosis; cell-cell signaling; tumor necrosis factor receptor binding	1.82110465	1.26280989	0.56888294	1.59160549	0.76353241	0.610304864
TNFSF4	Tumor necrosis factor (ligand) superfamily, member 4 (tax-transcription ally activated glycoprotein 1, 34 kDa)	Signal transduction; immune response; positive regulation of cell proliferation; integral to plasma membrane; cell-cell signaling; tumor necrosis factor receptor binding	0.85561258	0.69247961	0.36739997	0.43476557	0.49852032	0.189416647
TNFSF5	Tumor necrosis factor (ligand) superfamily, member 5 (hyper-IgM syndrome)	Signal transduction; immune response; anti-apoptosis; inflammatory response; integral to plasma membrane; soluble fraction; tumor necrosis factor receptor binding; CD40 receptor binding; B-cell proliferation; isotype switching; leukocyte cell adhesion; platelet activation	0.56401054	1.26443741	1.05460831	0.86632133	1.70793484	0.636554156
TNFSF6	Tumor necrosis factor (ligand) superfamily, member 6	Signal transduction; extracellular; immune response; induction of apoptosis; apoptosis; positive regulation of I-kappaB kinase/NF-kappaB cascade; integral to plasma membrane; cell-cell signaling; tumor necrosis factor receptor binding	2.55725879	1.74148221	1.18322445	0.98551507	0.89388262	0.877481578
TNFSF7	Tumor necrosis factor (ligand) superfamily, member 7	Cell proliferation; signal transduction; immune response; apoptosis; integral to plasma membrane; cell-cell signaling; tumor necrosis factor receptor binding	1.79076183	1.86714014	1.11393837	0.96722807	0.66632963	1.07371487
TNFSF8	Tumor necrosis factor (ligand) superfamily, member 8	Cell proliferation; signal transduction; immune response; induction of apoptosis; integral to plasma membrane; cell-cell signaling; tumor necrosis factor receptor binding	16.6031255	16.1111832	4.28095125	11.2155931	4.62559223	5.704360903
TNFSF9	Tumor necrosis factor (ligand) superfamily, member 9	Cell proliferation; signal transduction; immune response; apoptosis; integral to plasma membrane; cell-cell signaling; tumor necrosis factor receptor binding	0.42683846	0.7782374	0.43051526	0.49961541	0.43147894	0.387778271
VEGF	Vascular endothelial growth factor	Cell proliferation; signal transduction; extracellular; regulation of cell cycle; positive regulation of cell proliferation; homophilic cell adhesion; membrane; soluble fraction; heparin binding; growth factor activity; response to stress; vascular endothelial growth factor receptor binding; angiogenesis	0.34493609	0.43924221	0.48077441	0.27217503	0.29507061	0.282455082
PUC18	PUC18 plasmid	–	N/A	N/A	N/A	N/A	N/A	N/A
VEGFB	Vascular endothelial growth factor B	Cell proliferation; regulation of cell growth; signal transduction; extracellular; regulation of cell cycle; positive regulation of cell proliferation; membrane; heparin binding; growth factor activity; vascular endothelial growth factor receptor binding	0.59763504	0.75386562	1.02955742	0.6218807	0.85459142	0.466257659
BLANK	–	–	N/A	N/A	N/A	N/A	N/A	N/A
AS1R2	Artificial sequence 1 related 2 (80% identity) (48/60)	–	1.5539686	1.22295593	1.32009457	0.98773529	0.52224864	0.591712439
AS1R1	Artificial sequence 1 related 1 (90% identity) (56/60)	–	0.70736887	1.57696778	1.45720527	1.17608313	0.63025505	0.741073004
AS1	Artificial sequence 1	–	1.91282061	1.79068382	1.16574042	1.28525566	0.18929651	0.40325771
GAPD	Glyceraldehyde-3-phosphate dehydrogenase	Cytoplasm; oxidoreductase activity; glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity; glucose metabolism; glycolysis	0.56074995	0.98897125	1.07548536	0.9254465	1.22393987	0.759341513
B2M	Beta-2-microglobulin	Extracellular; immune response	0.74661134	0.89668368	0.95250045	0.94572891	0.98168609	0.84607305
HSPCB	Heat shock 90 kDa protein 1, beta	ATP binding; protein binding; cytoplasm; heat shock protein activity; protein folding; TPR domain binding; nitric-oxide synthase regulator activity; positive regulation of nitric oxide biosynthesis; unfolded protein binding; response to unfolded protein; ATP binding; protein binding; cytoplasm; heat shock protein activity; protein folding; TPR domain binding; nitric-oxide synthase regulator activity; positive regulation of nitric oxide biosynthesis; unfolded protein binding; response to unfolded protein	0.72168097	0.90224818	1.1021151	0.77865346	1.05306214	0.792455408
ACTB	Actin, beta	–	0.99999138	0.99998711	0.99999156	1	1	0.999990471
BAS2C	Biotinylated artificial sequence 2 complementary sequence	–	1.16477022	1.05712391	1.10977505	1.04786113	0.40201837	0.545022077
BAS2C	Biotinylated artificial sequence 2 complementary sequence	–	0.92847709	1.0361002	1.04182707	1.01571793	0.62977964	0.72145099

INSIP, idiopathic nonspecific interstitial pneumonia; IPF, idiopathic pulmonary fibrosis; N/A, not applicable.