Rheumatol Int (2015) 35:1343–1350
DOI 10.1007/s00296-015-3276-3

Rheumatology INTERNATIONAL

ORIGINAL ARTICLE - GENES AND DISEASE

Screening of candidate genes in fibroblasts derived from patients with Dupuytren's contracture using bioinformatics analysis

Haoyu Liu[1] · Weitian Yin[1] · Biao Liu[1] · Yan Liu[1] · Baofeng Guo[1] · Zhuang Wei[1]

Received: 12 January 2015 / Accepted: 23 April 2015 / Published online: 12 May 2015

Abstract Our study aimed to identify candidate genes associated with Dupuytren's contracture (DC) and elucidate their roles in DC development. The microarray data of GSE21221 were downloaded from Gene Expression Omnibus database, including six samples from carpal tunnel-derived fibroblasts and six samples from DC-derived fibroblasts. The differentially expressed genes (DEGs) in DC samples were screened using limma package. GO annotation and KEGG pathway analyses were performed by DAVID online tool. Protein–protein interaction network and expression correlation network were constructed to identify crucial relationships between DEGs. Finally, candidate DC-associated genes were predicted based on comparative toxicogenomics database. A total of 529 DEGs (138 up- and 391 down-regulated) in DC-derived fibroblasts were screened and compared with carpal tunnel-derived fibroblasts. Only ten DC-associated genes, such as neurotrophin 3 (NTF3) and protein kinase C, epsilon (PRKCE), were further screened. In addition, NTF3 was significantly enriched in MAPK signaling pathway, in which other DEGs, such as nuclear receptor subfamily 4, group A, member 1 (NR4A1), fibroblast growth factor 22 (FGF22) and BDNF, were enriched. Besides, NTF3 could co-express with fibrillin 2 (FBN2), and PRKCE could co-express with zinc finger protein 516 (ZNF516), solute carrier organic anion transporter family, member 2A1 (SLCO2A1), chromosome 10 open reading frame 10 (C10orf10) and Kelch domain containing 7A (KLHDC7A). Our study indicates that these DEGs, including NTF3, FBN2, NR4A1, FGF22, BDNF, PRKCE, ZNF516, SLCO2A1, C10orf10 and KLHDC7A, may play important roles in DC development and serve as candidate molecular targets for treating DC.

Keywords Dupuytren's contracture · Bioinformatics analysis · Differentially expressed genes · Pathway analysis · Expression correlation network

Introduction

Dupuytren's contracture is the most common connective tissue disorder of humans [1]. It is a disabling fibroproliferative condition in which an abnormal amount of normal collagen is produced [2]. The disease has a clear-cut family predisposition [3], but its exact etiology remains unclear. The mainstay of treatment for DC is surgery, and numerous surgical techniques have been described. However, all of these surgical techniques have a high incidence of complications and none has proven to be more effective than others [4]. Thus, exploring effective alternative therapies is urgent.

Although the pathogenesis of DC has not been fully investigated, various predisposing factors are being disclosed, such as smoking and diabetes mellitus, and are proved to contribute to DC development [5, 6]. Additionally, one dominant factor is genetic susceptibility, which is shown to play an important role in DC progression [7]. One study of Bayat et al. [8] has identified a heteroplasmic mitochondrial mutation strongly associated with DC. Hu et al. [9] suggested that autosomal dominant inheritance with incomplete penetrance of this disease was mapped to the long arm of chromosome 16.

Haoyu Liu and Weitian Yin have contributed equally to this work.

✉ Zhuang Wei
zhuangweizwzw@163.com

[1] Department of Hand Surgery, China-Japan Union Hospital of Jilin University, 126 Xiantai Street, Changchun 130031, China

Besides, several studies have dedicated to investigate the molecular pathogenesis of DC and have identified a large number of candidate molecules that may potentially be involved in its pathogenesis via gene expression profiling, proteomic profiling and cytogenetic profiling [10–12]. For example, transforming growth factor β is thought to stimulate fibroblast proliferation and extracellular matrix deposition and is found to be involved in DC [13]. Epidermal growth factor has been implicated in the pathogenesis of Dupuytren's disease palmar contracture [14]. Moreover, Zf9 transcription factor which increases TGF-β1 expression is considered as a candidate susceptibility gene for investigating hereditary predisposition to Dupuytren's disease [13]. Although the expanded efforts have been made to explore the genetic basis of DC, our understanding of the molecular pathogenesis of this disease is still limited, as well as good diagnostic and prognostic therapy.

Fibroblasts and myofibroblasts are considered to play an important role in the genesis of DC [15]. Previously, investigators have compared the gene expression profiles of fibroblasts derived from DC patients to identify key genes associated with the progression of DC [6] or to screen contrary genes in DC [16]. In contrast to these, bioinformatics approach was employed in the current study to identify the differentially expressed genes (DEGs) in DC-derived fibroblasts and subsequently perform functional analysis for DEGs. In addition, protein–protein interaction network and expression correlation network were constructed to identify crucial relationships between DEGs. Besides, candidate DC-associated genes were predicted. Our study aimed to provide insight into the pathogenesis of DC and discover candidate molecular targets for prevention or treatment of this disease.

Methods

Microarray data and preprocessing

The gene expression profile data GSE21221 deposited by Satish et al. [6] was downloaded from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). A total of 12 samples consisting of six samples from carpal tunnel-derived fibroblasts and six samples from DC-derived fibroblasts were used for the development of array data.

The annotation information of chip was downloaded based on the platform of GPL2507 Sentrix Human-6 Expression BeadChip (Illumina Inc., SanDiego, CA, USA) for further analysis. The ID number of original probe in CEL files was converted into Entrez gene symbol. If multiple probes mapped to the same gene symbol, the mean value was calculated as the expression value of this gene. Finally, the expression matrixes of total 16,642 genes were acquired.

DEGs screening

The DEGs in DC-derived fibroblasts compared with carpal tunnel-derived fibroblasts were analyzed using linear models for microarray data (limma, available at http://www.bioconductor.org/packages/release/bioc/html/limma.html) [17] package in Bioconductor in R. The |log2 fold change| > 1 and P value <0.05 were defined as the cutoff value.

Functional enrichment analyses for DEGs

Gene ontology (http://www.geneontology.org) [18] is used for the unification of biology according to a large scale of gene annotation information, which is mainly classified into biological process, molecular function and cellular component. Kyoto encyclopedia of genes and genomes (available at http://www.genome.ad.jp/kegg/) [19] database is used for enriching pathways based on predicted and known information of molecular interaction networks.

In our study, GO BP annotation and KEGG pathway analysis for DEGs were performed by Database for annotation, visualization and integrated discovery (http://david.abcc.ncifcrf.gov/) [20] online tool. A P value <0.05 was considered significant.

PPI network construction and module selection

The search tool for the retrieval of interacting genes (STRING, http://www.bork.embl-heidelberg.de/STRING/) [21] is an effective database for annotating functional interactions between screened DEGs and other genes via calculating their combined score. In this study, PPI relationships with a combined score >0.4 were considered significant. PPI network was then constructed using Cytoscape (available at http://cytoscapeweb.cytoscape.org/) [22] software [28] for visualization. In order to identify crucial relationships in the PPI network, the densely connected or possibly overlapping modules were subsequently identified using the ClusterONE [23] package in the Cytoscape software. A P value <4E−5 was defined as the significant threshold.

Expression correlation network construction

The genes with similar expression pattern in samples are considered as co-expressed genes, which always have the common regulatory function and high degree of correlation [24]. In our study, we performed correlation analysis for DEGs expression using Pearson correlation in CoExpress software (http://www.bioinformatics.lu/CoExpress/). Pearson correlation coefficient >0.9 of the expression value

indicated strongly positive correlation between genes, while correlation coefficient less than −0.9 indicated negative correlation between genes. The gene pairs with Pearson correlation coefficient >0.9 were regarded as co-expressed gene pairs. All the gene pairs with |Pearson correlation coefficient| >0.9 were considered significant. Then correlation network with all significant gene pairs was visualized using Cytoscape software. Then the overrepresented GO BP functional annotation for genes in the network was performed using biological networks gene ontology (BiNGO) tool [25]. A *P* value was calculated by hypergeometric test in BiNGO, then the false discovery rate (FDR) was carried out with the application of Benjamini–Hochberg method. FDR < 0.05 was set as the cutoff value.

Prediction of candidate DC-associated genes

The comparative toxicogenomics database (CTD, freely available at http://ctdbase.org/) is a public resource that provides the gene–disease relationships from the literature and is widely used to predict possible relationships using different types of associated data [26]. Thus, we predicted candidate DC-associated genes by mapping DEGs into CTD database.

Results

DEGs screening

A total of 529 DEGs in DC-derived fibroblasts compared with carpal tunnel-derived fibroblasts were screened using limma package, including 138 up-regulated and 391 down-regulated DEGs. The results showed that the number of down-regulated genes was more than up-regulated ones.

Functional enrichment analysis of DEGs

We performed GO BP enrichment analysis for up- and down-regulated DEGs to annotate their functions (Table 1). The overrepresented GO BP terms of up-regulated genes mainly included heterophilic cell–cell adhesion, neuron

Table 1 Significantly enriched GO BP terms and KEGG pathways ($P < 0.05$)

	GO ID or KEGG ID	Name	Counts	*P* values
A: The enriched GO BP terms				
Up-regulated	GO:0007157	Heterophilic cell–cell adhesion	5	3.46E−06
	GO:0031102	Neuron projection regeneration	4	6.67E−05
	GO:0045165	Cell fate commitment	9	8.20E−05
	GO:0007389	Pattern specification process	12	8.77E−05
	GO:0098609	Cell–cell adhesion	8	9.22E−05
	GO:0007267	Cell–cell signaling	55	2.20E−08
Down-regulated	GO:0033993	Response to lipid	38	3.58E−08
	GO:0009605	Response to external stimulus	76	5.75E−08
	GO:0023052	Signaling	168	7.00E−08
	GO:0044700	Single organism signaling	168	7.00E−08
B: The enriched KEGG pathways				
Up-regulated	04514	Cell adhesion molecules (CAMs)	7	4.28E−05
	04730	Long-term depression	3	1.43E−02
	04310	Wnt signaling pathway	4	2.37E−02
	05143	African trypanosomiasis	2	2.68E−02
	04916	Melanogenesis	3	3.73E−02
	04940	Type I diabetes mellitus	2	3.92E−02
	04010	MAPK signaling pathway	5	4.47E−02
	00071	Fatty acid metabolism	7	9.22E−05
Down-regulated	00980	Metabolism of xenobiotics by cytochrome P450	8	4.11E−04
	00350	Tyrosine metabolism	6	5.41E−04
	04730	Long-term depression	7	1.93E−03
	00140	Steroid hormone biosynthesis	6	2.85E−03

GO ID or KEGG ID represents the identification number of enriched GO terms or KEGG pathways. BP is the abbreviation of biological process. Description represents the name of GO terms or KEGG pathways; counts represent the number of up- or down-regulated genes enriched in GO terms or KEGG pathway. The smaller the *P* value is, the more significant GO terms or KEGG pathways are

projection regeneration and cell fate commitment. The enriched BP GO terms of down-regulated genes were mainly related to cell–cell signaling, response to lipid and response to external stimulus. Notably, the results showed that as many as 168 down-regulated genes were significantly enriched in signaling and single organism signaling.

In addition, the significantly enriched pathways of up- and down-regulated DEGs are shown in Table 1. Results showed that the up-regulated genes were significantly enriched in cell adhesion molecules, long-term depression, Wnt signaling pathway, type I diabetes mellitus and MAPK signaling pathway. Down-regulated genes were mainly enriched in fatty acid metabolism, metabolism of xenobiotics by cytochrome P450, tyrosine metabolism, long-term depression and steroid hormone biosynthesis.

PPI network analysis and module selection

Based on the information of STRING database and a combined score of PPI relationships >0.4, totally 281 nodes and 526 relationships were identified. The PPI network was then constructed (Fig. 1a). The top five nodes with the higher degrees were prostaglandin-endoperoxide synthase 2 (PTGS2, degree = 21), insulin-like growth factor 1 (IGF1, degree = 21), glucagon (GCG, degree = 20), phospholipase C, beta 1 (PLCB1, degree = 19) and brain-derived neurotrophic factor (BDNF, degree = 17).

In addition, functional module analysis was performed to analyze the functions of DEGs in DC-derived fibroblasts. Only one module was significantly screened in the PPI network, which contained 216 nodes and 63 relationships (Fig. 1b). Moreover, this module was significantly associated with cytokine–cytokine receptor interaction, chemokine signaling pathway, rheumatoid arthritis and Toll-like receptor signaling pathway.

Expression correlation network analysis

As shown in Fig. 2, correlation network of DEGs expression was constructed. A total of 222 co-expressed gene pairs (Pearson correlation coefficient >0.9) and 120 gene pairs (Pearson correlation coefficient less than −0.9) were identified. These gene pairs with Pearson correlation coefficient less than −0.9 were up-regulated gene–down-regulated gene pairs. The overrepresented GO BP functional correlation network of these DEGs with strong correlation is shown in Fig. 3. The results showed that these DEGs in the correlation network were significantly enriched in digestive system process, regulation of systemic arterial blood pressure and acute inflammatory response.

Prediction of candidate genes associated with DC

Based on the information of CTD database, only ten DC-associated genes were screened from 529 DEGs. Among them, neurotrophin 3 (NTF3) and purinergic receptor P2Y, G-protein coupled, 12 (P2RY12) were up-regulated genes, while others, such as androgen receptor (AR) and protein kinase C, epsilon (PRKCE), were down-regulated genes. The mean values of expression of ten DC-associated genes in DC-derived fibroblasts group and control group are shown in Fig. 4. In addition, NTF3 was significantly enriched in MAPK signaling pathway, in which other DEGs, such as nuclear receptor subfamily 4, group A, member 1 (NR4A1), fibroblast growth factor 22 (FGF22) and BDNF, were enriched. Besides, eight co-expressed

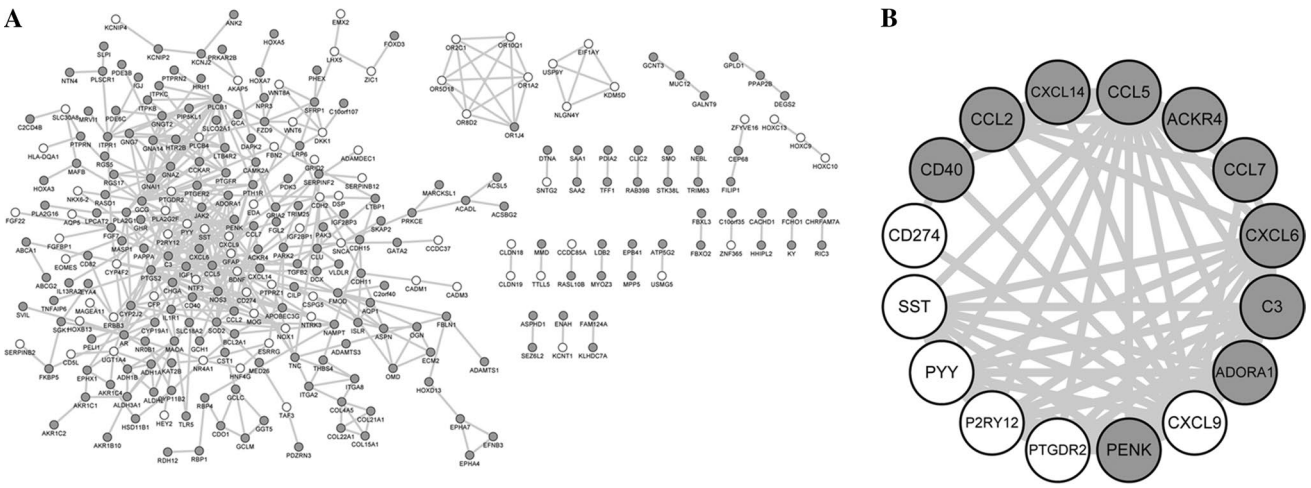

Fig. 1 Protein–protein network of differentially expressed genes (DEGs) (**a**) and significant module (**b**). *White nodes* represent up-regulated genes, and *gray nodes* represent down-regulated genes

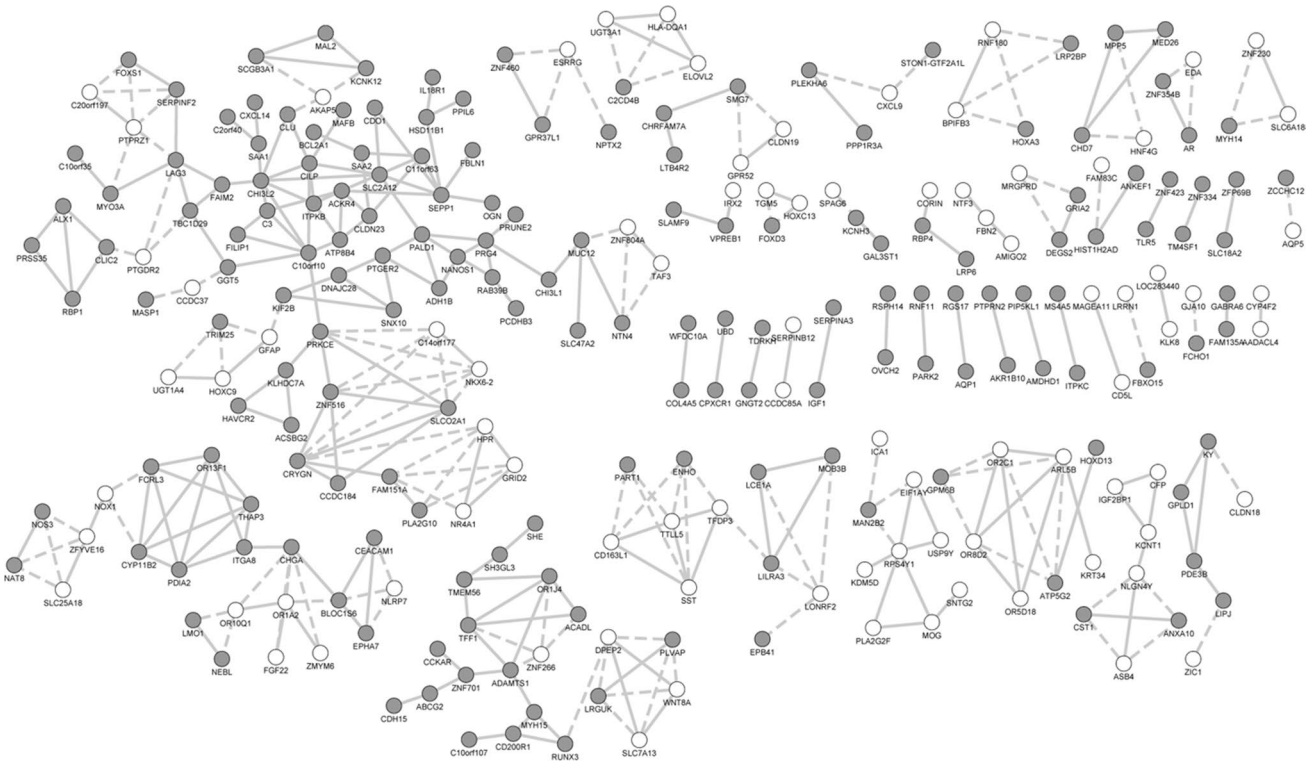

Fig. 2 Correlation network of DEGs expression. *White nodes* represent up-regulated genes, and *gray nodes* represent down-regulated genes. The *solid line* represents correlation coefficient between genes >0.9, while the *dotted line* represents correlation coefficient between genes less than −0.9

genes with these ten DC-associated genes were identified (Table 2). Thereinto, NTF3 could co-express with fibrillin 2 (FBN2), and PRKCE could co-express with zinc finger protein 516 (ZNF516), solute carrier organic anion transporter family, member 2A1 (SLCO2A1), chromosome 10 open reading frame 10 (C10orf10) and Kelch domain containing 7A (KLHDC7A).

Discussion

In the present study, bioinformatic methods were employed to identify candidate DC-associated genes that may be differentially expressed in DC. A total of 138 up- and 391 down-regulated DEGs in DC-derived fibroblasts compared with carpal tunnel-derived fibroblasts were screened. Additionally, only ten DC-associated genes were screened from 529 DEGs. Among them, NTF3 and P2RY12 were up-regulated genes, while other genes, such as AR and PRKCE, were down-regulated. Notably, NTF3 could co-express with FBN2 and was significantly enriched in MAPK signaling pathway, in which other DEGs, such as NR4A1, FGF22 and BDNF, were also enriched in. PRKCE could co-express with ZNF516, SLCO2A1, C10orf10 and KLHDC7A. Therefore, these DEGs are more likely to contribute to the development of DC.

DC is shown to be characterized by myofibroblast development and increased cytokines, such as TGF-β1 [27, 28]. TGF-β1 is identified to stimulate the collagen production during differentiation of cardiac fibroblasts to myofibroblasts [29], and its function blocking prevents myocardial fibrosis in rats [30]. A work of Horiguchi et al. indicated that negative regulation of TGF-β1 could be mediated by FBN2 [31]. FBN2 is a component of connective tissue microfibrils and is shown to be involved in elastic fiber assembly [32]. FBN2 is identified as potential predictors for radiation-induced fibrosis [33] and is strongly associated with congenital contractural arachnodactyly [34]. Thus, FBN2 may play an important role in DC via regulating TGF-β1. In addition, NTF3 is a member of the neurotrophin family, which controls survival and differentiation of mammalian neurons [35]. The promoter regions of NTF3 contain a number of predicted nuclear factor-κB (NF-κB) binding sites, and up-regulated NTF3 may activate the transcriptional activity of NF-κB [36]. The activation of NF-κB was found to be required for transformation of fibroblasts [37], and fibroblasts are considered to play an important role in the genesis of DC. In our study, NTF3

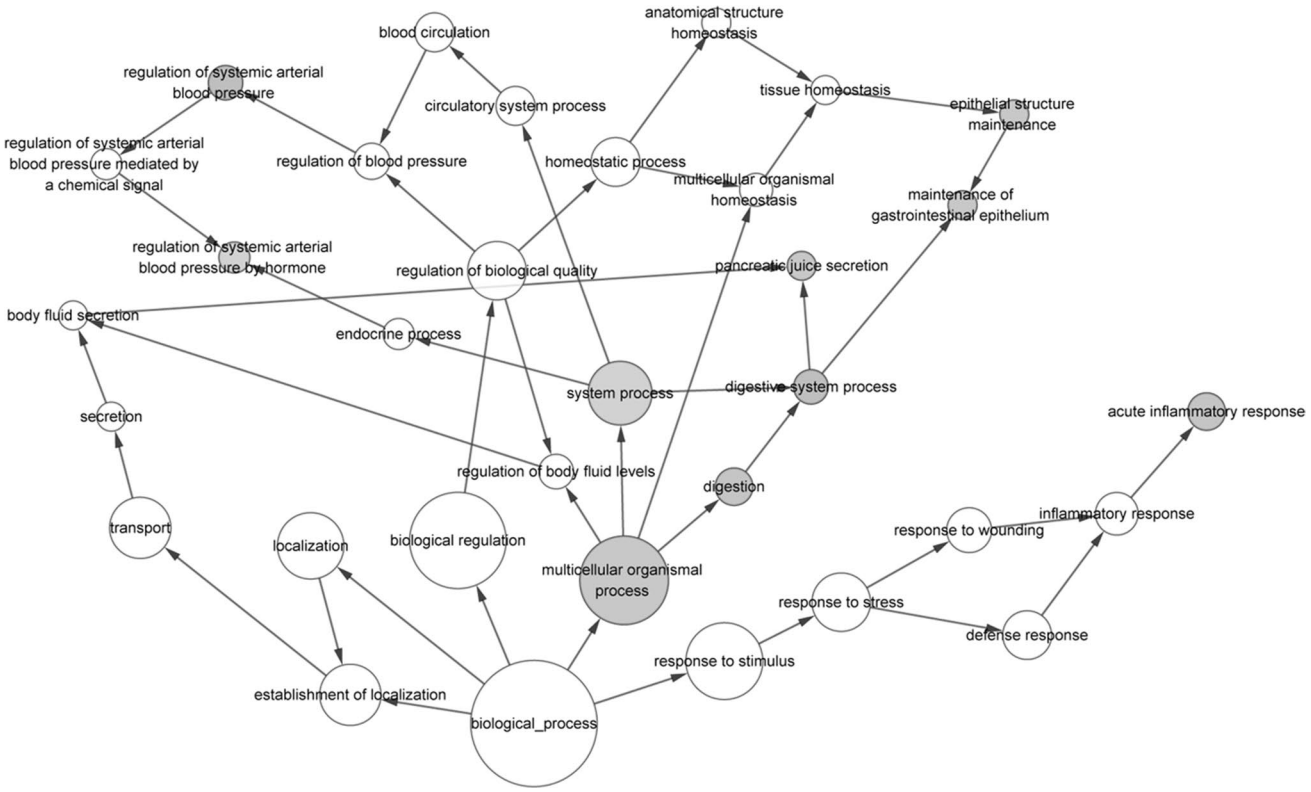

Fig. 3 GO BP branch network of DEGs with strong correlation. The *arrow* points to branch. *White nodes* represent GO BP terms, and *gray nodes* represent significantly enriched GO BP terms

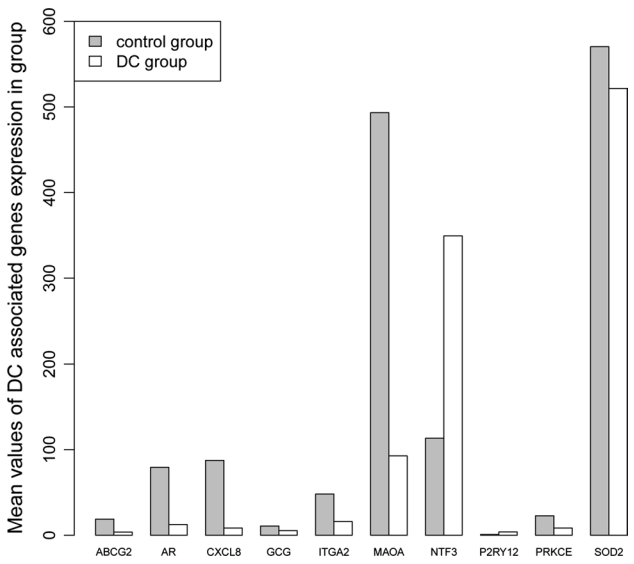

Fig. 4 Mean values of ten DC-associated genes expression in DC-derived fibroblasts group and control group. *White histograms* represent DC-derived fibroblasts group, and *gray histograms* represent control group

Table 2 Co-expressed gene pairs of disease-associated genes

Disease-associated genes	Co-expressed genes	Co-expressed coefficient
AR	ZNF354B	0.95327
PRKCE	ZNF516	0.9449
PRKCE	SLCO2A1	0.9417
ABCG2	CDH15	0.92363
PRKCE	C10orf10	0.9228
NTF3	FBN2	0.91843
ABCG2	ZNF701	0.9052
PRKCE	KLHDC7A	0.9013

could co-express with FBN2 directly. Therefore, our results suggest that NTF3 and FBN2 may play an important role in promoting development of DC via interaction between them.

In addition, NTF3 was significantly enriched in MAPK signaling pathway, in which other DEGs, such as NR4A1, FGF22 and BDNF, were also enriched in. MAPK signaling

pathway is shown to mediate growth factor-dependent cell survival [38]. MAPK signaling pathway is also involved in fibroblast-mediated wound contraction which is regulated by heat shock protein 27 [39], and has been shown to be strongly linked with DC [40]. Moreover, the constitutively active MAPK signaling could increase NR4A1 expression via direct modulation of the immediate promoter regions [41]. NR4A1 can regulate TGF-β signaling and promote the growth of fibrous [42]. FGF22 belongs to fibroblast growth factor family which has a role in the fibrotic process leading to the development of myofibroblast in Peyronie's disease, and this disease is related to DC [43]. BDNF is a potential marker and may react positively on hepatic myofibroblasts [44]. MAPK signaling pathway is proved to be the major intracellular signaling network activated by BDNF [45]. Considering the important roles of MAPK signaling pathway, we speculate that our results are in line with previous findings and suggest that NTF3, NR4A1, FGF22 and BDNF may promote myofibroblast development in DC via involving in this pathway.

Besides, our study also found that PRKCE was the predicted DC-associated genes and could co-express with several DEGs, such as ZNF516, SLCO2A1, C10orf10 and KLHDC7A. PRKCE belongs to protein kinase C (PKC) family members and can mediate β1-integrins activation in cardiac fibroblasts [46] or cell growth in thrombin-induced myofibroblasts [47]. ZNF516 play important roles in various cellular functions and its promoters frequently methylated in cervical neoplasia [48]. SLCO2A1 encodes a prostaglandin transporter and a novel mutation in the SLCO2A1 gene may cause pachydermoperiostosis with myelofibrosis in primary hypertrophic osteoarthropathy [49]. C10orf10 is identified to be differentially expressed in Hutchinson–Gilford progeria syndrome fibroblasts [50]. KLHDC7A can mediates cell–cell adhesion and is a key molecule for promoting diabetic retinopathy [51] and diabetes is proved to be a predisposing factor which may contribute to the development of DC [52]. Thus, KLHDC7A may be involved in DC due to the association between DC and diabetes. Although the roles of these DEGs in myofibroblast development in DC have not been fully discussed, we speculate that they may function as important molecules involved in the progression of this disease.

In conclusion, our study has shed new light on the mechanism and treatment of DC. As a result of this preliminary study, we confirm that these DEGs, including NTF3, FBN2, NR4A1, FGF22, BDNF, PRKCE, ZNF516, SLCO2A1, C10orf10 and KLHDC7A, may play a role in the DC development and could be candidate molecular targets for the treatment of DC. In addition, MAPK signaling pathway may play important roles in promoting development of DC. Further investigation in these genes and pathways may aid in better elucidation of mechanisms of DC pathogenesis and provide a new strategy in the therapy of this disease. However, no experimental validations are performed in our study, and more studies are still needed to confirm our hypothesis.

References

1. Wilbrand S, Flodmark C, Ekbom A, Gerdin B (2003) Activation markers of connective tissue in Dupuytren's contracture: relation to postoperative outcome. Scand J Plast Reconstr Surg Hand Surg 37:283–292
2. Beldner S (2013) Dupuytren's contracture. Orthopedics 36:929–930. doi:10.3928/01477447-20131120-05
3. Ryhanen J, Forsman M (2012) Dupuytren's contracture. Duodecim 128:421–429
4. Krüger-Sayn M, Porzberg G, Paschmeyer H (1998) Does the open palm technique for surgery of Dupuytren's contracture extend treatment and disability duration? A retrospective study. [Handchirurgie, Mikrochirurgie, plastische Chirurgie: Organ der Deutschsprachigen Arbeitsgemeinschaft fur Handchirurgie: Organ der Deutschsprachigen Arbeitsgemeinschaft fur Mikrochirurgie der Peripheren Nerven und Gefasse]. Organ der V 30:269–271
5. Shaw RB Jr, Chong AK, Zhang A, Hentz VR, Chang J (2007) Dupuytren's disease: history, diagnosis, and treatment. Plast Reconstr Surg 120:44e–54e
6. Satish L, LaFramboise WA, O'Gorman DB et al (2008) Identification of differentially expressed genes in fibroblasts derived from patients with Dupuytren's contracture. BMC Med Genomics 1:10. doi:10.1186/1755-8794-1-10
7. Pan D, Watson HK, Swigart C, Thomson JG, Honig SC, Narayan D (2003) Microarray gene analysis and expression profiles of Dupuytren's contracture. Ann Plast Surg 50:618–622
8. Bayat A, Walter J, Lambe H, Watson JS, Stanley JK, Marino M, Ferguson MW, Ollier WE (2005) Identification of a novel mitochondrial mutation in Dupuytren's disease using multiplex DHPLC. Plast Reconstr Surg 115:134–141
9. Hu F, Nystrom A, Ahmed A et al (2005) Mapping of an autosomal dominant gene for Dupuytren's contracture to chromosome 16q in a Swedish family. Clin Genet 68:424–429
10. Shih B, Wijeratne D, Armstrong DJ, Lindau T, Day P, Bayat A (2009) Identification of biomarkers in Dupuytren's disease by comparative analysis of fibroblasts versus tissue biopsies in disease-specific phenotypes. J Hand Surg Am 34:124–136
11. Kraljevic Pavelic S, Sedic M, Hock K et al (2009) An integrated proteomics approach for studying the molecular pathogenesis of Dupuytren's disease. J Pathol 217:524–533
12. Casalone R, Mazzola D, Meroni E et al (1997) Cytogenetic and interphase cytogenetic analyses reveal chromosome instability but no clonal trisomy 8 in Dupuytren contracture. Cancer Genet Cytogenet 99:73–76
13. Bayat A, Watson JS, Stanley JK, Ferguson MW, Ollier WE (2003) Genetic susceptibility to dupuytren disease: association of Zf9 transcription factor gene. Plast Reconstr Surg 111:2133–2139
14. Augoff K, Kula J, Gosk J, Rutowski R (2005) Epidermal growth factor in Dupuytren's disease. Plast Reconstr Surg 115:128–133
15. Hinz B, Gabbiani G (2012) The role of the myofibroblast in Dupuytren's disease: fundamental aspects of contraction and therapeutic perspectives. In: Dupuytren's disease and related hyperproliferative disorders. Springer, Basel, Switzerland, pp 53–60
16. Ji X, Tian F, Tian L (2015) Identification and function analysis of contrary genes in Dupuytren's contracture. Mol Med Rep 12:482–488

17. Smyth GK (2005) Limma: linear models for microarray data. In: Bioinformatics and computational biology solutions using R and bioconductor. Springer, New York, pp 397–420
18. Ashburner M, Ball CA, Blake JA et al (2000) Gene Ontology: tool for the unification of biology. Nat Genet 25:25–29
19. Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30
20. Alvord G, Roayaei J, Stephens R, Baseler MW, Lane HC, Lempicki RA (2007) The DAVID gene functional classification tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 8:R183
21. Franceschini A, Szklarczyk D, Frankild S et al (2013) STRING v9. 1: protein–protein interaction networks, with increased coverage and integration. Nucleic Acids Res 41:D808–D815
22. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504
23. Nepusz T, Yu H, Paccanaro A (2012) Detecting overlapping protein complexes in protein–protein interaction networks. Nat Methods 9:471–472
24. Janaki C, Joshi RR (2004) Motif detection in arabidopsis: correlation with gene expression data. In Silico Biol 4:149–161
25. Maere S, Heymans K, Kuiper M (2005) BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. Bioinformatics 21:3448–3449
26. Davis AP, King BL, Mockus S, Murphy CG, Saraceni-Richards C, Rosenstein M, Wiegers T, Mattingly CJ (2011) The comparative toxicogenomics database: update 2011. Nucleic Acids Res 39:D1067–D1072
27. Vaughan MB, Howard EW, Tomasek JJ (2000) Transforming growth factor-β1 promotes the morphological and functional differentiation of the myofibroblast. Exp Cell Res 257:180–189
28. Al-Qattan MM (2006) Factors in the pathogenesis of Dupuytren's contracture. J Hand Surg Am 31:1527–1534
29. Petrov VV, Fagard RH, Lijnen PJ (2002) Stimulation of collagen production by transforming growth factor-β1 during differentiation of cardiac fibroblasts to myofibroblasts. Hypertension 39:258–263
30. Kuwahara F, Kai H, Tokuda K, Kai M, Takeshita A, Egashira K, Imaizumi T (2002) Transforming growth factor-β function blocking prevents myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. Circulation 106:130–135
31. Horiguchi M, Ota M, Rifkin DB (2012) Matrix control of transforming growth factor-β function. J Biochem 152:321–329
32. Wagenseil JE, Mecham RP (2007) New insights into elastic fiber assembly. Birth Defects Res C Embryo Today 81:229–240
33. Forrester HB, Li J, Leong T, McKay MJ, Sprung CN (2014) Identification of a radiation sensitivity gene expression profile in primary fibroblasts derived from patients who developed radiotherapy-induced fibrosis. Radiother Oncol 111:186–193
34. Nishimura A, Sakai H, Ikegawa S et al (2007) FBN2, FBN1, TGFBR1, and TGFBR2 analyses in congenital contractural arachnodactyly. Am J Med Genet A 143:694–698
35. Durany N, Michel T, Zöchling R, Boissl KW, Cruz-Sánchez FF, Riederer P, Thome J (2001) Brain-derived neurotrophic factor and neurotrophin 3 in schizophrenic psychoses. Schizophr Res 52:79–86
36. Howe EN, Cochrane DR, Cittelly DM, Richer JK (2012) miR-200c targets a NF-κB up-regulated TrkB/NTF3 autocrine signaling loop to enhance anoikis sensitivity in triple negative breast cancer. PLoS ONE 7:e49987
37. Dolcet X, Llobet D, Pallares J, Matias-Guiu X (2005) NF-kB in development and progression of human cancer. Virchows Arch 446:475–482
38. Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg ME (1999) Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and-independent mechanisms. Science 286:1358–1362
39. Hirano S, Rees RS, Gilmont RR (2002) MAP kinase pathways involving hsp27 regulate fibroblast-mediated wound contraction. J Surg Res 102:77–84
40. Krause C, Kloen P, ten Dijke P (2011) Elevated transforming growth factor B and mitogen-activated protein kinase pathways mediate fibrotic traits of Dupuytren's disease fibroblasts. Fibrogenesis Tissue Repair 4:14
41. Smith AG, Lim W, Pearen M, Muscat GE, Sturm RA (2011) Regulation of NR4A nuclear receptor expression by oncogenic BRAF in melanoma cells. Pigment Cell Melanoma Res 24:551–563
42. Palumbo-Zerr K, Zerr P, Distler A et al (2015) Orphan nuclear receptor NR4A1 regulates transforming growth factor-[beta] signaling and fibrosis. Nat Med 21:150–158
43. Mulhall JP, Thom J, Lubrano T, Shankey TV (2001) Basic fibroblast growth factor expression in Peyronie's disease. J Urol 165:419–423
44. IJzer J, Roskams T, Molenbeek RF, Ultee T, Penning LC, Rothuizen J, Van den Ingh TS (2006) Morphological characterisation of portal myofibroblasts and hepatic stellate cells in the normal dog liver. Comp Hepatol 5:1–9
45. Nguyen N, Lee SB, Lee YS, Lee K-H, Ahn J-Y (2009) Neuroprotection by NGF and BDNF against neurotoxin-exerted apoptotic death in neural stem cells are mediated through Trk receptors, activating PI3-kinase and MAPK pathways. Neurochem Res 34:942–951
46. Stawowy P, Margeta C, Blaschke F, Lindschau C, Spencer-Hänsch C, Leitges M, Biagini G, Fleck E, Graf K (2005) Protein kinase C epsilon mediates angiotensin II-induced activation of β1-integrins in cardiac fibroblasts. Cardiovasc Res 67:50–59
47. Bogatkevich GS, Gustilo E, Oates JC, Feghali-Bostwick C, Harley RA, Silver RM, Ludwicka-Bradley A (2005) Distinct PKC isoforms mediate cell survival and DNA synthesis in thrombin-induced myofibroblasts. Am J Physiol Lung Cell Mol Physiol 288:L190–L201
48. Ribas J, Michailidi C, Perez J, Soudry E, Tapiaz O, Guzman P, Munoz S (2014) Genome-wide methylation profiling reveals Zinc finger protein 516 (ZNF516) and FK-506-binding protein 6 (FKBP6) promoters frequently methylated in cervical neoplasia, associated with HPV status and ethnicity in a Chilean population. Epigenetics 9:1–10
49. Zhang Z, Xia W, He J et al (2012) Exome sequencing identifies SLCO2A1 mutations as a cause of primary hypertrophic osteoarthropathy. Am J Hum Genet 90:125–132
50. Wang J, Robinson JF, O'Neil CH, Edwards JY, Williams CM, Huff MW, Pickering JG, Hegele RA (2006) Ankyrin G overexpression in Hutchinson–Gilford progeria syndrome fibroblasts identified through biological filtering of expression profiles. J Hum Genet 51:934–942
51. Vidhya G, Anusha B (2014) Diaretinopathy database—a gene database for diabetic retinopathy. Bioinformation 10:235
52. Townley W, Baker R, Sheppard N, Grobbelaar A (2006) Dupuytren's contracture unfolded. BMJ 332:397–400