DEGs and Biological Process Profiling to screen the novel biomarkers associated with both Uterine Leiomyomas and Uterine leiomyosarcomas

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Abstract

Background

Uterine Leiomyomas (ULM) or Uterine fibroid are benign lesion of unspecified aetiology and still there is dearth of prognostic biomarkers for diagnosis. The aim of this present study is to explore the novel biomarkers to be associated with Uterine Leiomyomas (ULM) and Uterine leiomyosarcomas (ULMS) that were responsible for their pathogenicity.

Methods

The microarray dataset (GSEID:GSE64763) was retrieved from the Gene Expression Omnibus database. Data preprocessing and differential gene expression analysis was performed. Principal Component Analysis (PCA) plot and heat map for ULM and ULMS were constructed for respective differentially expressed genes. The DEGs were further intersected to find the common DEGs in ULM and ULMS. Based upon STRING v 10.5, protein- protein interaction network was constructed. Further, Gene Ontology (GO) and KEGG pathway enrichment analysis were also performed to dissect out possible function and pathways.

Results

A total of 50 significant DEGs for ULM while 321 DEGs for ULMS have been identified with their official gene symbol. Between ULM and ULMS, total 14 common DEGs were identified of which 8 were up-regulated while 6 were down-regulated. Comparison of DEGs list with annotated gene list obtained from OMIM and Gene Cards, lead to identification of only 3 known disease genes (RAD51B, ESR1 and PDGFRA) while SHOX2, TNN and COL11A1 genes were found to be novel biomarkers in ULM and ULMS both. Gene ontology and KEGG pathway enrichment analysis of common novel and known candidate genes led to the identification of several important processes and pathways like ECM receptor interactions and Focal adhesion.

Conclusions

SHOX2, TNN and COL11A1 are the novel biomarkers related to both ULM and ULMS disease and have been found to be associated with ECM receptor interactions and Focal adhesion like pathways and hence can serve as novel diagnostic as well as therapeutic targets.

Background

The uterus derived from paramesonephric organogenesis is an essential supportive organ for prenatal growth and development in Eutherians. Histologically, the uterus has an inner mucosal

layer(Endometrium) and outer muscularis layer (Myometrium) [1]. Myometrium composed of highly vascularized smooth muscles cells which helps in inducing contraction during childbirth [2]. Uterine Leiomyomas (ULM)or Uterine fibroid are benign lesion of unspecified aetiology which mainly arise from Myometrium [3].These lesions composed of smooth muscles including the extracellular matrices are commonly found in pelvic area of those women bearing their reproductive ages[4].Uterine leiomyosarcomas (ULMS) a rare malignant tumor known for hematogenous transmission leading to recurrence at both native and distant areas of uterine smooth muscles[5].

According to NIH India, and different case studies which revealed that 25% of Indian women found to be suffering with ULM [6]. Nearly 25% of cases were found to be carriers of different symptoms which includes excessive bleeding, pain in pelvic region, pregnancy related complications, menstrual cramps [7]. Even ULMS cases were found to be 25–36% near 50–60 years of age period [8]. Various predisposing factors like obesity, stress, smoking, age, race which is highly prevalent in African-American women, hormonal (like estrogen) imbalance are associated with leiomyoma occurrence. Even some genetic factors too are found to be associated with the diseases[9].

Till date, these tumors have been found to be resistant to various chemotherapeutic agents and still adjuvant therapy does not hold a promising role in treatment of these tumors [10]. So, in attempts of knowing pathobiology of these lesions comparison study was done with normal myometrium. Also, very few genes were found to be associated with Uterine Fibroid and ULMS that were responsible for their pathogenicity of the diseases. So, to search more candidate genes and to disclose their mechanism at molecular level inclusive *in silico* approach using different bioinformatics softwares were applied. The purpose of the present study is to screen potent biomarkers of ULM and ULMS diseases. The present study includes the gene expression dataset (ID:GSE64763) analysis to identify differential gene expressions (DEGs).The construction of PPI(protein-protein interaction) networks were performed based upon the combined score. Enrichment and functional analysis of DEGs was also performed.

Methods

Retrieval of Microarray gene expression profile.

Using NCBI Gene expression Omnibus (GEO) datasets (https://www.ncbi.nlm.nih.gov/geo/) [11] the raw gene expression profile (ID:GSE64763) [12] dataset was retrieved. The sample dataset were obtained for ULMS,ULM and NL tissue specimens. In this dataset RNA were hybridized to HG-U133A_2] Affymatrix Human Genome U133A2.0Array at GPL571 platform. Different bioinformatics tools were used for the study of differential genes expressions in ULMS, NM and ULM samples.

Preprocessing dataset and screening DEGs.

The preprocessing of retrieved raw datasets were performed. In this the values of gene expression of probes related to specific genes were averaged and then using BiGGEsTs software [13] selection of up regulated and down regulated genes were made. GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) tool

was used for converting the probe level symbols into gene level symbols. The selected DEGs have < 0.05 adjusted p values and threshold logFC values > 0.1 for up regulated and <-0.1 for down regulated genes.

Generation of Principal component analysis and heatmap plots

Using the online tool ClustVis [14], heatmap and Principal component analysis (PCA) plot was generated for DEGs. This tool can support upto maximum 2MB of file size thus it was impossible to generate PCA plot for total gene expression dataset.

PPI network and subnetwork construction

To predict functional interactions among proteins, an online tool STRING v 10.5 [15] (https://www.string-db.org/) is used. This online tool provides combined scores between gene pairs for protein-protein interactions. For present study, the DEGs which were identified were uploaded to this online database and combined score > 0.4 was set as the parameter for analysis. Then Cytoscape v 3.2.1(http://www.cytoscape.org) [16], an in silico software package was used for different network and sub networking creation. Degree and edge betweenness criteria were employed for constructing networks.

DEGs functional analysis

DAVID (Database for Annotation, Visualisation And Integrated Discovery) software [17] (https://david.abcc.ncifcrf.gov) integrates an extensive set of functional annotation of large sets of genes record. Gene Ontology (GO) enrichment analysis involves molecular function (MF), cellular component (CC) and biological process (BP) which by using DAVID v 6.8 and STRING v 10.5 tools were performed. Depending upon the hypergeometric distribution, DAVID uses a whole set of genes based upon the similar or closely associated functions.

Results

Selection of DEGs between for ULM and ULMS

Microarray data of ULM, ULMS and control specimens were normalized (Fig. 1) using GEO2R. A total of 50 significant DEGs for ULM while 321 DEGs for ULMS have been identified with their official gene symbol. In ULM, out of total DEGs, 29 were up-regulated and 21 were down-regulated while in ULMS, 154 were up-regulated and 167 were down-regulated (Fig. 2A and 2C) (Supplementary file 1). Among total DEGs, 8 up-regulated DEGs while 6 down-regulated DEGs were found to be common between ULM and ULMS (Fig. 2C). The p-value < 0.05 and $|\log_2 FC| > 0.1$ were used as selection criteria. On the basis of average gene expression value DEGs were selected. Further, 2 DEGs in ULMS and 1 DEGs in ULM were found to be common in OMIM and Gene Cards.

Principal component and hierarchical clustering analysis of DEGs

Principal Component Analysis for ULM and ULMS reveals a scatter plot showing total variance of 50.6% and 44.9% corresponding to the principal component 1 (x-axis) while 7.3% and 7.4% corresponding to principal component 2 (y-axis) respectively (Fig. 3A and 3B). Heat-map shows a data matrix where coloring gives an overview of the numeric differences. Two separate heat map for ULM and ULMS were constructed for respective differentially expressed genes (Fig. 4A and 4B).

The Protein-Protein Interaction Network

For protein-protein interaction network, all DEGs with combined score > 0.4 (283 gene pairs out of 371 DEGs) was used which yielded one main network having 266 nodes and 883 edges (Fig. 5) while a separate network of DEGs with combined score > 0.9 was extracted separately (Fig. 6). A total of 110 DEGs with a combined score > 0.9 were included in network (red node-for up regulated and blue node-for down-regulated) (Fig. 6).

Known Disease Genes and candidate genes to ULM and ULMS

Comparison of DEGs related to ULM and ULMS reveals 14 common DEGs of which 8 were up-regulated while 6 were down-regulated. However, out of these common DEGs, only 10 DEGs were found to have combined score > 0.4 and hence included in interaction network (Fig. 5). Furthermore, we were interested to know the genes which have already been validated. For this, we compared our DEGs list with annotated gene list for obtained from OMIM and Gene Cards (Fig. 7A)which lead to identification of only 3 known disease genes (2 for ULMS and 1 for ULM; represented as a red and blue triangle in the network, Fig. 6). Direct neighbours of these three known genes were considered as candidate genes related to ULM and ULMS; this yields a total of 4 candidate genes which are shown in Fig. 6. All the common as well as known candidate genes related to ULM and ULMS both (Fig. 7B) were found to be significantly altered when plotted against average gene expression value (Fig. 7C). Out of 13 common as well as known candidate genes, 9 genes namely KIF5C(Kinesin Family Member 5C) with significant p value 1.95E-10, ZNF365(Zinc Finger Protein 365) with significant p value 4.29E-08, EPYC(Epiphycan precursor) with significant value 3.39E-03, COL11A1(COLLAGEN, TYPE XI, ALPHA-1) with significant p value 5.96E-08, SHOX2(Short Stature Homeobox 2) with significant p value 9.59E-11, MMP13(Matrix metalloproteinase 13) with significant p value 1.29E-03, TNN (Tenascin N) with significant p value 5.16E-02, RNF128(Ring Finger Protein 128) with significant p value 1.21E-03, RAD51B(RAD51 Paralog B) with significant p value 1.35E-08 were up-regulated while 3 genes namely GATA2 (GATA Binding Protein 2) with significant p value 7.06E-13, GPM6A (Glycoprotein M6A) with significant p value 1.35E-08, ESR1 (Estrogen Receptor 1) with significant p value 9.57E-02 and PDGFRA(platelet-derived growth factor receptor alpha) with significant p value 3.85E-01 were down-regulated.

Functional enrichment analysis

Gene ontology enrichment analysis for DEGs of ULM and ULMS was performed and significantly enriched functions, processes, and cellular components (p-value < 0.05) were listed in Table 1 (For ULM DEGs) and Table 2 (For ULMS DEGs). Major significant (p-value < 0.05) processes enriched for ULM were

regulation of cell death, regulation of apoptosis, cell-cell adhesion and cell morphogenesis (Fig. 8A) while extracellular matrix organization, response to steroid hormone stimulus, regulation of cell proliferation, blood-vessel morphogenesis, cell motility and cell cycle phase were significant processes (p-value < 0.05) for ULMS (Fig. 8B).

Table 1
GO enrichment analysis for ULM

Category	Term	PValue
GOTERM_BP_FAT	GO:0030182 ~ neuron differentiation	6.93E-04
GOTERM_BP_FAT	GO:0048666 ~ neuron development	0.001469
GOTERM_BP_FAT	GO:0000902 ~ cell morphogenesis	0.001822
GOTERM_BP_FAT	GO:0048812 ~ neuron projection morphogenesis	0.001913
GOTERM_BP_FAT	GO:0007601 ~ visual perception	0.002014
GOTERM_BP_FAT	GO:0050953 ~ sensory perception of light stimulus	0.002014
GOTERM_BP_FAT	GO:0032989 ~ cellular component morphogenesis	0.002928
GOTERM_BP_FAT	GO:0048858 ~ cell projection morphogenesis	0.003177
GOTERM_BP_FAT	GO:0032990 ~ cell part morphogenesis	0.003718
GOTERM_BP_FAT	GO:0031175 ~ neuron projection development	0.003718
GOTERM_BP_FAT	GO:0007155 ~ cell adhesion	0.007299
GOTERM_BP_FAT	GO:0022610 ~ biological adhesion	0.007349
GOTERM_BP_FAT	GO:0001501 ~ skeletal system development	0.008057
GOTERM_BP_FAT	GO:0007409 ~ axonogenesis	0.012363
GOTERM_BP_FAT	GO:0030030 ~ cell projection organization	0.013121
GOTERM_BP_FAT	GO:0051216 ~ cartilage development	0.01479
GOTERM_BP_FAT	GO:0048667 ~ cell morphogenesis involved in neuron differentiation	0.015297
GOTERM_BP_FAT	GO:0000904 ~ cell morphogenesis involved in differentiation	0.022985
GOTERM_BP_FAT	GO:0016337 ~ cell-cell adhesion	0.031559
GOTERM_BP_FAT	GO:0001503 ~ ossification	0.033675
GOTERM_BP_FAT	GO:0060348 ~ bone development	0.038069
GOTERM_BP_FAT	GO:0002062 ~ chondrocyte differentiation	0.044313
GOTERM_BP_FAT	GO:0050804 ~ regulation of synaptic transmission	0.04565
GOTERM_BP_FAT	GO:0001502 ~ cartilage condensation	0.046718
GOTERM_BP_FAT	GO:0042981 ~ regulation of apoptosis	0.048735
GOTERM_BP_FAT	GO:0007600 ~ sensory perception	0.050046

Category	Term	PValue
GOTERM_BP_FAT	GO:0043067 ~ regulation of programmed cell death	0.050487
GOTERM_BP_FAT	GO:0010941 ~ regulation of cell death	0.051154
GOTERM_BP_FAT	GO:0051969 ~ regulation of transmission of nerve impulse	0.052463
GOTERM_BP_FAT	GO:0031644 ~ regulation of neurological system process	0.056324
GOTERM_BP_FAT	GO:0043066 ~ negative regulation of apoptosis	0.058531

Table 2
GO enrichment analysis for ULMS

Category	Term	PValue
GOTERM_BP_FAT	GO:0022403 ~ cell cycle phase	2.68E-05
GOTERM_BP_FAT	GO:0007548 ~ sex differentiation	5.38E-05
GOTERM_BP_FAT	GO:0045137 ~ development of primary sexual characteristics	6.11E-05
GOTERM_BP_FAT	GO:0016477 ~ cell migration	7.65E-05
GOTERM_BP_FAT	GO:0007049 ~ cell cycle	1.34E-04
GOTERM_BP_FAT	GO:0000279 ~ M phase	1.62E-04
GOTERM_BP_FAT	GO:0051674 ~ localization of cell	2.47E-04
GOTERM_BP_FAT	GO:0048870 ~ cell motility	2.47E-04
GOTERM_BP_FAT	GO:0001568 ~ blood vessel development	2.90E-04
GOTERM_BP_FAT	GO:0001944 ~ vasculature development	3.68E-04
GOTERM_BP_FAT	GO:0006259 ~ DNA metabolic process	4.07E-04
GOTERM_BP_FAT	GO:0043627 ~ response to estrogen stimulus	4.18E-04
GOTERM_BP_FAT	GO:0006928 ~ cell motion	4.88E-04
GOTERM_BP_FAT	GO:0022402 ~ cell cycle process	6.54E-04
GOTERM_BP_FAT	GO:0007160 ~ cell-matrix adhesion	7.92E-04
GOTERM_BP_FAT	GO:0046546 ~ development of primary male sexual characteristics	8.09E-04
GOTERM_BP_FAT	GO:0048608 ~ reproductive structure development	0.00139
GOTERM_BP_FAT	GO:0031589 ~ cell-substrate adhesion	0.001398
GOTERM_BP_FAT	GO:0046661 ~ male sex differentiation	0.001491
GOTERM_BP_FAT	GO:0006260 ~ DNA replication	0.001539
GOTERM_BP_FAT	GO:0000278 ~ mitotic cell cycle	0.00168
GOTERM_BP_FAT	GO:0003006 ~ reproductive developmental process	0.001771
GOTERM_BP_FAT	GO:0008406 ~ gonad development	0.002999
GOTERM_BP_FAT	GO:0001501 ~ skeletal system development	0.003182
GOTERM_BP_FAT	GO:0048514 ~ blood vessel morphogenesis	0.0033
GOTERM_BP_FAT	GO:0042127 ~ regulation of cell proliferation	0.003979
GOTERM_BP_FAT	GO:0007017 ~ microtubule-based process	0.004052

Category	Term	PValue
GOTERM_BP_FAT	GO:0007155 ~ cell adhesion	0.004063
GOTERM_BP_FAT	GO:0022610 ~ biological adhesion	0.004131
GOTERM_BP_FAT	GO:0000280 ~ nuclear division	0.004443
GOTERM_BP_FAT	GO:0007067 ~ mitosis	0.004443
GOTERM_BP_FAT	GO:0030900 ~ forebrain development	0.00446
GOTERM_BP_FAT	GO:0048754 ~ branching morphogenesis of a tube	0.004908
GOTERM_BP_FAT	GO:0000087 ~ M phase of mitotic cell cycle	0.005027
GOTERM_BP_FAT	GO:0048545 ~ response to steroid hormone stimulus	0.005596
GOTERM_BP_FAT	GO:0040012 ~ regulation of locomotion	0.005596
GOTERM_BP_FAT	GO:0051270 ~ regulation of cell motion	0.005786
GOTERM_BP_FAT	GO:0048285 ~ organelle fission	0.005859
GOTERM_BP_FAT	GO:0007389 ~ pattern specification process	0.00604
GOTERM_BP_FAT	GO:0008283 ~ cell proliferation	0.007655
GOTERM_BP_FAT	GO:0030334 ~ regulation of cell migration	0.00832
GOTERM_BP_FAT	GO:0001763 ~ morphogenesis of a branching structure	0.008467
GOTERM_BP_FAT	GO:0030198 ~ extracellular matrix organization	0.008615
GOTERM_BP_FAT	GO:0051129 ~ negative regulation of cellular component organization	0.010756
GOTERM_BP_FAT	GO:0051726 ~ regulation of cell cycle	0.011261
GOTERM_BP_FAT	GO:0051301 ~ cell division	0.012264
GOTERM_BP_FAT	GO:0007018 ~ microtubule-based movement	0.01266
GOTERM_BP_FAT	GO:0046128 ~ purine ribonucleoside metabolic process	0.012796
GOTERM_BP_FAT	GO:0042278 ~ purine nucleoside metabolic process	0.012796
GOTERM_BP_FAT	GO:0010564 ~ regulation of cell cycle process	0.013179
GOTERM_BP_FAT	GO:0032355 ~ response to estradiol stimulus	0.013255
GOTERM_BP_FAT	GO:0006261 ~ DNA-dependent DNA replication	0.016877
GOTERM_BP_FAT	GO:0003002 ~ regionalization	0.0195
GOTERM_BP_FAT	GO:0016337 ~ cell-cell adhesion	0.019788

Category	Term	PValue
GOTERM_BP_FAT	GO:0010628 ~ positive regulation of gene expression	0.02067
GOTERM_BP_FAT	GO:0009719 ~ response to endogenous stimulus	0.021243
GOTERM_BP_FAT	GO:0035239 ~ tube morphogenesis	0.021344
GOTERM_BP_FAT	GO:0045765 ~ regulation of angiogenesis	0.022199
GOTERM_BP_FAT	GO:0043583 ~ ear development	0.022922
GOTERM_BP_FAT	GO:0009725 ~ response to hormone stimulus	0.023152
GOTERM_BP_FAT	GO:0008585 ~ female gonad development	0.023373
GOTERM_BP_FAT	GO:0000904 ~ cell morphogenesis involved in differentiation	0.023513
GOTERM_BP_FAT	GO:0007126 ~ meiosis	0.025804
GOTERM_BP_FAT	GO:0051327 ~ M phase of meiotic cell cycle	0.025804
GOTERM_BP_FAT	GO:0042698 ~ ovulation cycle	0.027118
GOTERM_BP_FAT	GO:0048568 ~ embryonic organ development	0.027718
GOTERM_BP_FAT	GO:0051321 ~ meiotic cell cycle	0.027849
GOTERM_BP_FAT	GO:0030539 ~ male genitalia development	0.029527
GOTERM_BP_FAT	GO:0045446 ~ endothelial cell differentiation	0.029527
GOTERM_BP_FAT	GO:0030155 ~ regulation of cell adhesion	0.02957
GOTERM_BP_FAT	GO:0046660 ~ female sex differentiation	0.029802
GOTERM_BP_FAT	GO:0046545 ~ development of primary female sexual characteristics	0.029802
GOTERM_BP_FAT	GO:0060173 ~ limb development	0.031103
GOTERM_BP_FAT	GO:0051329 ~ interphase of mitotic cell cycle	0.031103
GOTERM_BP_FAT	GO:0048736 ~ appendage development	0.031103
GOTERM_BP_FAT	GO:0030951 ~ establishment or maintenance of microtubule cytoskeleton polarity	0.033716
GOTERM_BP_FAT	GO:0030952 ~ establishment or maintenance of cytoskeleton polarity	0.033716
GOTERM_BP_FAT	GO:0051325 ~ interphase	0.034588
GOTERM_BP_FAT	GO:0006468 ~ protein amino acid phosphorylation	0.035765
GOTERM_BP_FAT	GO:0007162 ~ negative regulation of cell adhesion	0.03637

Category	Term	PValue
GOTERM_BP_FAT	GO:0045935 ~ positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	0.037712
GOTERM_BP_FAT	GO:0032989 ~ cellular component morphogenesis	0.039122
GOTERM_BP_FAT	GO:0001655 ~ urogenital system development	0.039596
GOTERM_BP_FAT	GO:0000226 ~ microtubule cytoskeleton organization	0.039648
GOTERM_BP_FAT	GO:0001525 ~ angiogenesis	0.040762
GOTERM_BP_FAT	GO:0000902 ~ cell morphogenesis	0.041229
GOTERM_BP_FAT	GO:0046700 ~ heterocycle catabolic process	0.042066
GOTERM_BP_FAT	GO:0009119 ~ ribonucleoside metabolic process	0.043126
GOTERM_BP_FAT	GO:0031328 ~ positive regulation of cellular biosynthetic process	0.044459
GOTERM_BP_FAT	GO:0042060 ~ wound healing	0.044885
GOTERM_BP_FAT	GO:0006690 ~ icosanoid metabolic process	0.045507
GOTERM_BP_FAT	GO:0048839 ~ inner ear development	0.045516
GOTERM_BP_FAT	GO:0051173 ~ positive regulation of nitrogen compound metabolic process	0.04854
GOTERM_BP_FAT	GO:0009891 ~ positive regulation of biosynthetic process	0.049902
GOTERM_BP_FAT	GO:0042048 ~ olfactory behavior	0.050147
GOTERM_BP_FAT	GO:0045944 ~ positive regulation of transcription from RNA polymerase II promoter	0.051839
GOTERM_BP_FAT	GO:0033559 ~ unsaturated fatty acid metabolic process	0.055667
GOTERM_BP_FAT	GO:0048806 ~ genitalia development	0.057626
GOTERM_BP_FAT	GO:0048858 ~ cell projection morphogenesis	0.057997
GOTERM_BP_FAT	GO:0045941 ~ positive regulation of transcription	0.058249
GOTERM_BP_FAT	GO:0008584 ~ male gonad development	0.058362
GOTERM_BP_FAT	GO:0010604 ~ positive regulation of macromolecule metabolic process	0.058378
GOTERM_BP_FAT	GO:0043062 ~ extracellular structure organization	0.059855

Co-enrichment analysis of common and known candidate genes related both to ULM and ULMS led to the identification of several important processes. A separate biological processes network was created for those genes (Fig. 9). UP-regulated genes like KIF5C, ZNF365, EPYC, COL11A1, SHOX2, MMP13, TNN,

RNF128, RAD51B were found to be involved in the regulation of cell proliferation, cell adhesion, response to estrogen stimulus (Fig. 8A). Major processes regulated by down-regulated genes (GATA2, GPM6A, ESR1 and PDGFR1A) were regulation of transcription,cell morphogenesis and cell differention,cell projection,extracellular matrix organization.(Fig. 8B). Hence, 10 DEGs identified in this study were estimated to be candidate disease genes of ULM and ULMS both.

Pathway enrichment analysis

KEGG pathway enrichment analysis for DEGs of ULM and ULMS revealed a total of 8 significantly enriched pathways (p value < 0.05). Small cell lung cancer, cell cycle, vascular smooth muscle contraction, focal adhesion, Cell Adhesion Molecules (CAMs), ECM receptor interaction were pathways identified for ULM while ECM receptor interaction and focal adhesion are significant pathways associated with ULMS (Fig. 10).

Discussion

Uterine Leiomyoma, or uterine fibroid (ULM), is a benign lesion which arises commonly in the muscular areas of the uterine wall [18]. Uterine leiomyosarcoma (ULMS) is a smooth muscles malignancy that arises in the smooth muscles areas of the uterus [19]. Approximately, among every 1000 women having fibroid, one to five women were found with ULMS too. The prevalence of their occurrence is increasing and till date no effective treatments were found [20]. So, in order to find more efficient methods for treatment, several studies have suggested different pathways and particular genes that are associated with the development of ULMS and ULM. Recently, different bioinformatics studies were used for finding the molecular mechanism of uterine leiomyomas and uterine leiomyosarcoma disease. In this present in silico analysis, the highly efficient screening of gene expressions dataset was performed which revealed a total of 371 DEGs (50 ULM and 321 ULMS genes).

On the basis of GO cluster, the main biological processes of DEGs involve cell adhesion, cell motility, cell differentiation, localization of cell in ULMS and cell adhesion, apoptosis, neuronal development, cell morphogenesis in ULM. Major DEGs that formed the hub nodes were eight up regulated genes (KIF5C,ZNF365,EPYC,COL11A1,SHOX2,MMP13,TNN,RNF128) and six down regulated genes(ABLIM1,GRAMD3,GATA3,ABCA8,GPM6A,LMBRD1).

ZNF365 gene was found to be involved in maintenance of stable genome, repairing damaged DNA. Moreover, ZNF365 also promotes recovery of stalled replication fork in order to provide genomic stability which were detected in both hereditary and sporadic cancer types [21]. Variations in ZNF365 gene may increase the risk of having breast cancer through affecting the dense tissues proportion in breast [22]. According to YJhang et al. ZNF365 loss leads to delay in progression of mitosis and this also results in exit due to stress in replication process which leads to increase in aneuploidy, centrosome reduplication and disruption of cytokinesis process [21]. However, this gene mechanism in the case of ULM and ULMS has yet not been identified and since, we speculate that ZNF365 may be closely related with DNA repairing and genome stability thus could be a potent target for ULM and ULMS treatment.

KIF5C gene encodes motor proteins that belong to the kinesin superfamily involved in eukaryotic cell motilities [23]. Tsibris *et al.*, 2003 found the KIF5C gene to be one of the up-regulated genes in uterine fibroid [24]. Artur Padzik et al. revealed that KIF5C protein phosphorylated by JNK alters its cell motility and transport of microtubules loaded with cargoes. Wei Wang et al. suggested from their experiment that among 4 linear transcripts mRNAs KIF5C was an up regulated gene in ULM [25]. However, till date no studies could find the KIF5C gene role in ULMS case. Further, KIF5C gene is associated with cell motility like features and hence may prove to be a novel target for these both ULM and ULMS.

Lisowska et al. recognized EPYC genes associated with LOX were found in disease free survivability with overall survival and disease-free survival in ovarian cancer [26]. EPYC genes encodes for proteoglycan. These help in regulating fibrillogenesis. EPYC gene was found to be involved in breast, uterine, colorectal cancer [27]. Radosław Januchowski et al. found EPYC gene to be upregulated in both cell lines (A2780DR1, A2780DR2) that were DOX resistant [28]. However, till date no studies have reported its function in ULM and ULMS. Since, in different cancers EPYC gene was found to be up regulated and in this present study this gene was found to be up regulated which may help to provide a novel lead for treatment of these uterine tumors.

SHOX2 gene is used to regulate transcription processes and its DNA methylation was found to be the biomarker of lung cancer [29].In breast cancer, S. Hong et al. investigated induction of EMT through SHOX2 overexpression [30]. B. Schmidt et al. identified that methylation of SHOX2 DNA was found as biomarker for lung cancer [31]. Fubiao Ye etal. investigated cell apoptosis and cell proliferation, extracellular matrix formation as major roles of SHOX2 on nucleus pulposus cells [32]. However, SHOX2 role in ULMS and ULM diseases was not found till now. Since in different carcinomas cases its involvement in cell apoptosis and cell proliferation, extracellular matrix formation like processes may provide a biomarker for the treatment of both ULMS and ULM also.

TNN gene encodes proteins involved in cell migration [33]. In tumors it stimulates angiogenesis of endothelial cells. It was also found to be one of the biomarkers for breast cancer [34].According to Leif E.Peterson et al., the TNN gene is involved in cell matrix adhesion in lung adenocarcinoma [35]. Baolin Liu et al investigated that cancer genes like TNN were found to be involved in extracellular matrix interactions like pathways [36]. However, TNN gene was not identified in ULM and ULMS like cases and since it helps in cell matrix adhesion, so it may serve as a promising target for these both cases.

MMP13 encodes protein produced from stromal fibroblast that are involved in degradation of different ECM components and induces angiogenesis by increasing protein levels of VEGF and VEGFR2 [37].According to Sunil K Halder et al. high expression of MMP13 in uterine leiomyoma pathogenesis was detected [38].Though it was not found in ULMS cases. And according to Guillaume E Courtoy et al. MMP13 encoded proteins were involved in apoptosis, cell proliferation in myoma [39]. And this may provide a potential lead for treatment ULMS also.

GPMA6 gene encodes protein involved in neuronal differentiation and development. These encoded proteins helps in neuronal stem cells migration [40]. GPMA6 gene was found to be novel target gene

involved in proliferation, promoting tumor survival and development in thyroid carcinomas [41]. And these features of GPMA6 gene would help to provide novel candidate for both ULM and ULMS.

According to Xuhui Liu et al., the COL11A1 gene was identified as a marker for uterine fibroid via gene expression analysis. COL11A1 gene encoding proteins was found in focal adhesion and extracellular matrix receptor interactions which suggests to be involved as biomarkers in leiomyoma cases [42]. However, it was not found to be involved in leiomyosarcomas. However the features of focal adhesion and ECM receptor interactions of this gene may help to identify a potent marker for ULMS.

RNF128 (also called as Grail) is ubiquitin E3 ligase and plays a vital role in producing cytokines [43]. Yi-Ying Lee et al. suggested that RNF128 downregulation was involved in urothelial cancer [44]. Miika Mehine et al. investigated through integrated ULM dataset analysis that RNF128 to be one of the markers for ULM [45]. Though none of the studies revealed its connection with ULMS but being p53 interacting glycoprotein and under stress conditions becomes crucial for apoptosis induced by p53 may also help to identify a key biomarker for ULMS cases.

GATA2 was found to be involved in cell proliferation and cell cycle regulation. Recently Shan Yu et al. found that GATA2 as one of the markers for breast cancer [46]. Shun Sato et al. through their bioinformatics studies found that GATA2 was a biomarker of uterine fibroid [47]. Veronica Rodriguez-Bravo et al. have reported GATA2 gene aggressiveness in prostate cancer due to its overexpression leads to increase in proliferation, invasiveness [48]. Since no any study revealed the GATA2 role in ULMS hence GATA2 gene can be thought to play an important role in cell proliferation and can be considered as a novel candidate for ULMS cases.

Lisa Golmard et al. suggested that RAD51B mutation correlated with breast and ovarian cancer. These genes were found to be involved in DNA repair mechanism [49]. Zehra Orduluet al. revealed RAD51B to be one of the biomarkers for uterine fibroid [50]. Javier A. Arias-Stella et al. identified RAD51B as one of the biomarkers in uterine myxoid leiomyosarcomas [51]. Since, RAD51B correlation with both ULM and ULMS may prove to be a potent marker for their treatment.

According to Niko Välimäk et al. found ESR1 as one of the potential markers in uterine fibroid diseases [52]. Mostafa A. Borahay et al. revealed the different estrogen signalling pathways involving ESR1 to be responsible in uterine leiomyoma diseases [53]. Recent studies of Adi Zundelevich et al. reported correlation of ESR1 mutation and breast cancer [54]. Heather Miller et al. also investigated estrogen receptor involvement in uterine leiomyosarcomas [55]. Thus, ESR1 correlation with both ULM and ULMS cases may provide potential key markers for their treatment.

Madhura Joglekar-Javadekar et al. suggested that PDGFRA mutation leads to hepatocellular carcinoma, leukemias, gastrointestinal stromal tumors (GISTs) and glioblastoma [56]. Guangli Suo et al. found the PDGFRA gene to be involved in different signalling pathways and in growth of uterine smooth muscles [57]. Its overexpression may lead to uterine leiomyoma. Juhasz-Böss also reviewed PDGFRA correlation with ULMS. So, PDGFRA gene might provide a biomarker for treatment of both ULM and ULMS.

In this microarray analysis NM, ULM and ULMS tissues has been used which provides an integrated approach to study the synergistic effect of differential gene expression on several biological processes and pathways to reveal their mechanism at molecular level.

Conclusion

We conclude that RAD51B,ESR1 and PDGFRA genes were found to be common reported biomarkers both in ULM and ULMS treatment through participating into several pathways and also associated with ECM receptor interactions and Focal adhesion like pathways which was revealed through our studies. Additionally, SHOX2, TNN and COL11A1 might be the novel biomarkers related both with ULM and ULMS disease which were also found to be associated with ECM receptor interactions and Focal adhesion like pathways which were revealed through our findings. The present study provides us a new perspective to detect the potent biomarkers responsible for both ULM and ULMS but still in vitro and in vivo experiments are still needed to verify the results.

Abbreviations

ULM: Uterine Leiomyoma; ULMS: Uterine Leiomyosarcoma; PCA: Principal Component Analysis; DEGs: Differentially Expressed Genes; GO: Gene Ontology; GEO: Gene expression Omnibus; PPI: Protein-Protein Interaction; NM: Normal Myometrium; ECM :Extra Cellular Matrix; DAVID: Database for Annotation, Visualization And Integrated Discovery; MF: molecular function; CC: Cellular Component; BP: Biological Process; KIF5C:Kinesin Family Member 5C; ZNF365:Zinc Finger Protein 365;EPYC:Epiphycan precursor; COL11A1:COLLAGEN, TYPE XI, ALPHA-1; SHOX2:Short Stature Homeobox 2; MMP13:Matrix metalloproteinase 13; TNN: Tenascin N; RNF128:Ring Finger Protein 128; RAD51B:RAD51 Paralog B; GATA2:GATA Binding Protein 2;GPM6A:Glycoprotein M6A;ESR1:Estrogen Receptor 1; PDGFRA: Platelet-Derived Growth Factor Receptor Alpha.

Declarations

Ethics approval and consent to participate

The present work is totally computational and hence does not require any ethical approval and consent of participation.

Consent for publication

Not applicable

Availability of data and material

Request for additional materials can be addressed to the Ravi Bhushan. All the relevant data are enclosed in the manuscript and provided as supplementary file. The RNA-seq. raw data were retrieved from NCBI's

Gene Expression Omnibus and are accessible through GEO accession number i.e. GSE64763 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64763).

Competing interests

The authors declare that they have no any conflict of interest.

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Authors' contributions

SU: Methodology, Investigation, Writing - review & editing RB: Conceptualization, Data curation, Writing-review & editing. DG: Visualization, Investigation, Validation. PKD: Review & editing. All Authors have read and approved the manuscript.

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Figures

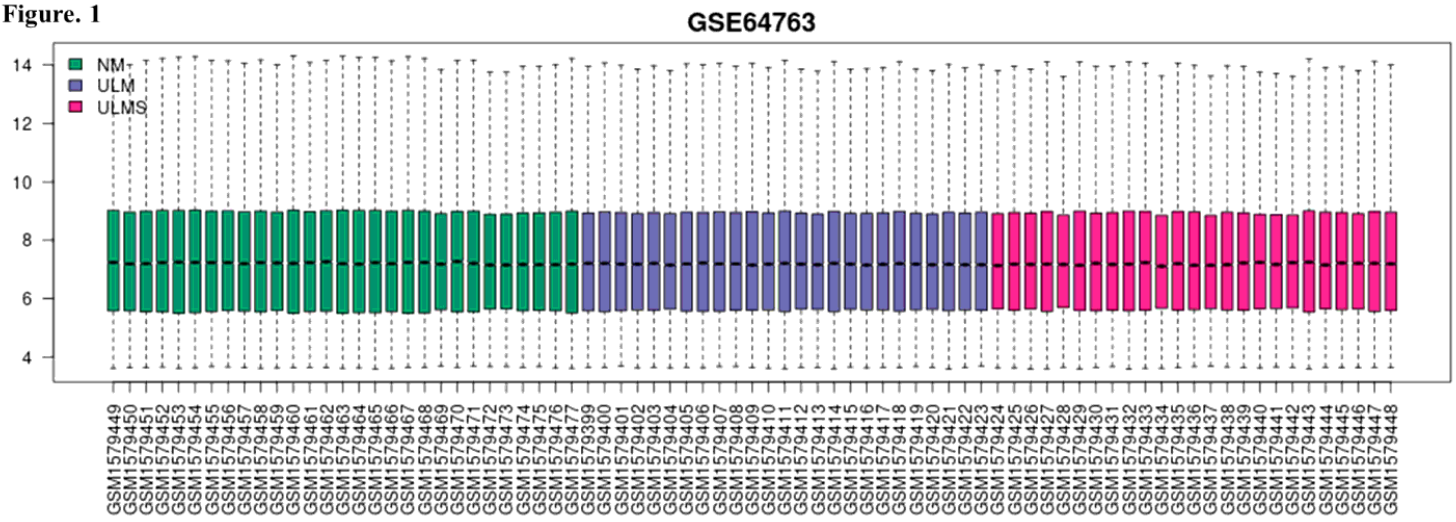
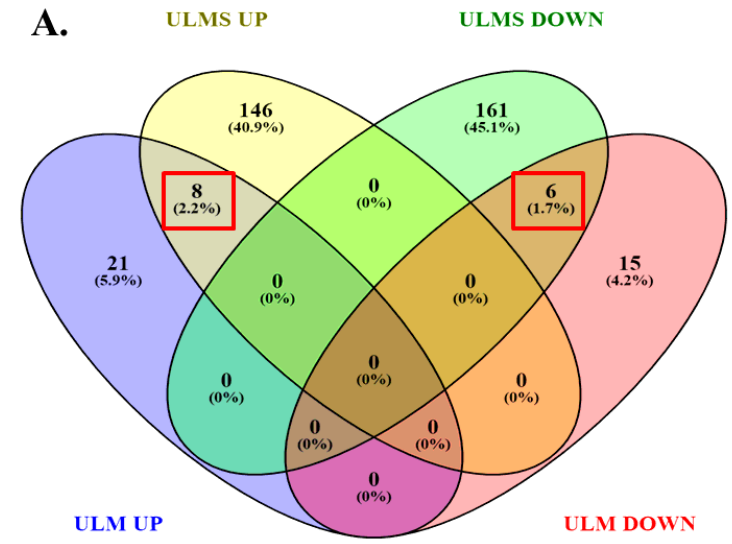


Figure 1

Microarray data normalization. Box plot showing the distribution of values data for the selected samples. The lines in the box are coincident, indicating that these chips have been highly normalized.

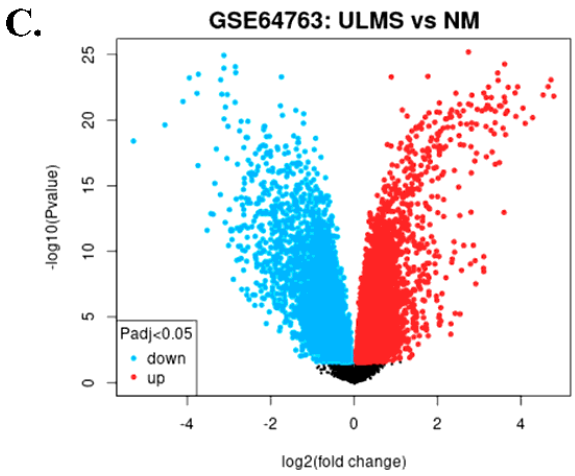
Figure. 2



B.

DEGs of ULM	50
DEGs of ULMS	321
ULM UP Genes	29
ULM Down Genes	21
ULMS UP Genes	154
ULMS Down Genes	167

UP Genes	Down Genes
KIF5C	ABLIM1
ZNF365	GRAMD3
EPYC	GATA2
COL11A1	ABCA8
SHOX2	GPM6A
MMP13	LMBRD1
TNN	
RNF128	



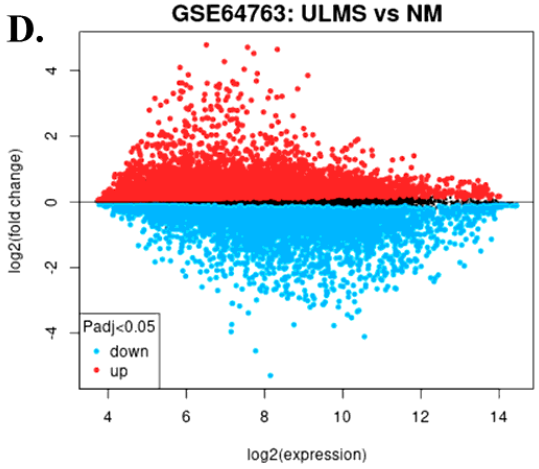


Figure 2

Venn diagram showing common DEGs in ULM and ULMS. Total DEGs with up-regulated and down-regulated genes. The red rectangle highlights the UP and DOWN regulated genes common in both cases. Venny tool v 2.1.0 was used to draw the venn diagram.

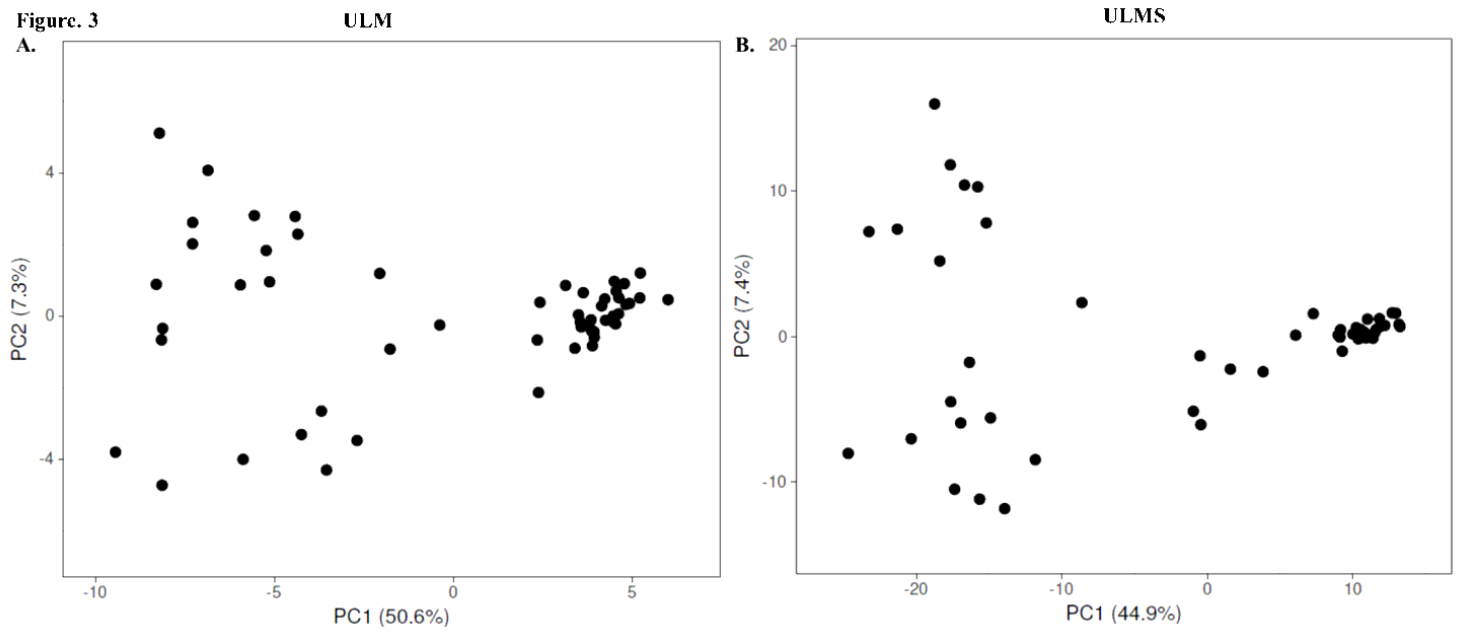


Figure 3

Principal component analysis of dataset GSE64763. PCA plot shows a scatter plot with principal component 1 (x-axis) and principal component 2 (y-axis). 3A: Showing total variance of 50.6% to principle component 1 and 7.3% to principle component 2. 3B: Showing total variance of 44.9% corresponding to the principal component 1 (x-axis) and 7.4% corresponding to principal component 2 (y-axis) respectively. ClustVis tool was used for this.

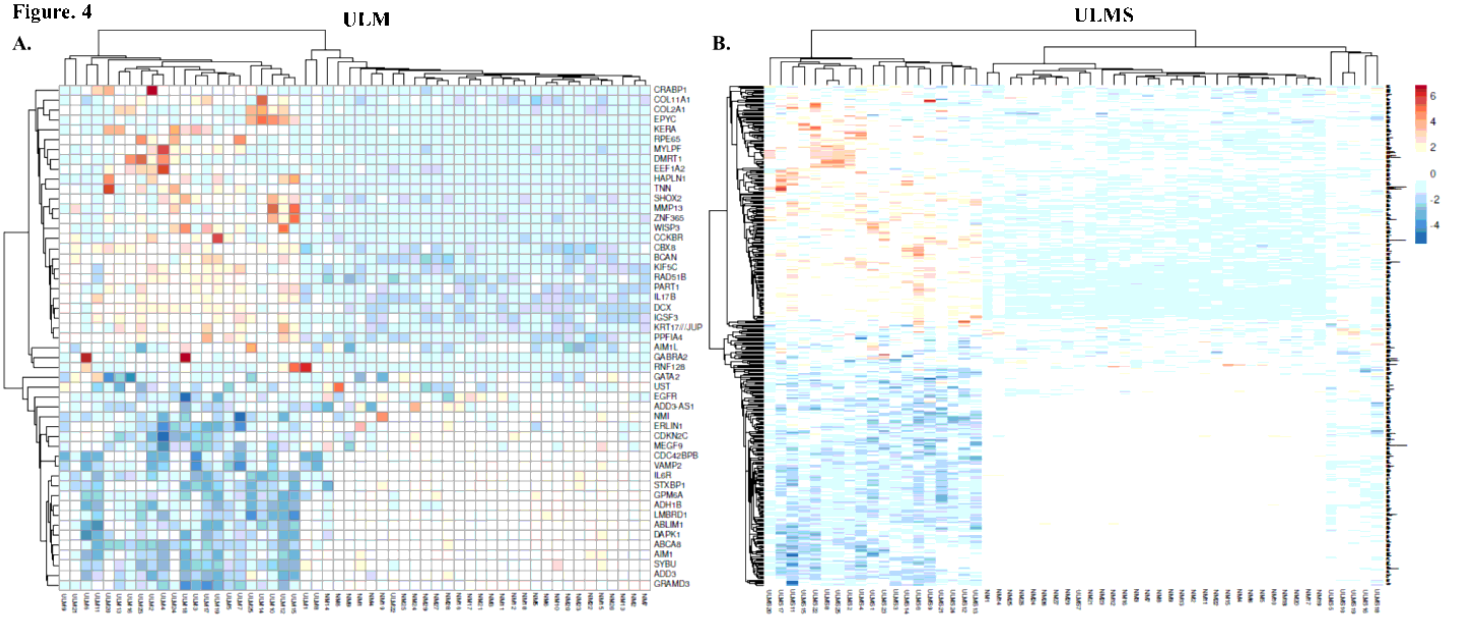


Figure 4

Heat map of differentially expresses gene sets. Heat map showing the average gene expression of differentially expressed genes (DEGs) A. among Uterine Leiomyoma (ULM) and normal myometrium

(NM) and B. among Uterine Leiomyosarcomas (ULMS) and normal myometrium (NM) . The blue to orange gradation represents the gene expression values change from small to large. ClustVis tool was used to draw heat map.

Figure. 5

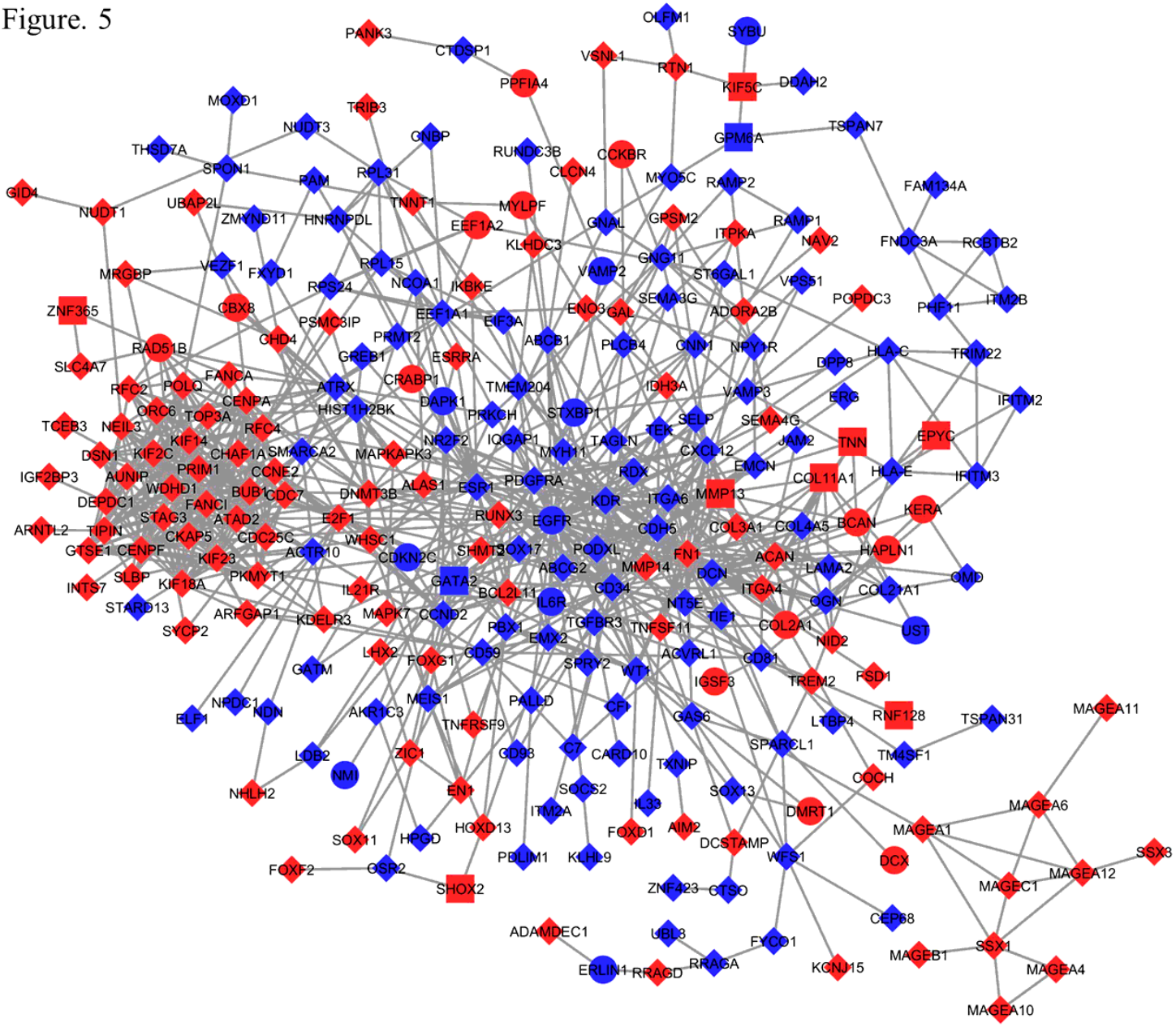


Figure 5

Venn diagram showing the common as well as known Uterine fibroids. The red rectangle highlights the common genes between ULM and ULMS aas well as UF related candidate genes. Venny tool v 2.1.0 was used to draw the venn diagram.

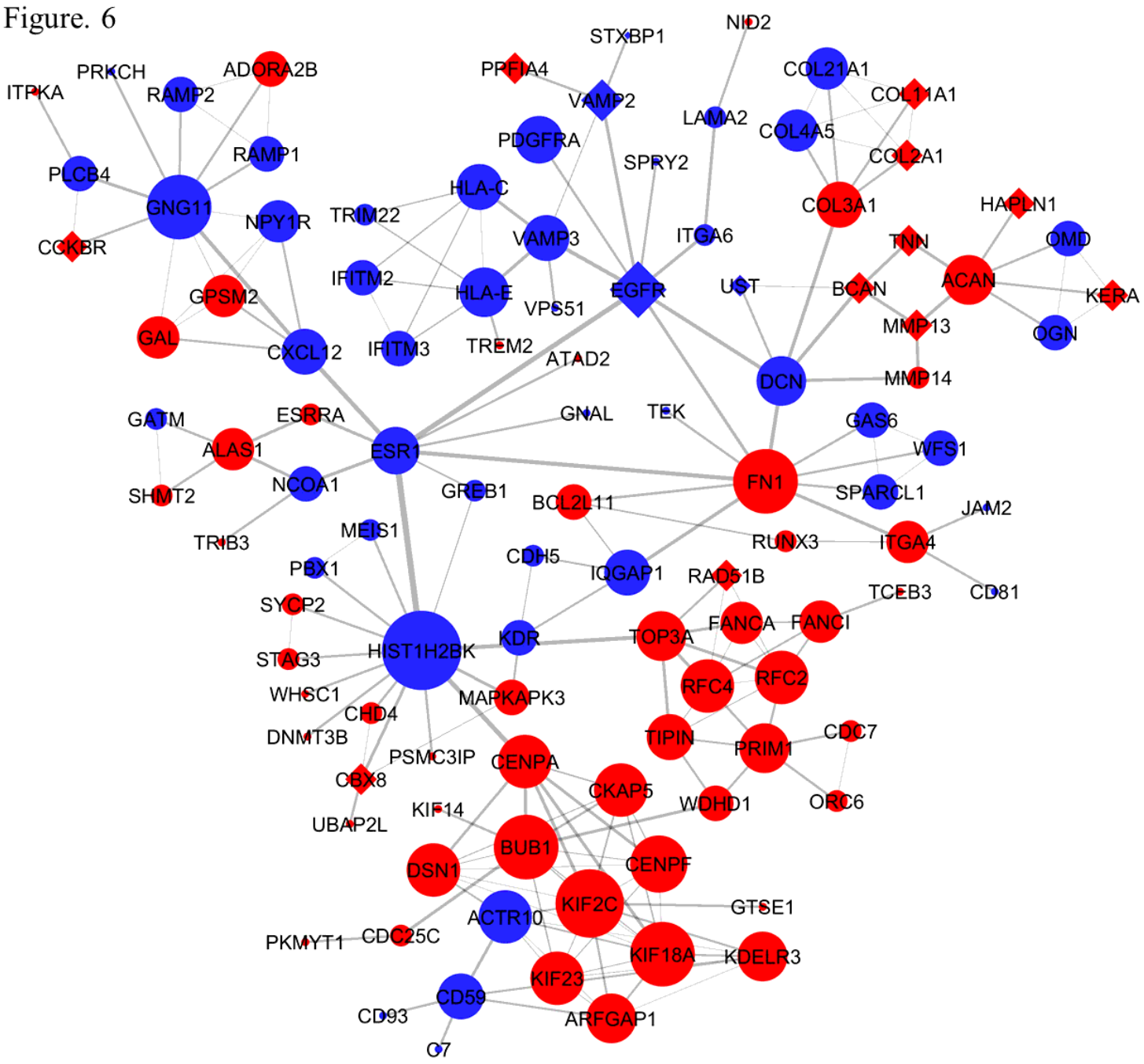
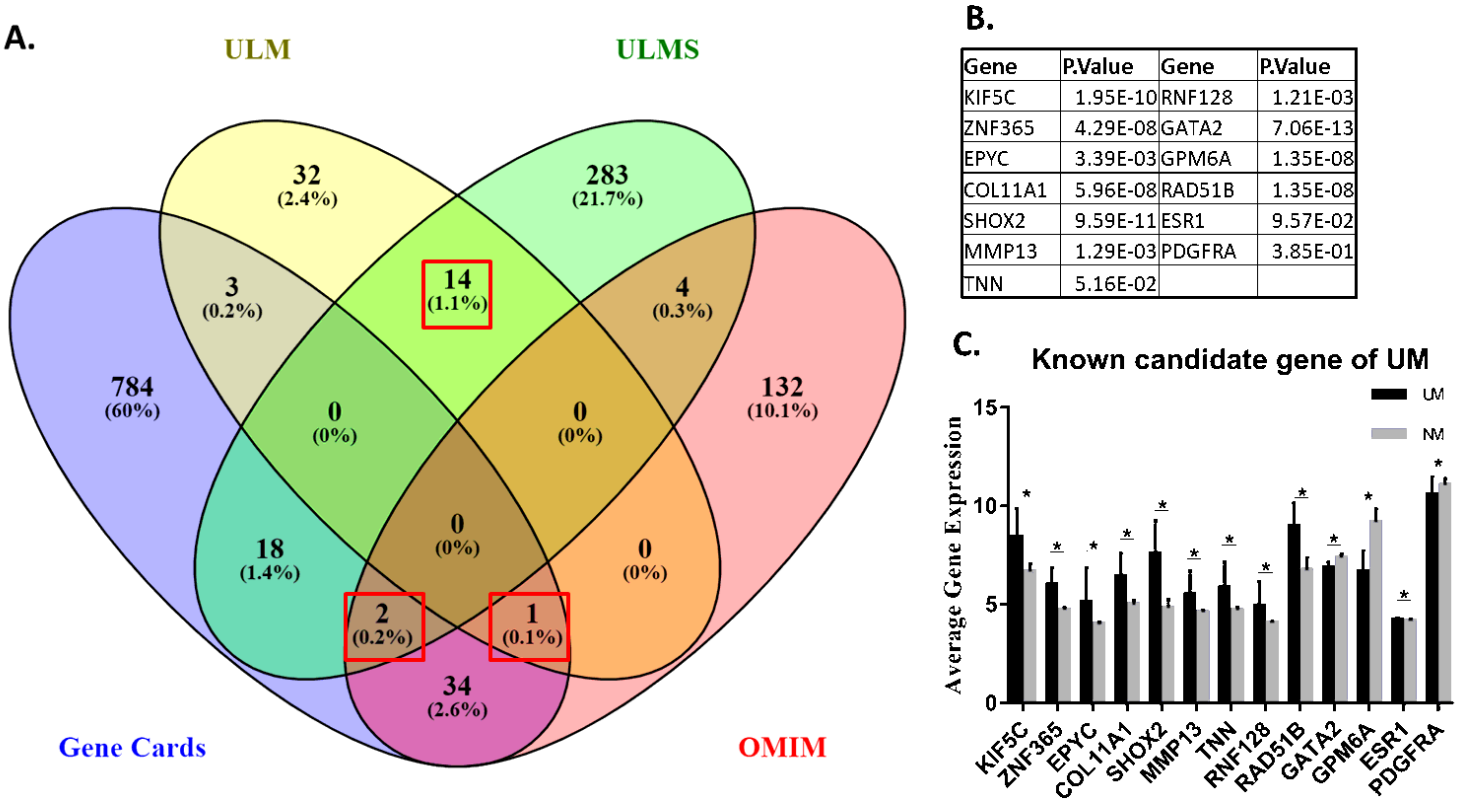


Figure 6

Protein-Protein interaction (PPI) of differentially expressed genes. Circle DEGs related to ULM, Diamond genes related to ULMS, Rectangle Common genes in ULM and ULMS. Red for up regulated genes Blue for down-regulated genes. Lines the correlation between genes Thickness of lines (edges) is proportional to the combined score. Cytoscape v 3.2.1 was used to construct the network.

Figure. 7



B.

Gene	P.Value	Gene	P.Value
KIF5C	1.95E-10	RNF128	1.21E-03
ZNF365	4.29E-08	GATA2	7.06E-13
EPYC	3.39E-03	GPM6A	1.35E-08
COL11A1	5.96E-08	RAD51B	1.35E-08
SHOX2	9.59E-11	ESR1	9.57E-02
MMP13	1.29E-03	PDGFRA	3.85E-01
TNN	5.16E-02		

Figure 7

Protein-Protein interaction (PPI) of differentially expressed genes. Red Circle and Red Diamond up-regulated genes, Blue Circle and Blue Diamond down-regulated genes. Lines the correlation between genes Thickness of lines (edges) is proportional to the combined score. Cytoscape v 3.2.1 was used to construct the network.

Figure. 8A

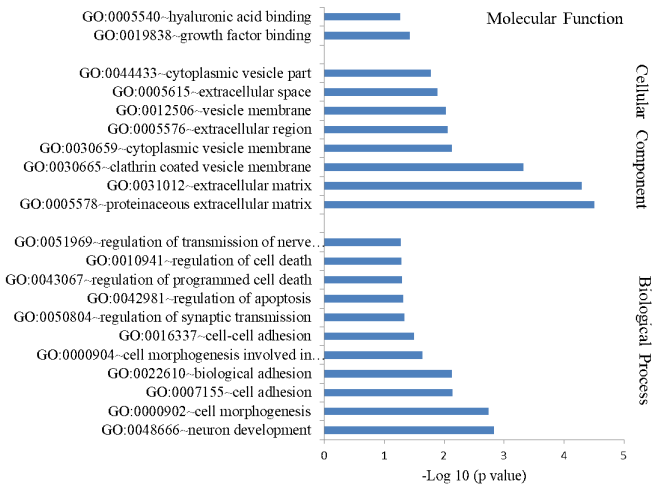


Figure. 8B

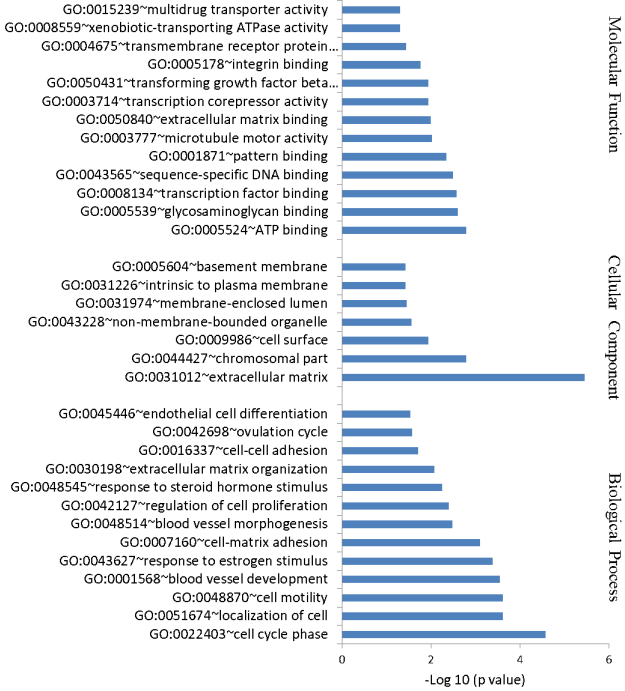


Figure 8

A: Gene Ontology analysis for ULM related DEGs in PPI network in uterine fibroids. Bar graph showing significant processes, function and cellular component enriched in diabetic mothers for up-regulated genes. DAVID v 6.7 was used for annotation. B: Gene Ontology analysis for ULMS related DEGs in PPI network in uterine fibroids. Bar graph showing significant processes, function and cellular component enriched in diabetic mothers for up-regulated genes. DAVID v 6.7 was used for annotation.

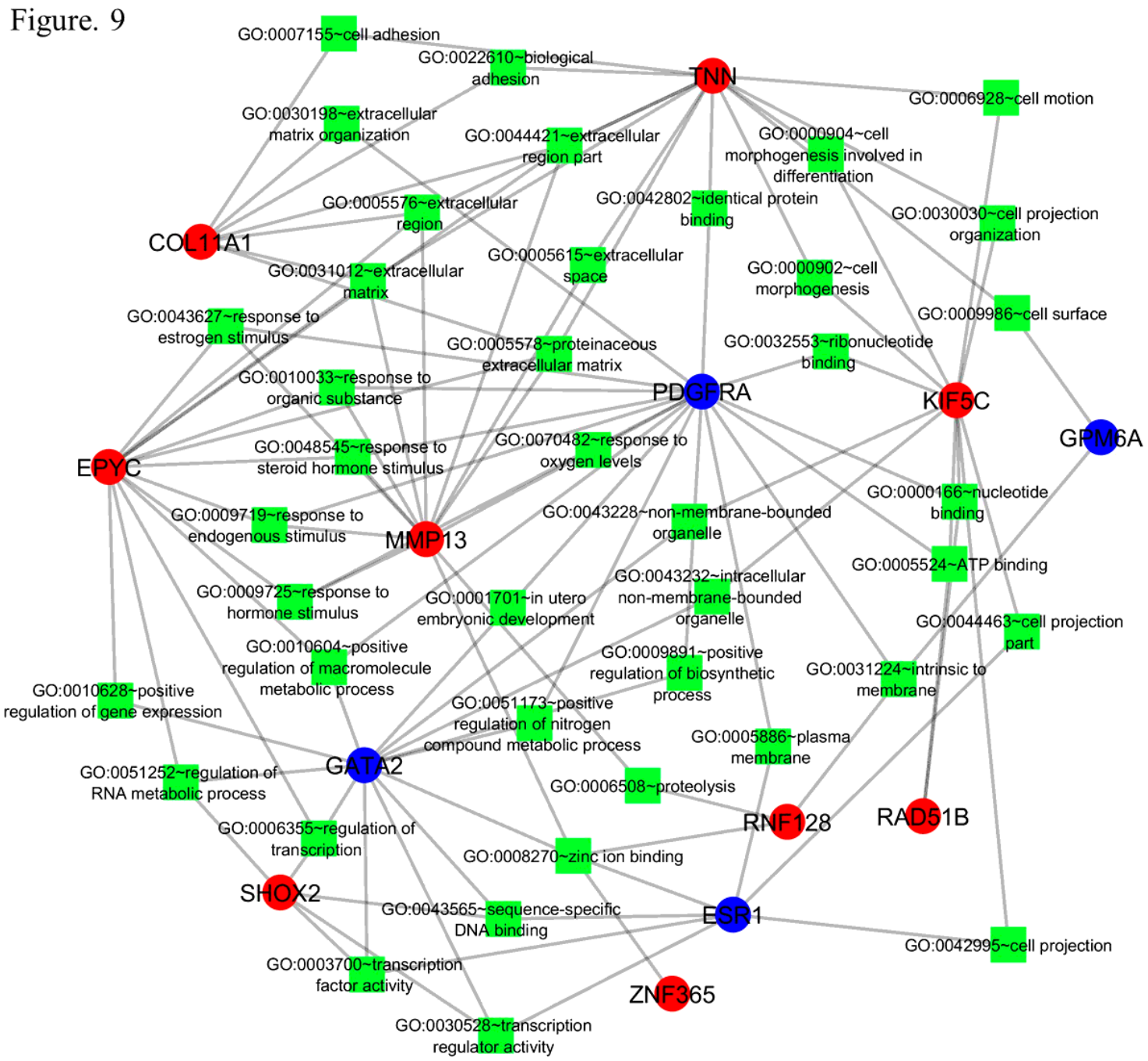


Figure 9

Functional analysis of common as well as known UF genes. Functional analysis uncover many significant processes being regulated by the candidate and known UF related genes. Red circle up-regulated genes, Blue circle up-regulated genes, Light green rectangle biological processes.

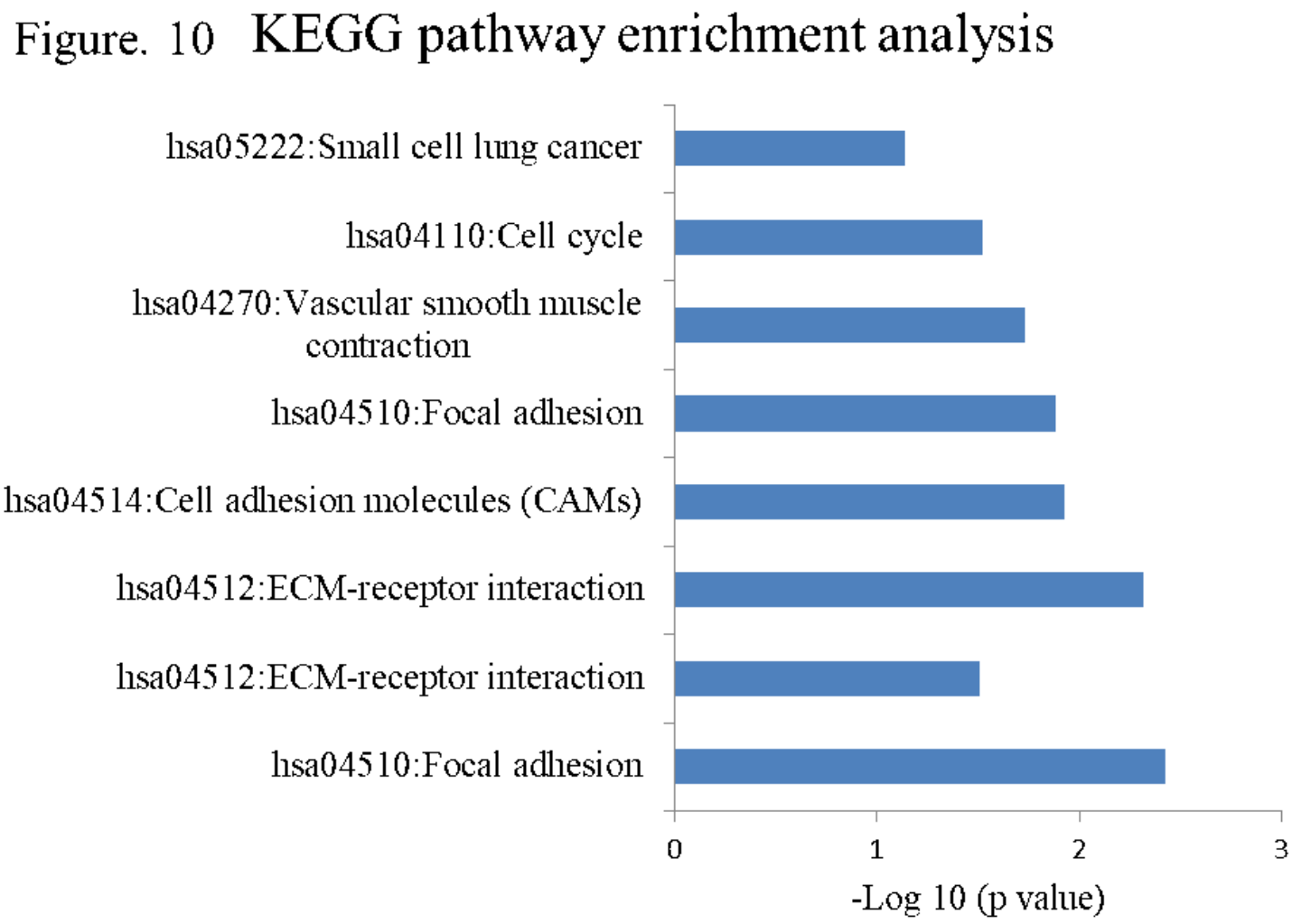


Figure 10

KEGG Pathway analysis for Differentially Expressed Genes in uterine fibroids. Pathway enrichment for DEGs lead to identification of 6 significant pathways for ULMS- while 2 significant pathways for ULM related DEGs. DAVID v 6.7 was used for annotation.