Brief Report

Molecular Biomarkers in Lymphangioleiomyomatosis (LAM)

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Article

Molecular Biomarkers in Lymphangioleiomyomatosis (LAM)

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Abstract: Lymphangioleiomyomatosis (LAM) is a cystic pulmonary disorder that has been known to primarily affect women of childbearing age. The disorder can develop with mutations in the tuberous sclerosis (TSC) genes TSC1 & TSC2 but may arise by other irregular cases in the lack of these mutations. LAM can manifest in a wide variety of ways and generally compounded in symptoms of other lung disorders such as bronchitis, emphysema, & asthma. However, due to lack of effective diagnostic methods, biomarkers of LAM leading to a treatment for the disorder are currently unmet. Using integrative dating analytics and bioinformatics approaches; this study used publicly available microarray data to identify biomarkers in LAM. The results showed important differential expressed genes (DEGs) including: CDH2, CXCL6, ANXA10, MFAP5, RPS4Y1, DCBLD2, PAPPA, TFPI2, SERPINE1, LOX, MYH11, RGS1, SEPP1, TMOD1, ID1, SFTPB, CDH1, HTR2B, OGN, and IGLC1. Furthermore, gene – network and gene ontology (GO) analysis were conducted to show the importance of these genes from a molecular understanding. Taken together, the results revealed could serve as diagnostic and prognostic biomarkers with LAM patients.

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Introduction

Lymphangioleiomyomatosis (LAM) is a rare disease that affects approximately 3.4 to 7.8 per million women worldwide. It is characterized by the intrusion and proliferation of abnormal smooth muscle – like LAM cells which leads to the destruction of lung parenchyma and formation of cysts on the lung [1,2]. LAM can be connected with the tuberous sclerosis complex (TSC-LAM) or sporadic (S-LAM) in which patients do not have TSC gene mutations or its clinical manifestations. Estrogen may play an important role in LAM cell survival, destructive, and proliferation potential [1–3]. Estrogen regulated gene transcription may modulate signaling to activate mTOR. Various studies have shown that estrogen and estrogen disruptors may promote various transcriptional regulators such as ID proteins to be involved in various molecular activities such as vascular remodeling, stem cell differentiation, and pulmonary dysfunction [3–11]. While genes such as TSC1 and TSC2 are known to be involved with LAM development [1–5], very little is known about other genes that are associated with the disorder. Through this integrative study, we explore important genes and gene – networks that may open up opportunities to explore LAM development. Overall, this data can be used to focus on novel diagnostic and therapeutic areas.

Methods

Publicly accessible data was used from NCBI Gene Expression Omnibus (GEO) for this study [12]. Dataset GSE12027 [13] consists of 14 LAM and 11 controls samples (25 total samples). The Limma-Voom R package was used to identify differentially expressed mRNAs from the featureCounts output [14]. The level for statistical significance was set at $p < 0.05$ and Log2 fold change values were calculated by comparing expression levels in patient samples to those in control

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samples for each mRNA [15,16]. String, a database used for predicted gene – gene/protein - protein interactions was used to show the gene-network for the top 20 DEGs. The gene interactions originate from data collected from supplementary databases, computational predictions, and data transfer between organisms [17]. Furthermore, gene ontology was used to show important molecular processes connected to the genes. ChEA3 is a web – based TF enrichment analysis (TFEA) tool that outputs enrichment results and helped show important molecular functions within the 20 DEGs and associated genes [18].

Results

We acquired NCBI GEO dataset GSE12027 [13] and used the Limma-Voom R package [14] to examine key DEGs and their roles in LAM. The top up – and down – regulated genes in LAM are shown in Table 1 and include: CDH2, CXCL6, ANXA10, MFAP5, RPS4Y1, DCBLD2, PAPPA, TFPI2, SERPINE1, LOX, MYH11, RGS1, SEPP1, TMOD1, ID1, SFTPB, CDH1, HTR2B, OGN, and IGLC1. We also showed the expression fold changes and p - values for each gene. Figures 1 and 2 show the important gene networks and gene ontology with connected molecular processes.

Table 1. Top 20 up – and down – regulated genes in LAM.

Gene Symbol	Gene Title	Log2 (Fold Change)	p – value
CDH2	cadherin 2	7.6295995	3.73E-12
CXCL6	C-X-C motif chemokine ligand 6	7.2061993	3.26E-14
ANXA10	annexin A10	6.7952473	5.57E-13
MFAP5	microfibrillar associated protein 5	5.7810303	5.57E-13
RPS4Y1	ribosomal protein S4, Y-linked 1	5.6284047	1.57E-15
DCBLD2	discoidin, CUB and LCCL domain containing 2	5.5517299	9.99E-13
PAPPA	pappalysin 1	5.5260289	1.43E-11
TFPI2	tissue factor pathway inhibitor 2	5.4304952	2.63E-13
SERPINE1	serpin family E member 1	5.3529649	3.70E-11
LOX	lysyl oxidase	5.0978044	2.85E-12
MYH11	myosin heavy chain 11	-12.0887684	1.11E-17
RGS1	regulator of G-protein signaling 1	-9.9510615	4.09E-13
SEPP1	selenoprotein P, plasma, 1	-9.5071713	2.18E-14
TMOD1	tropomodulin 1	-9.2792733	1.57E-15
ID1	Inhibitor of differentiation - 1	-9.126583	1.32E-06
SFTPB	surfactant protein B	-8.471653	4.91E-08
CDH1	cadherin 1	-8.3548182	2.27E-13
HTR2B	5-hydroxytryptamine receptor 2B	-7.6577741	4.34E-13
OGN	osteoglycin	-7.6373228	1.53E-12
IGLC1	immunoglobulin lambda constant 1	-7.6012966	1.12E-07

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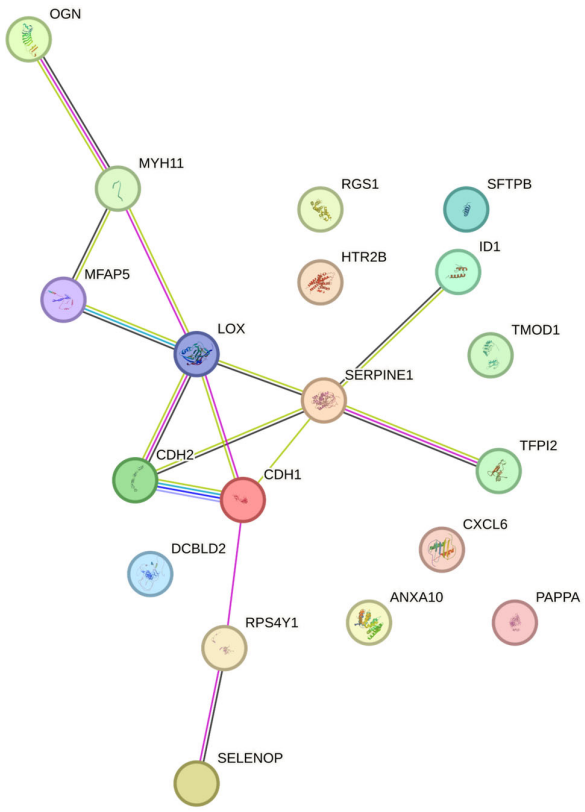

Figure 1. Gene-networks between the top 20 DEGs. The interactions include direct (physical) and indirect (functional) associations.

Figure 2. Gene enrichment (GO) of the top 20 up – and down – regulated genes. The dots represent the top 20 DEGs from Table 1 and important associated genes. Molecular processes are listed corresponding to each of the different color-coded dots (genes).

Discussion

The integrative analysis allowed us to map out key gene – networks, DEGs, and gene ontology of LAM. By using NCBI GEO, the R – package, String, and ChEA3; we discovered valuable

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information in the gene and molecular processes involved in LAM. It has been previously determined that genes such as TSC1 and TSC2 have been involved in LAM [1–3], but these new results can help add to the growing amounts of information. Limitations in our study includes a small sample size, which can be addressed by conducting studies with much larger sample sizes.

Conclusions

Through the combination of bioinformatics and data analytics, we have shown significant DEGs, gene networks, and gene ontology components within LAM. Key genes highlighted in the study include: CDH2, CXCL6, ANXA10, MFAP5, RPS4Y1, DCBLD2, PAPPA, TFPI2, SERPINE1, LOX, MYH11, RGS1, SEPP1, TMOD1, ID1, SFTPB, CDH1, HTR2B, OGN, and IGLC1. Overall, further gene expression and gene network analysis on LAM should be conducted to open up more opportunities for improved diagnostic and preventative tools.

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Data Availability Statement: The data used for the analysis is deposited at NCBI GEO (GSE12027). Further inquiries can be directed to the corresponding author.

Conflict of Interest: The author has no conflict of interest to declare.

Statement of Ethics: An ethics statement was not required because this study is based on publicly deposited and accessible data.

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