Hernia (2020) 24:1211–1219
https://doi.org/10.1007/s10029-020-02213-7

ORIGINAL ARTICLE

Comprehensive analysis reveals a six-gene signature and associated drugs in mimic inguinal hernia model

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Received: 13 April 2020 / Accepted: 4 May 2020 / Published online: 18 May 2020

Abstract

Purpose Inguinal hernia often occurs in elderly men, and more than one in five men will undergo inguinal hernia repair during their lifetime. Nevertheless, the underlying molecular mechanisms of the pathogenesis behind hernia formation is still unclear. The aims in this study are finding out the potential gene markers and available drugs.

Methods Firstly, we re-analyzed the GSE92748 datasets, including four high and four low expressions of humanized aromatase transgenic mice, which refers to mimic humanized hernia, to identify differentially expressed genes (DEGs) in Arom^{hum}H group compared with Arom^{hum}L group by the criteria: fold change ≥ 1.4 and adjust P value < 0.05. Secondly, the gene ontology and signaling pathway enrichment analyses of these DEGs were performed through online databases. In addition to the protein and protein interaction networks among these DEGs were constructed and the significant gene modules were chosen for further gene-drug interaction analysis. Lastly, the existing drugs target to these module genes were screen to explore the therapeutic effect for treatment of hernia.

Results We have identified 64 DEGs, which were associated with muscle system process, actomyosin structure organization etc. Moreover, the significant module genes in PPI networks were *Cmya1, Casq2, Cmya5, Ttn, Csrp3* and *Actc1*, and one existing drug, DEXAMETHASONE, have targeted to *Actc1* gene.

Conclusions In the paper, we identified 6 potential genes and one existing drug for inguinal hernia, which might be used as targets and drugs for the study of inguinal hernia.

Keywords Inguinal hernia · Mimic hernia model · Bioinformatics · Genetic biomarkers

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Introduction

Inguinal hernia is a prevalent malady in elderly man, and hernia repair is the most commonly performed general surgical procedure around the world. An inguinal hernia occurs in the abdomen near the groin area. It is a protrusion of contents that originated from the abdominal-cavity through the inguinal canal [1]. Though the pathogenesis is weakly understood, the probabilities of the lifetime risk of inguinal hernia are very higher in men than that in women [2]. What's more, the symptoms of inguinal hernia included that the bulges can appear to increase in size when standing up or cough, in addition, it also comprised pain, burning sensations, swelling of the scrotum in men. There are many risks for inguinal hernia, which include: heredity, having a prior inguinal hernia, being male, premature birth, being overweight or obese, pregnancy, cystic fibrosis, chronic cough, and chronic constipation [3, 4].

Currently, an inguinal hernia has classified into indirect or direct, incarcerated, or strangulated categories [5, 6]. The former type of hernia can occur at any of time during your lifetime. However, a direct inguinal hernia more likely occurs in adults owing to their ages. Moreover, when tissue in the groin becomes stuck and can't be reducible, an incarcerated inguinal hernia could be happened. Besides, a strangulated inguinal hernia is the most severe type of inguinal hernia, usually could be life-threatening and require emergency medical care [7].

To date, the diagnosing an inguinal hernia usually depends on a physical exam, such as asking you to cough while standing so that the doctor could check the hernia. However, there is no much researches about genetics of hernia that has been focused on the molecular mechanisms of pathogenesis, and the specific molecular mechanisms have not been disclosed.

In this study, using comprehensive bioinformatics analyses is a proper strategy to uncover the potential genes and signaling pathways in the mimic human inguinal hernia model. Firstly, we downloaded the GSE92748 gene expression datasets [8], which contained humanized aromatase transgenic mice to mimic human inguinal model, from the National Center for Biotechnology Information (NCBI) [9]. Then dysregulated genes were identified between different groups. Furthermore, gene ontology, signaling pathway enrichment annotation and protein–protein interaction were performed among these dysregulated genes through different bioinformatics methods [10, 11]. Finally, we could find the potential gene biomarkers and correlated pathways, which might be associated with inguinal hernia and could be give us a new sight to explore the molecular mechanism of inguinal hernia hidden.

Methods and materials

Microarray data analysis

GSE92748 expression profile (*.txt* format files) and correlated clinical information data (*.soft* format file) have been acquired from NCBI-GEO website [9, 12], which was done on the GPL6887 platform. GSE92748 datasets contain 4 high expression of humanized aromatase transgenic mice (marked Arom^{hum}H), 4 low expression of humanized aromatase transgenic mice (marked Arom^{hum}L), and 4 wild type mice (marked WT). We filter out 8 samples from the GSE92748 to identify different expression genes (DEGs) between Arom^{hum}H and Arom^{hum}L groups.

Data preprocessing

Probe identification numbers were transformed into official gene symbols based on the information built in GPL6887 platform, the mRNA probes were retained and the other non-mRNA probes were abandoned, and the multiple probes to the same gene were assigned the significant value as the gene expression level. Then, using limma package to detect gene expression matrix, processed by affy, affyPLM packages, and obtain differentially expressed genes (DEGs) in Arom^{hum}H group and Arom^{hum}L group [13–16]. DEGs with the fold change (FC) ≥ 1.4 & adjust P value < 0.05, corrected by the Benjamini–Hochberg method [17], as the cut-off criteria were selected for the follow-up analyses.

Gene ontology and pathway enrichment analysis

The gene ontology (GO) analysis is a general and useful method for annotating gene products and their characteristics of functional features [10]. Gene Ontology annotation is defined into three classes (biological process, cellular component, molecular function). The Kyoto Encyclopedia of Genes and Genomes (KEGG) database is an open access informatic source from Japan for interpreting biological function and characteristics of the organic system, produced by the microarray and RNA-seq experiments [11]. The GO and KEGG enrichment of DEGs were analyzed using an online tool DAVID, a functional annotation bioinformatics microarray analysis website, used to gene annotation, visualization. FDR (false discovery rate) < 0.05 was considered as statistically significance [18, 19].

Protein interaction and module analysis

The online database STRING (version 11.0), covering about 24.6 M proteins and more than 3.1 billion interactions originated from 5.09 K organisms, was known as the primary

source to describe and display the interaction among various proteins, encoded by corresponding genes [20]. Firstly, we uploaded DEGs into the STRING website, and the minimum interaction score > 0.4 (low confidence) was recognized as significant. Then the *TSV* format file of protein–protein interaction (PPI) information was downloaded, and PPI networks were constructed through Cytoscape software [21]. Subsequently, the Molecular Complex Detection (MCODE) and STRING app built in Cytoscape was used to classify the significant gene modules (clusters), which have highly interconnected clusters in the PPI network [22, 23]. All parameters in MCODE were executed by default. The genes/nodes in gene modules were performed drug-gene interaction analysis.

Drug-gene interaction and functional analysis of potential genes

To get interaction between genes and the existing drugs and explore the potential application of the new drug indications for human hernia. The drug-gene interaction database (DGIdb: https://www.dgidb.org) is an open-source and supports searching, browsing and filtering of information on drug-gene interactions based on over thirty trusted sources [24]. The module genes, as the potential targets, were pasted into the drug-gene database to search for existing drugs or compounds. These potential genes which have matched drugs were obtained and also performed functional enrichment analysis.

Statistics analysis

The moderate t-test was applied to identify DEGs; Fisher's Exact test was used to analyzed GO and KEGG annotation enrichments [25]. All statistical analyses were executed in R version 3.6.1 software Fig. 1.

Results

Identification of DEGs

There are 64 DEGs identified in Arom^{hum}H compared with Arom^{hum}L group, according to the criteria: fold change (FC) ≥ 1.4 & adjust *P* value < 0.05. Among them, 43 up-regulated genes and 21 down-regulated genes (Table 1).

Gene ontology and pathway enrichment analysis

To outline gene ontology and signal pathway enrichments of DEGs, we used DAVID website to visualize functional annotations. As shown in Fig. 2 and Table 2, it showed that the significant enrichment terms for BP, CC of DEGs. In BP annotation, it was mainly involved in the muscle system process, actomyosin structure organization, and muscle structure development. In CC annotation, it was significantly involved in the extracellular exosome, extracellular vesicle, and extracellular organelle. There no signal pathway enrichment terms can be available with *FDR* < 0.05.

As for up-regulated DEGs, the GO annotation was significantly involved in the muscle system process, extracellular exosome etc. (Table 3), while the down-regulated DEGs haven't annotated any GO and signaling pathway terms.

Protein interaction and module analysis

The 64 DEGs were input into the STRING database and then analyzed with STRING APP built in Cytoscape software. A total of 29 genes/nodes with 38 edges were participated in the construction of the PPI networks, and 35 genes haven't fallen into the PPI networks (Fig. 3a). Furthermore, a significant gene module was selected to cluster all genes using the MCODE APP built in Cytoscape. Module 1 consists 6 genes/nodes with 12 edges/interactions, which 4 up-regulated genes (*Cmya1, Casq2, Csrp3* and *Actc1*) and 2 down-regulated genes (*Cmya5, Ttn*) (Fig. 3b).

Drug-gene interaction and functional analysis of potential genes

The 6 potential genes clustered in the significant gene module 1 were selected for drug-gene interaction analysis. In human species, we found that there was just *ACTC1* target to one potential existing drug, namely DEXAMETHASONE.

Discussion

Inguinal hernia as one of the common symptoms often occurs in the groin in elderly men. Some symptoms can affect the quality of people's life. As you found out, you can't prevent the birth defect that makes you vulnerable to an inguinal hernia. We can, however, reduce strain on our abdominal muscles and tissues. Such as keep moving and sustain a healthy weight.

Currently, a large number of researches have revealed that inguinal hernial have associated with the status of muscle tissues. In this paper, we used Arom^{hum} mice to mimic the human inguinal model and expected to discover potential gene markers and some existing drugs. Arom^{hum} mice represent a pathologically and unique relevant experimental model to study the molecular mechanism behind inguinal hernia. Comprehensive analyses of gene expression profiling allowed us to identify a number of potential molecular biomarkers (*Cmya1, Cmya5, Casq2, Csrp3*, *Ttn*

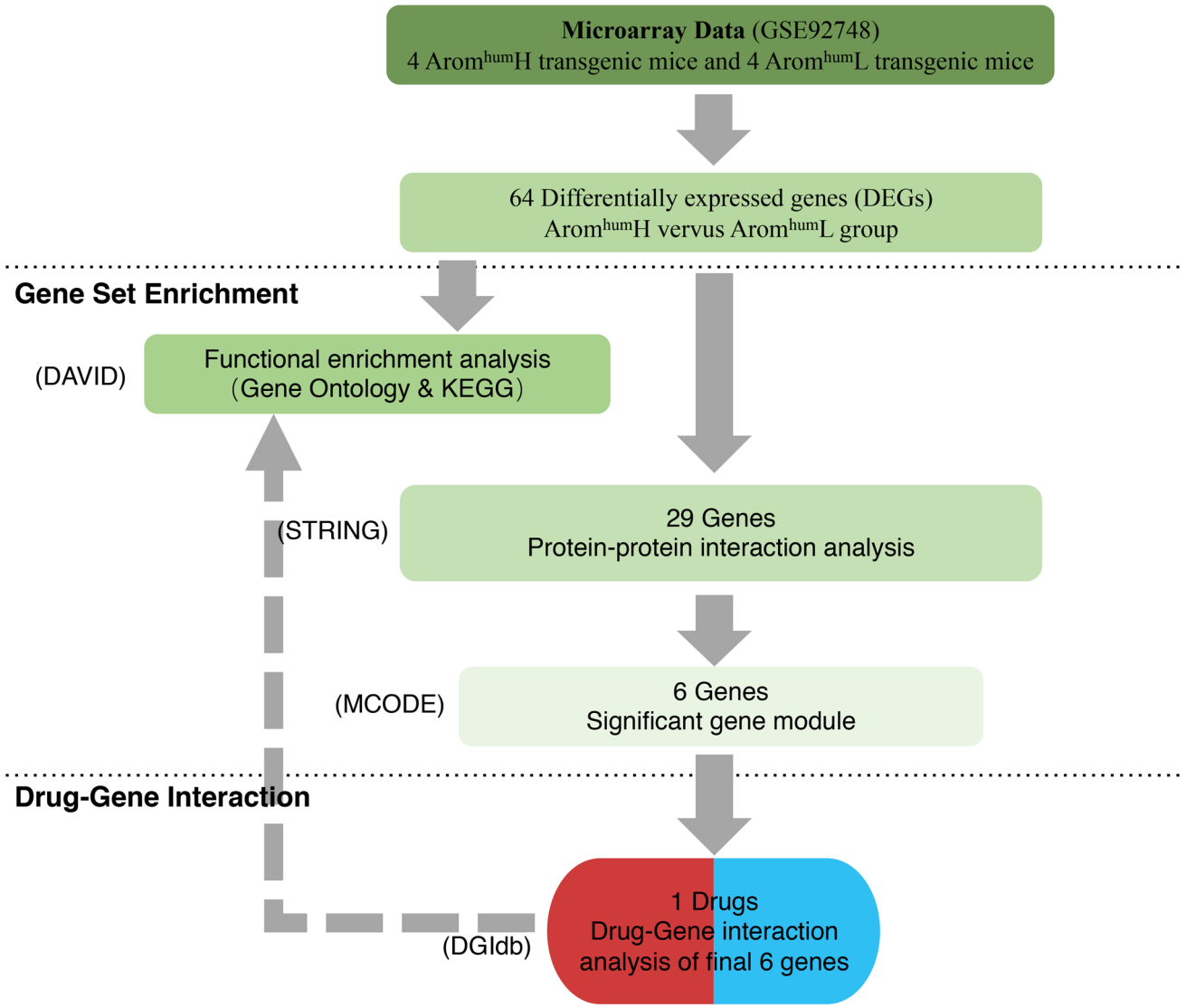

Fig. 1 The framework of data analyses

and Actc1) and new drug indications of the existing drug (DEXAMETHASONE).

Cmya1 (Cardiomypathy-associated gene 1) have involved in embryonic cardiac development, postnatal cardiac remodeling and myocardial injury repair, and its abnormal gene expression have correlated with cardiac hyperplasia and primary myocardiopathy [26]. *Cmya5* (Cardiomyopathy-associated gene 5) encodes myospryn, could be recognized as a biomarker for some diseases affecting striated muscle and associated with aschizophrenia [27, 28]. *Casq2* (Calsequestrin 2), generally referring to its mutation, is considered to be the crucial sarcoplasmic reticulum (SR) Ca2 + storage protein needed for SR Ca2 + release in the mammalian heart [29, 30]. *Crsp3* (cardiac LIM protein cysteine and glycine-rich protein 3) is thought to crucial component mediating cardiac mechanotransduction and stress responses within cells [31]. *Ttn* (titin) is familiar with its high expression in human cardiomyocytes and necessary for normal sarcomere function [32].

Based on the above illustration, we found that most of these genes are related to cardiac events. It is well known that muscle tissues include myocardial tissue and skeletal muscle tissue, and the components of the heart are myocardial tissue. Nevertheless, abdominal muscle is composed of skeletal muscle and the occurrence of hernia is closely associated with the status of muscle tissues. Studies have shown that *Cyma1* and *Cyma5* represent high expression levels in myocardial and skeletal muscle, as well as in injury muscle [27, 33]. *Casq2* has involved in cardiovascular physiology, skeletal muscle phenotypes, hematopoiesis and metabolism

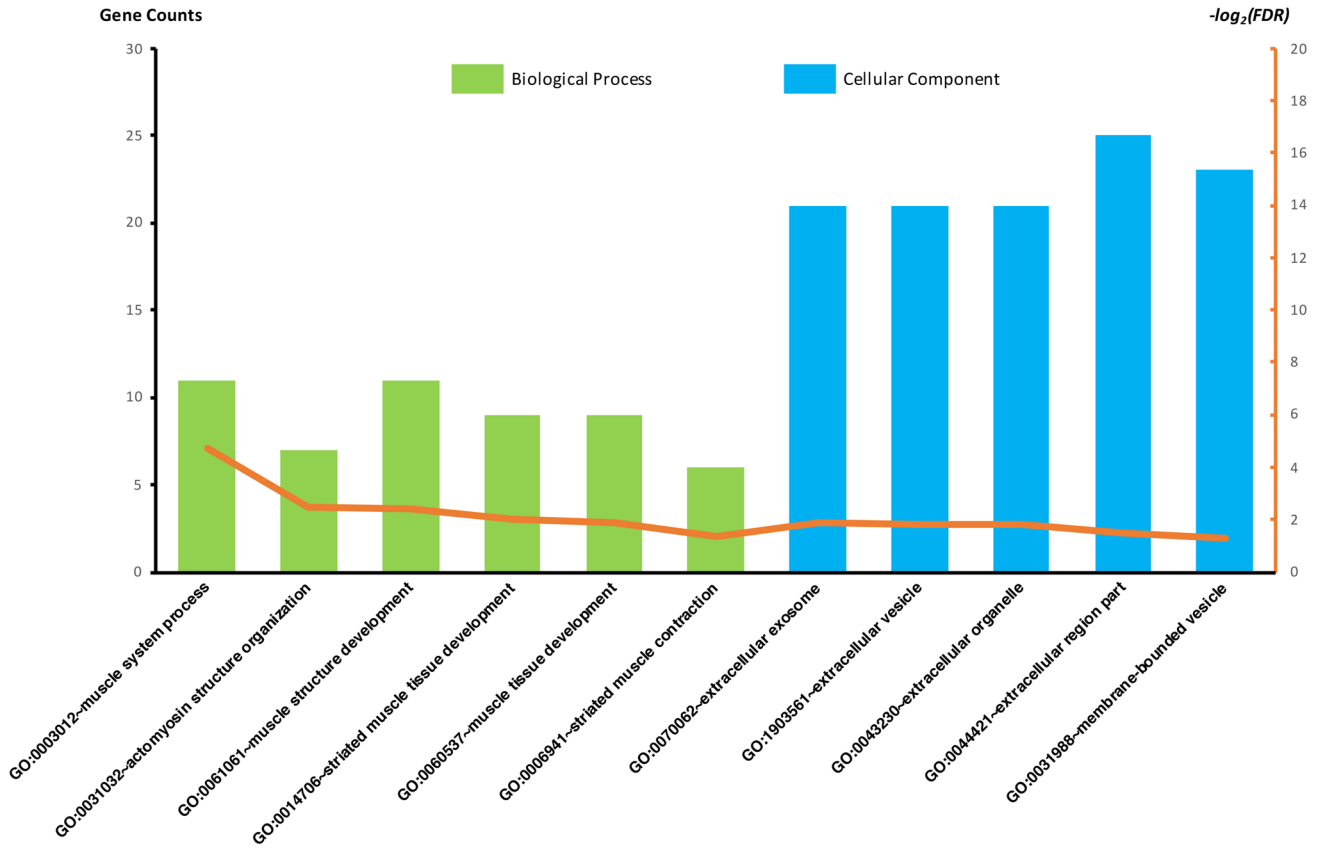

Fig. 2 All available significant gene ontology enrichment terms of the differentially expressed genes (DEGs)

[34]. *Csrp3* is also a skeletal muscle-specific LIM-only factor expressed in skeletal muscle, and crucial to maintain the structure and function of skeletal normal muscle [35]. The variants of *Ttn* could be recognized as the current classification criteria to *Ttn*-associated skeletal muscle disorders [36]. As a result, we come to the conclusion that these five genes are linked to skeletal muscle. On the one hand, these facts gave us reason to believe that they could be treated as potential targets of inguinal hernia for further research. On the other hand, we also proposed another hypothesis, whether the inguinal hernia is a systemic disease, which is a supplement to the previous point: a locally occurring disease.

As to Actc1 (actin alpha cardiac muscle), Mazzarotto et al. have suggested that Actc1 has associated with Dilated cardiomyopathy (DCM) and recognized as a diagnostic testing biomarker [37]. Meanwhile, Alliot-Licht et al. have shown that dexamethasone, target to Actc1, increased the proportion of multipotential mesenchymal progenitor cells [38]. Inder et al. have described that dexamethasone drug administration inhibits skeletal muscle expression of androgen receptor (AR), which have associated with fibrosis, skeletal muscle atrophy, and the development of hernias [8, 39]. However, the expression levels of androgen receptor target genes in the lower abdominal muscle of the inguinal hernia were decreased [8]. These studies seem to remind us that dexamethasone might play some unknown role in the occurrence of the inguinal hernia.

Up to date, all these genes and drugs haven't been found in the research and application of inguinal hernia. Though we used comprehensive analyses to identify 6 genes and one existing drug through the mimic mice model of humanized inguinal hernia, it is needed some molecular experiments to support our results. What's more, the humanized inguinal hernia datasets were not as easily acquired and collected as cancer datasets from the online database and reality, so this paper gives us a clue to explore the molecular mechanism of the inguinal hernia.

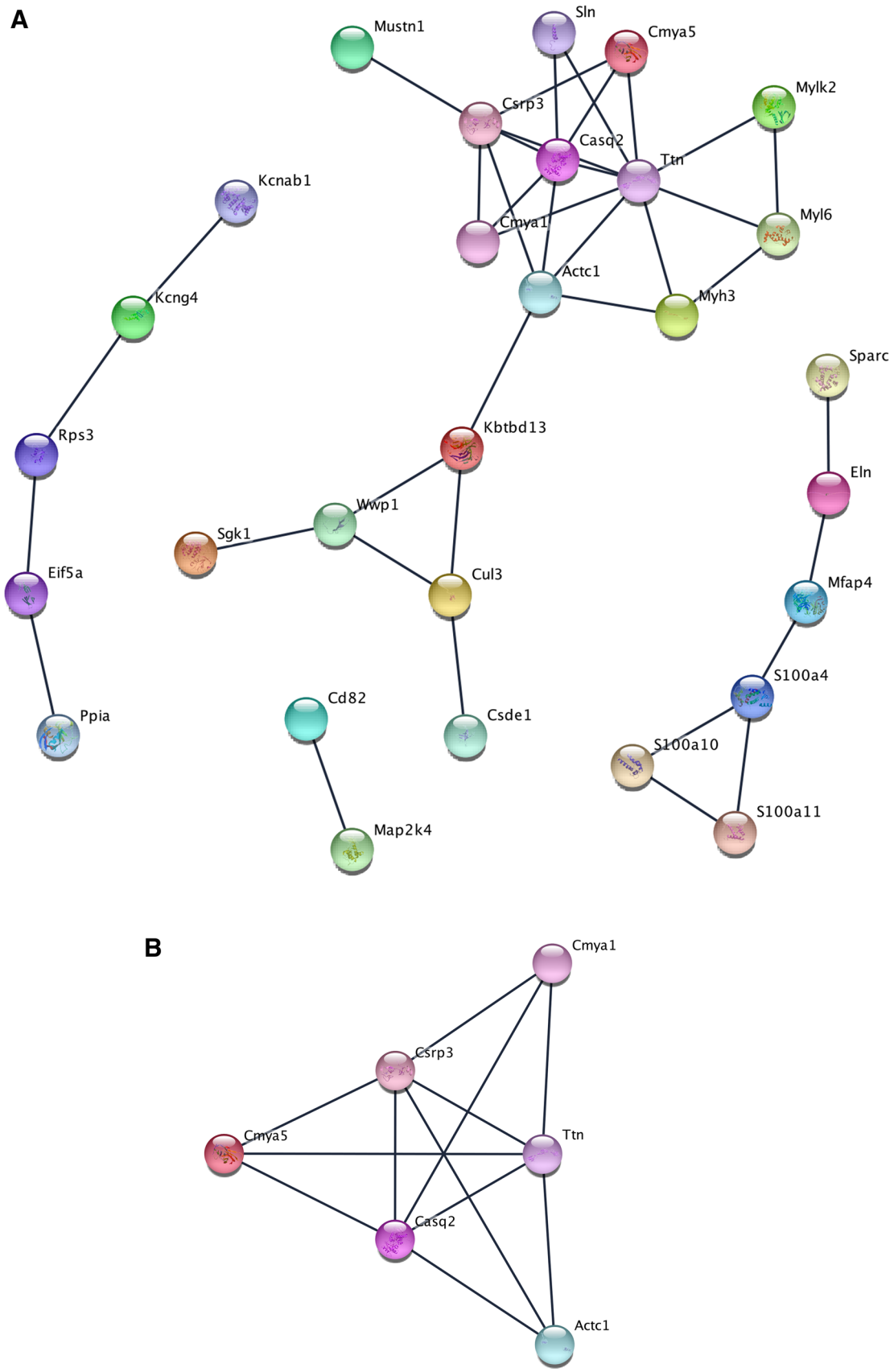

Fig. 3 **a** The protein–protein interaction (PPI) networks of differentially expressed genes (DEGs); **b** the significant gene module in the PPI networks

Table 1 The DEGs in Arom^{hum}H versus Arom^{hum}L transgenic mice group

DEGs	Gene Name
Up-regulated	*Csrp3, LOC381365, Dnclc1, S100a4, Casq2, Fez1, Dynll1, Actc1, Cd82, Mfap4, Ihpk3, C920004C08Rik, Mustn1, A930003A15Rik, LOC277856, S100a11, Lmcd1, Ankrd46, Myl6, Myh3, Arpp21, LOC100048037, Cmya1, Ncam1, Sln, S100a10, Sparc, Eln, Rarres2, LOC381649, Rps3, Clic1, Eif5a, Ppia, Ywhah, Prickle3, Lyve1, LOC269251, Cldn15, Mela, Carhsp1, Abcd3, LOC433546*
Down-regulated	*Kcnab1, Kcng4, Tigd2, Gdf1, Sgk1, AI595366, Mylk2, Cmya5, Zfp180, Rorc, Csde1, Map2k4, Cul3, A130092J06Rik, Ttn, 9630025H16Rik, Adi1, 5430433E21Rik, Wwp1, Cyp27a1, LOC240131*

Conclusions

Through applying a series of bioinformatics methods to gene expression profiling, we acquired 6 potential biomarkers (*Cmya1, Cmya5, Casq2, Csrp3*, *Ttn and Actc1*) and one existing drug (DEXAMETHASONE), which will provide insight for new study targets and new drug indications.

Table 2 The significant gene ontology and signal enrichment terms of DEGs. (* Terms that do not meet the FDR < 0.05 conditions are not shown)

Term	Category*	Description	FDR	Count
GO:0003012	BP	muscle system process	1.92E-05	11
GO:0031032	BP	actomyosin structure organization	0.00319923	7
GO:0061061	BP	muscle structure development	0.00372445	11
GO:0014706	BP	striated muscle tissue development	0.00888642	9
GO:0060537	BP	muscle tissue development	0.013662	9
GO:0006941	BP	striated muscle contraction	0.04333932	6
GO:0070062	CC	extracellular exosome	0.01329793	21
GO:1903561	CC	extracellular vesicle	0.01451113	21
GO:0043230	CC	extracellular organelle	0.01511119	21
GO:0044421	CC	extracellular region part	0.03016274	25
GO:0031988	CC	membrane-bounded vesicle	0.04987683	23

Table 3 The significant gene ontology and KEGG enrichment terms of Up-regulated and down-regulated DEGs, respectively.(*Terms that do not meet the FDR < 0.05 conditions are not shown)

Term	Category*	Description	FDR	Count
Up-regulated[1]				
GO:0003012	BP	muscle system process	0.03539252	7
GO:0070062	CC	extracellular exosome	0.00112087	18
GO:1903561	CC	extracellular vesicle	0.00121711	18
GO:0043230	CC	extracellular organelle	0.00126457	18
GO:004421	CC	extracellular region part	0.00165417	21
GO:0031988	CC	membrane-bounded vesicle	0.00819165	19
GO:0005576	CC	extracellular region	0.01681277	21
Down-regulated				
GO and signaling pathway enrichment terms can't available with FDR < 0.05				

Author contributions BZ do data analysis using bioinformatics tools. ZW, JLW, HYL, YYZ, WBC, XHZ, YW, LMX and YHZ participated in data analysis and discussion, BZ interpreted data and wrote the manuscript. YLZ organized and offered funding supports for the project. All authors read and approved the final manuscript.

Funding This study was supported by the National Natural Science Foundation of China (No. 81770294 & No. 81371698).

Compliance with ethical standards

Conflict of interests The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical statement We only re-analyzed the open source datasets, and no ethical approval needed.

Human and animal rights The human study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanjing Medical University and complied strictly with the national ethical guidelines of China.

Informed consent Written informed consent was obtained from all participants before inclusion in the study. All participants were identified by assigned numbers.

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