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RESEARCH ARTİCLE

Cisplatin treatment in pulmonary sarcoidosis: An *In silico* approach

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ABSTRACT

Background: Sarcoidosis is a complex disorder characterized by parenchymal lung involvement, intrathoracic lymphadenopathy, and extrapulmonary manifestations. Sarcoidosis can cause high mortality and morbidity. **Aims and Objectives:** It is needed computational methods to estimate potential miRNA-disease-drugrelationships in pulmonary sarcoidosis since experimental approaches are expensive and time consuming. **Materials and Methods:** In this study, we performed principal component analysis -based unsupervised feature extraction method to GSE34608 microRNA expression profile, which was downloaded from the Gene Expression Omnibus database that consists of peripheral blood samples of eight pulmonary sarcoidosis patients and eight healthy controls. **Results:** We detected a set of 100 microRNAs that could successfully discriminate pulmonary sarcoidosis patients from healthy controls with 81.2% accuracy. Among these miRNAs, we validated miR-15b by Human MicroRNA Disease Database and identified 22 pathways by miRpath and 20 approved drugs by PharmacomiR. When these pathways were integrated with drug-affected pathways detected by the database for annotation, visualization, and integrated discovery database, four overlapping pathways were determined, which were closely associated with cisplatin. **Conclusion:** This study provides novel insight into pulmonary sarcoidosis pathogenesis, and we identified potential drug candidate for this disease.

KEY WORDS: Pulmonary Sarcoidosis; microRNA; Principal Component Analysis -based Unsupervised Feature Extraction; Cisplatin

INTRODUCTION

Sarcoidosis is a multisystem granulomatous illness with an unknown etiology that can affect almost any organ.[1] The pulmonary involvements account for 90–95% of all cases.[2] Most deaths are caused by pulmonary sarcoidosis, in which pulmonary hypertension and/or pulmonary fibrosis develop,[3,4] but it usually progresses slowly across decades.[5]

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Despite increasing advances in studies, the diagnosis of sarcoidosis remains difficult with limited treatment options. Because of the lack of adequate experimental approaches, which are time consuming and expensive, improving bioinformatic techniques for defining potential miRNA-disease associations become essential. Various miRNA-associated datasets facilitate the improvement of database-based computational approaches to estimate the most likely miRNA-drug-disease associations.[6-9]

More innovative methods are required to overcome the diagnostic and therapeutic difficulties in sarcoidosis. We use the *in silico* methods to explore novel potential therapeutic drugs for pulmonary sarcoidosis. We analyzed the blood-based microRNA expression profiles of sarcoidosis patients with the principal component analysis (PCA)-based

unsupervised feature extraction (FE) technique; then, miRNA in pulmonary sarcoidosis was validated. We obtained drugs affected by this miRNA using the PharmacomiR database. Furthermore, miR-related pathways and drug-affected pathways were defined. The overlapping pathways were achieved by integrating these pathways. Subsequently, a drug that targets overlapping pathways was supposed to be a potential novel drug for pulmonary sarcoidosis therapy.

MATERIALS AND METHODS

Identification of Differentially Expressed miRNAs

We searched the Gene Expression Omnibus (GEO) database using the following keywords: "Sarcoidosis," "Expression profiling by array," and "Homo sapiens." The inclusion criteria were as follows: (1) Peripheral blood samples of sarcoidosis patients and healthy controls were compared, (2) adequate information to perform the analyzes. The GSE34608-GPL7731_series_matrix.txt.gz microRNA expression profile was downloaded from the GEO database, consisting of eight pulmonary sarcoidosis patients (four females and four males, aged 42 ± 16.4 years) and eight healthy controls (five females and three males, aged 50 ± 12.6 years). Since our *in silico* study does not include neither animal nor human materials, no ethical committee approval was required.

PCA is a mathematical data reduction method and the process of extracting relevant information from a large dataset. We used the PCA-based unsupervised FE method to determine a microRNA set that could discriminate pulmonary sarcoidosis from control groups. Contrary to standard PCA that integrates the samples, PCA-based unsupervised FE integrates the microRNAs that PC scores and PC loadings were imputed to genes and samples, respectively.[10] This method has been applied to gene expression data in our previous studies.[11] All statistical analyzes were done using R Studio open source software program.[12]

Human MicroRNA Disease Database (HMDD) (http://cmbi.bjmu.edu.cn/hmdd) was used to acquire pulmonary sarcoidosis-related miRNAs, which consists a set of experimentally verified miRNA and disease associations. This database was used to validate the miRNAs acquired by the PCA-based unsupervised FE method.

Identification of miRNA-Drug-Disease Interactions

miRNAs can define the effectiveness of drugs that have led to the so-called "miRNA pharmacogenomics," and these miRNAs have been called "pharmacomiRs." The validated miRNA was integrated into PharmacomiR which is used to identify and characterize potential pharmacomiRs in the relevant drug context.[13] miRNA targets are received from VerSe, miRecords,[14] and miRTarBase,[15] and gene-drug interactions are from VerSe and "overlapping associations" were selected.

Drug-affected Pathways and miRNA Pathway Analysis

The molecular pathways that may be affected by the differential expression of the validated miRNA were defined by Diana-miRPath software.[16] This tool performs enrichment analysis of many miRNA target genes by correlating any set of miRNA targets to each noted Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. miRNA was analyzed with TargetScan predicted target genes that were enriched in KEGG pathways with $P < 0.05$.

The Database for Annotation, Visualization, and Integrated Discovery database (DAVID), a tool for inquiring biological information from many genes, was used to conduct KEGG pathway analysis, which was acquired from PharmacomiR; $P < 0.05$ was considered as statistically significant.[17] Then, these two pathway analyzes were overlapped, and the common ones were chosen and estimated to be the most related ones.

RESULTS

Detection and Validation of Differentially Expressed miRNAs

In our study, the GSE34608 microRNA expression profile was downloaded from the GEO database. First, we applied PCA to pull dawn the number of predictor variables, with minimal loss of information, which was attained by 1st finding the direction that has the largest variance (PC1: 96.8%) and finding the following directions. Then, by applying "lm" R code, PC3 was selected with an adjusted $P = 0.0017$. After that, based on these PC scores, we calculated the p-values of microRNAs using the "pchisq" R code, and then, we achieved 100 microRNAs with an adjusted $P < 0.0001$ by "p.adjust" R code. Second, we performed PCA again by applying the "prcomp" which was achieved by 1st finding the direction that has the largest variance (PC1: 96.3%) and finding the following directions. Then, by applying "lm" R code to 100 microRNAs expression profile matrix, only PC3 was found significant among pulmonary sarcoidosis patients and healthy controls with an adjusted $P = 0.003$.

Then, 16 samples (patients and healthy controls) were discriminated into two classes using the "lda" R code. Linear discriminant analysis can successfully distinguish healthy controls from patients with pulmonary sarcoidosis. In a group of eight healthy controls, one case was misclassified as the patient, and in a group of eight pulmonary sarcoidosis, two cases were misclassified as control.

Discriminant score was used to produce receiver operating curve (ROC) and area under the ROC curve was calculated as 0.836 for distinguishing pulmonary sarcoidosis from healthy control samples with sensitivity and specificity values 75% and 87.5%, respectively [Figure 1], and the overall accuracy

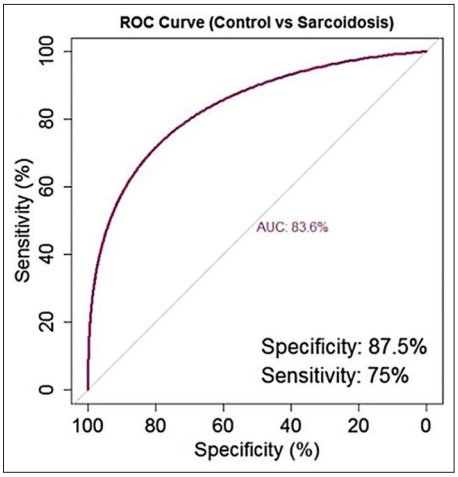

Figure 1: Receiver operating curve comparing patients with sarcoidosis and healthy controls

was found 0.812. In these 100 miRNAs, miR-15b was validated in pulmonary sarcoidosis using HMDD.

Pathway Enrichment and Drug Prediction of Validated miRNA

In this study, we submitted miR-15b to PharmacomiR to identify the targeted genes and targeted drugs. The majority of these drugs were vincristine, cisplatin, gemcitabine, doxorubicin, and docetaxel. The target genes and drugs are demonstrated in Table 1.

The target genes were put into DAVID to screen their biological functions in detail. The drug-related pathways were mostly enriched in PI3K-Akt signaling pathway, glioma, melanoma, small cell lung cancer, and prostate cancer.

Analysis of miR-15b through miRPath (v3.0) showed functional categories such as fatty acid biosynthesis, fatty acid metabolism, viral carcinogenesis, hippo signaling pathway, and adherens junction. These two analyzes were then integrated, and obtained overlapping pathways are shown in Table 2. These pathways were related to BCL2, CDK6, CDC25A, IGF1R, and CCND1 genes. Finally, it was identified that these genes were closely associated with cisplatin.

DISCUSSION

Sarcoidosis is a chronic granulomatous illness with an unknown etiology that occurs mostly in patients between the ages of 20 and 60.[18] Nearly 80% of patients require treatment, and therapy may still go on 5 years after diagnosis in almost half of the patients who need systemic treatment.[19,20] Corticosteroids have been the primary treatment for patients with progressive and symptomatic disease. There are some side effects in long-term corticosteroid therapy, which may stop the adrenal glands that produce the cortisol hormone.

Table 1: miRNA target gene-drug associations

miRNA	Gene	Drug	Gene-drug association scores
miR-15b	BDNF	Cocaine	1
miR-15b	CCND1	Genistein	1
miR-15b	CCND1	Nutlin	1
miR-15b	IGF1R	Sorafenib	1
miR-15b	IRS1	İnsulin	1
miR-15b	BCL2	Cisplatin	4
miR-15b	BCL2	Gemcitabine	2
miR-15b	BCL2	Daunorubicin	1
miR-15b	BCL2	Doxorubicin	2
miR-15b	BCL2	Paclitaxel	1
miR-15b	BCL2	Cytarabine	1
miR-15b	BCL2	Tamoxifen	1
miR-15b	BCL2	Docetaxel	2
miR-15b	BCL2	Etoposide	1
miR-15b	BCL2	Vincristine	5
miR-15b	BCL2	Mitomycin	1
miR-15b	BCL2	Fludarabine	1
miR-15b	BCL2	5-fluoroucil	1
miR-15b	BCL2	l-ohp	1
miR-15b	PIM1	Doxorubicin	1
miR-15b	BMI1	Cisplatin	1
miR-15b	BMI1	Doxorubicin	1
miR-15b	CDK6	Cisplatin	1
miR-15b	CDC25A	3,3’-diindolylmethane	1

Table 2: Overlapped miRNA drug-affected pathways

Category	Term	Genes
KEGG_PATHWAY	hsa05215 :Prostate cancer	IGF1R, CCND1, BCL2
KEGG_PATHWAY	hsa05200: Pathways in cancer	IGF1R, CCND1, BCL2, CDK6
KEGG_PATHWAY	hsa04110: Cell cycle	CCND1, CDK6, CDC25A
KEGG_PATHWAY	hsa05161: Hepatitis B	CCND1, BCL2, CDK6

KEGG: Kyoto Encyclopedia of Genes and Genomes

When corticosteroid use interrupted, it might take some time for the body to make cortisol at a normal level. Short-term corticosteroids usage is an effective therapy, but disruptions in sleep, mood, and appetite may occur.[21,22] Increasing mortality of this disease strongly supports the need to develop recent treatments and the need for disease-modifying anti-sarcoidosis therapies.

miRNAs are small non-coding RNAs of 17–22 nucleotides in length and have been determined that are involved in many physiological processes.[23,24] Lately, the use of microRNAs in the prognosis and diagnosis of multiple respiratory diseases

is a promising development.[25,26] Ascoli *et al*. showed a set of eight-microRNA signatures that distinguish sarcoidosis from controls with 74.8% accuracy.[27]

The study of approved drugs for new therapeutic applications in the drug discovery process is a timesaving approach.[28] We have successfully applied computational analysis of transcriptional responses of cells to genetic perturbations and chemical or in disease for preclinical investigations of additional drug indications. Revealing of miRNAs implicated in the modulation of the drug is growing into a remarkable research focus on diseases. miRNAs can identify the efficacy of drugs that have expanded to the term of "miRNA pharmacogenomics."[13] In this context, miRNAs are components of a triad consisting of miRNA target drug, which function to diminish the drug effect, by modulating miRNA, the target gene, or both.

In our study, miR-15b was detected to play a crucial role in pulmonary sarcoidosis through *in silico* approaches. Microarray, a high-throughput technology, has shown that miR-15b was generally overexpressed in different malignant tumors.[29] It was also shown that miR-15b was significantly dysregulated in multiple sclerosis patients.[30] When we overlapped miR-related pathways and drug-affected pathways, prostate cancer and pathways in cancer were determined to be mostly enriched pathways. We found these pathways to be significant in some other studies in concerning sarcoidosis.[27,31]

There are controversial studies concerning malignancy and sarcoidosis in the literature. Several studies reported that solid tumors and hematologic malignancies occur in sarcoidosis patients and that the new onset of sarcoidosis develops in oncologic patients.[32] Sarcoidosis potentially induces metastasis as it involves tumor-promoting and immune regulating cell subsets.[33] It was determined using imaging technology that sarcoidosis could mimic cancer recurrence or metastatic progress.[34,35]

CONCLUSION

In our study, we found through *in silico* analysis that cisplatin in the drug-pathway network can potential novel drug for sarcoidosis patients. Cisplatin has been used for 50 years, that is, a common prototypic platinum chemotherapeutic agent.[36]

According to the literature, it had evaluated the use of chemotherapeutic drugs with cisplatin in cases that have sarcoidosis and cancer together. However, there is no information on the effectiveness of the alone use of cisplatin in sarcoidosis patients. Our study suggests searching for the use of cisplatin as an alternate or combined therapy on sarcoidosis patients in future researches.

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