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Network Based Approach for Understanding the Chondrocyte Biology for Rheumatoid Arthritis

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ABSTRACT: The major component of cartilage connective tissue, chondrocytes plays major role in Rheumatoid arthritis (RA),which is a chronic auto-immune disease of unknown etiology characterized by series of process such as inflammation, synovial hyperplasia and cartilage destruction leading to bone erosion. In the present work, role of chondrocytes which maintains the balance between synthesis and degradation of cartilage by osteoblast and osteoclast are focused. The key regulatory molecules in chondrocytes associated with pathophysiology of RA are extracted by integrating Biological network analysis and gene expression profiling data analysis. The expression data retrieved from gene expression omnibus included normal donor and diseased data set.By applying analytical and statistical measureshighly correlated differentially expressed genes were obtained.Clusteringof up and down networks resulted in three meaningful modular networks with seed genes ABO, AMPD3 and COA1. These seed genes were highly connected with the neighboring genes. So the biological analysis of modular network opens more ways for unraveling further more interactions to produce novel proteins related to RA.

KEY WORDS: Rheumatoid arthritis, Chondrocytes, Gene Expression Profiling, Gene-gene Correlation, Biological network analysis

I. INTRODUCTION

Chondrocytesare the cells found in cartilage connective tissue which produce the cartilage matrix and maintains a stable balance between the synthesis and the degradation of matrix components (Fujisawa et al. 1999). The main function of cartilage is to reduce the friction between bones and hold them off from rubbing together(Otero andGoldring 2007). Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease that frequently affects synovial joints characterized by synovial hyperplasia, cartilage destruction and bone erosion (Rossol et al. 2013). The cells of synovium contain macrophages, chondrocytes and fibroblasts(McInnes and Schett 2007). The initial inflammatory process in RA takes place in the synovial lining of diarthrodial joints (Szekanecz and Koch 2007).The immune cells and inflammatory moleculeshave crucial role in pathophysiology of RA (Huber et al. 2006). Human chondrocyte secretes cytokines such as Monocyte Chemoattractant Protein (MCP-1) which are involved in cartilage degradation and rheumatic joint diseases (Röhner et al. 2012). Present work focuses on identification of key regulatory molecules involved in pathology of RA from chondrocytes.

Gene expression profiling with microarrays and network biology has emerged as a novel approach for finding principle signature molecules and biological pathways which are associated with complex diseases (Baueret al. 2009; Haupl et al. 2010; Chand and Alam 2012). To predict novel association and key regulatory molecule in complex disease, co-expressed genes are combined in biological network analysis. These techniques integrate biologically relevant information which isreliable to predict signature molecules from meaningful biological network (Tornow and Mewes 2003; Marcotte et al. 1999; Begley et al. 2002). In the present study, we analyze molecular expression profiles of human chondrocytes reported for RA to predict promising genes involved in its pathophysiology.

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II. MATERIALS AND METHODS

2.1 Collection of Dataset

The objective of the research work lied on the prediction of signature molecules from chondrocytes involved in the pathophysiology of RA. The datasets of chondrocytes related to RA were fetched from Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/).The dataset GSE10024 under Affymetrix platform were preferred which consisted of total of 6 samples. The dataset was related to comparison of Rheumatoid Arthritis Synovial Fibroblast (RASF) stimulated chondrocytes to Normal Donor Synovial Fibroblast (NDSF) stimulated chondrocytes by keeping unstimulated chondrocytes as baseline (Andreas et al. 2008).

2.2Data Pre-processing

The 6 samples of the series GSE10024 was imported to GeneSpring GX 12.6 (Chu et al. 2001) microarray data analysis tool. Using summarization algorithm, Robust Multi-array Average (RMA) the raw signal values of each probe set in the samples were normalized by baseline to median of control sample. RMA algorithm involves background correction of raw signal values, log2 transformation of back corrected values followed by quantile normalization (Irizarry et al. 2003).

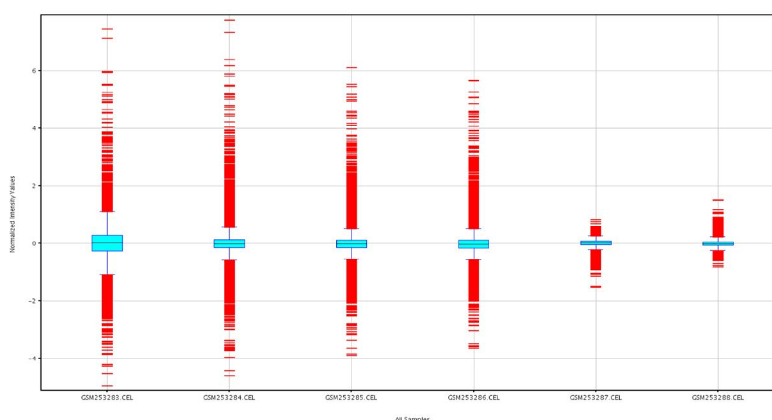

Fig. 1 Box plot of normalized value of the series GSE10024 using RMA algorithm

Experimental grouping and interpretation was created using GeneSpring GX 12.6. The samples in the datasets were grouped as RASF stimulated (2 samples), NDSF stimulated (2 samples) and unstimulated chondrocytes (2 samples).

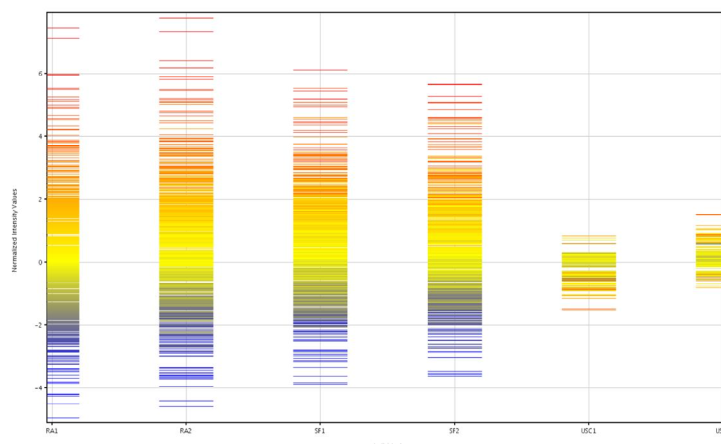

Fig. 2 Profile plot of normalized value of the series GSE10024 based on RMA algorithm

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2.3 Differentially Expressed Genes (DEGs)

In order to detect Differentially Expressed Genes (DEGs) statistical analysis was performed based on one-way analysis of variance (ANOVA) and Benjamini-Hochberg False Discovery Rate (FDR). Direct FDR with p-value cut off 0.05 and Fold Change (FC) value 1.5 ($\geq$ 1.5 and $\leq$ -1.5)was carried out to separate the genes into up-regulated and down-regulated genes.

2.4 Network Construction and analysis

Based on the Pearson correlation with centroid linkage clustering, gene-gene correlation map was constructed for both up and down regulated genes (Fig. 3, Fig. 4).The statistical relationship among the up and down regulated genes were developed based on Pearson correlation coefficient (R-value) (Ng et al. 2006). Symmetrical n x n matrix was generated with R value cutoff 0.8 and above. The data were pre-processed and made non redundant using PERL script. The pre-processed data with high correlation were incorporated in Cytoscape (Shannon et al. 2003) and co-expressed gene's networks were constructed for both up and down regulated genes.

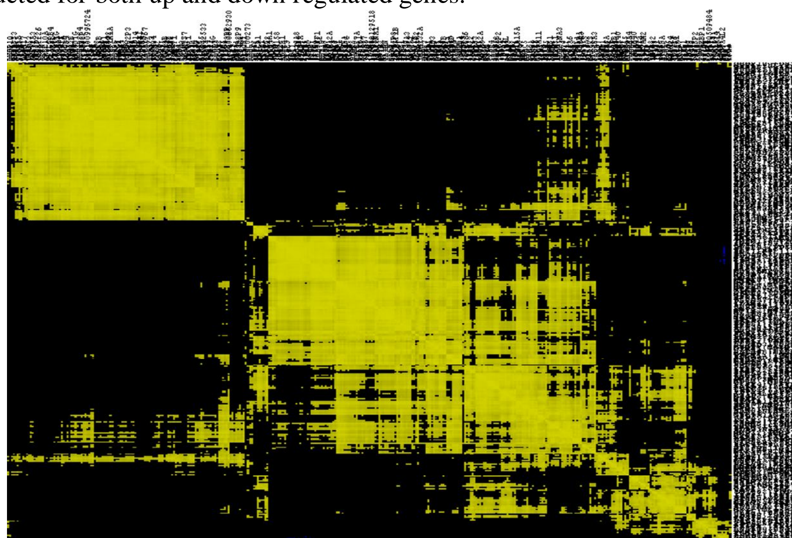

Fig. 3 Gene-gene correlation map for up-regulated genes

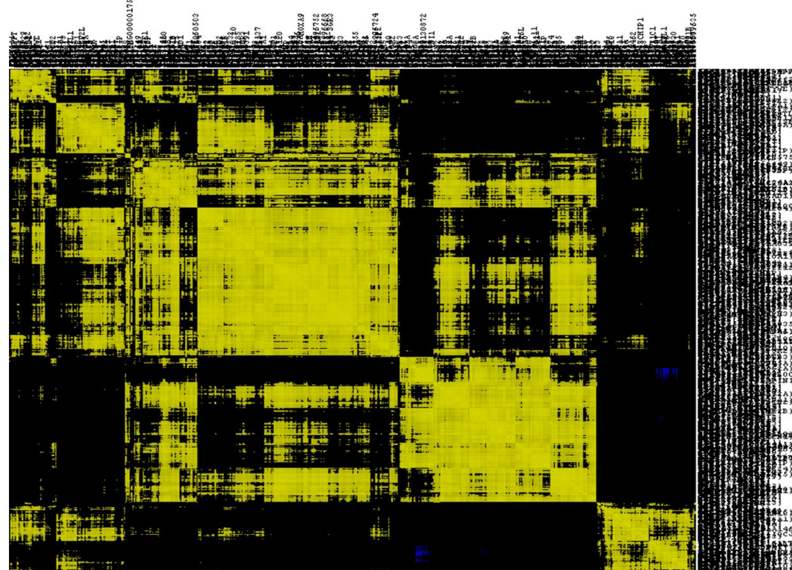

Fig. 4 Gene-gene correlation map for down-regulated genes

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Biological networks are scale free networks with functionally and statistically significant interacting molecules (Sengupta et al. 2009).Nodes represent genes or proteins and interactions between the nodes are represented by degrees which convey various pathways through which they are associated (Snijesh and Singh 2014). Cytoscape plugins-Network analyzer and Clusterviz were adopted for topological and clustering analysis (Cai et al. 2010). The plugin Clusterviz was used to develop dense clusters based on fast hierarchical agglomerative algorithm (FAG-EC) with a precise complex threshold. Functional characterization and Gene Ontology analysis were performed using Biointerpreter(http://www.biointerpreter.com/biointerpreterv3/)(Varatharajan et al. 2013) andWebGestalt (http://bioinfo.vanderbilt.edu/webgestalt/)(Zhang et al. 2005).

III. RESULTS AND DISCUSSION

3.1 Expression Characteristics

Differentially expressed genes in chondrocyte series GSE10024 were analyzed based on multiple hypothesis testing which resulted in 1073 DEGs (374 up and 699 down regulated genes).Higher correlation coefficient with Pearson centroid linkage algorithm resulted in 374 interactomes (edges=23160) for up-regulated and 699 (edges=93953) for down-regulated networks. Highly correlated co-expression network was clustered using clusterviz which gave total three sub-networks, (Table. 1) two for up-regulated and 1 for down-regulated with high modularity and in degree.

Up						
	Nodes	**Edges**	**Modularity**	**In degree**	**Out degree**	**Seed**
Rank1	242	13790	8.312	12131	1659	ABO
Rank2	129	7663	4.603	7663	1664	AMPD3
Down						
	Nodes	**Edges**	**Modularity**	**In degree**	**Out degree**	
Rank1	699	93953	∞	93953	0	COA1

Table 1.Clusters with high modularity in up and down regulated genes.

The seed nodes obtained through cluster analysis from the interactome were ABO, Adenosine Monophosphate Deaminase 3 (AMPD3) for up-regulated andCytochrome C Oxidase Assembly Factor 1(COA1) for down-regulated networks (Fig .5).Seed nodes are the highest scoring node in the cluster and link all other nodes in the cluster (Li et al. 2010). The functional characterization revealed that clusters of these genes in the entire network(Rank1 and Rank2 for up and Rank1 for down) were involved in angiogenesis, auto phagocytosis, cell adhesion, growth factors and regulation, immune response, inflammation and vasculogenesis.They also showed involvement in pathways such as cytokine-cytokine receptor interaction, Chemokine signaling pathway, Toll like receptor signaling pathway, Osteoclast differentiation, antigen processing pathway, TGF beta signaling pathway and glycosaminoglycan biosynthesis pathway which confirmed that these molecules may play crucial role in pathophysiology of RA.

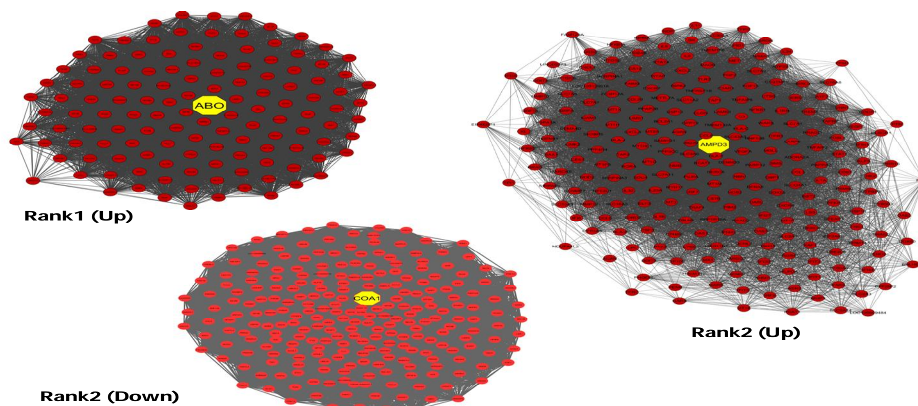

Fig. 5 Seed nodes with their interactomes for both up and down regulated network

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The seed genes in the up regulated network ABO and AMPD3 were reported to have crucial role in immune response and inflammation process (Michelis et al. 2014; Fessler et al. 2002).Cytochrome C Oxidase Assembly Factor 1which regulates COX assembly i.e prostaglandin endoperoxidase synthesis of different units. The inhibition and down regulation of COA1 shows that the immune system itself tries to down regulate the COA1 protein for inhibiting the COX assembly proteins. The interacting gene of these down regulated network shows the silencing of the genes during pathogenesis of RA. Majority of the genes present in these clusters showed their cellular localization mainly in the nucleus followed by membrane region (Fig. 6).

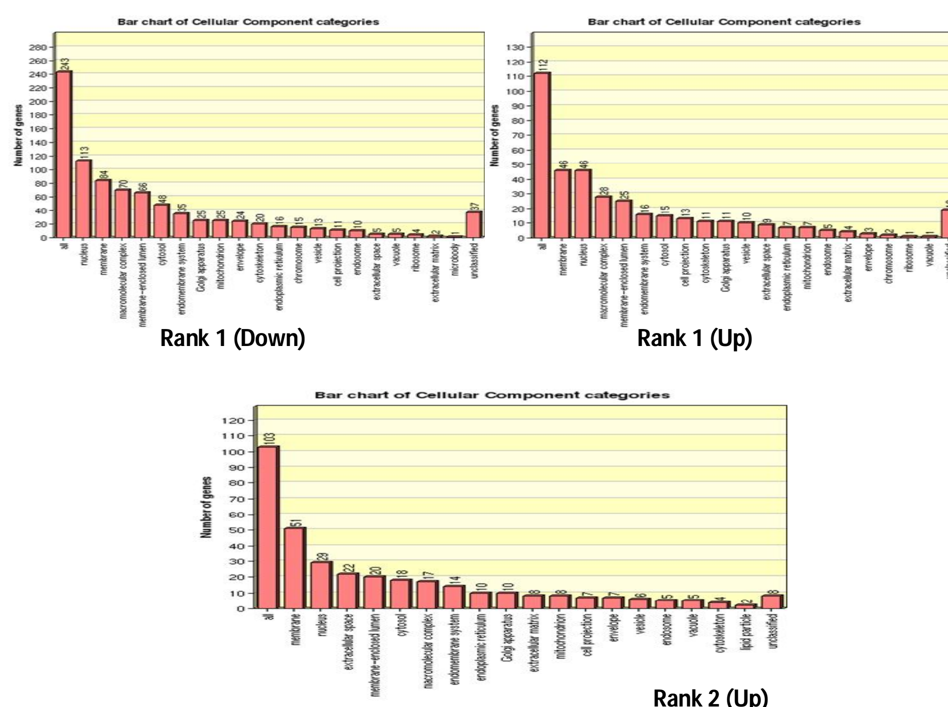

Fig. 6 Cellular localization of clustered genes

IV. CONCLUSIONS

Expression data analysis of chondrocyte related to RA gave differentially expressed genes at the fold change level +/- 1.5. The up and down regulated genes were represented with their seed nodes which were connected with high indegree ranging from (93953- 7663). The indegree reports that all the information flows from upper hub nodes to inner core node. The understanding of these three seed genes gave an insight to explore more seed networks for identifying significant pathways and proteins which may play role in pathophysiology of RA.

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