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Computational systems biology of oxLDL induced macrophage foam cell formation-A multilayer regulatory network analysis of atherogenic process

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ABSTRACT

Atherosclerosis, a chronic inflammatory disease of the vascular system, presents significant challenges to developing effective molecular diagnostics and novel therapies. A systems biology approach integrating data from large-scale measurements (e.g. transcriptomics, proteomics and genomics) is successfully contributing to deciphering regulatory networks underlying the response of many different cellular systems to perturbations. In this study we have used novel systems biology tools to analyze the pathogenic process of macrophage foam cell formation when exposed to oxidized low density lipoprotein (oxLDL). Our network-driven integrative analysis not only identified the pathogenic network behind atherogenic process but also found the multi layer relationship behind macrophage foam cell formation.

Key words: Atherosclerosis; Systems biology; Foam cell; Microarray; Macrophage.

INTRODUCTION

Systems biology provides a framework for assembling models of biological systems from systematic measurements. Since the field was first introduced a decade ago, considerable progress has been made in technologies for global cell measurement and in computational analyses of these data to map and model cell function [1]. A key concept in systems biology is that of emergent properties important features of biologic systems that can best be identified by examining the system as a whole. A particularly important goal of systems biology is to construct networks sets of genes or proteins or metabolites that act in concert in a common biologic process [2].

Recently, the employment of microarray technology has rapidly produced vast catalogs of gene expression activities. The immense data highlights the need for a systematic tool to identify and analyze the underlying gene regulatory networks. Several computational methods for the inference of transcriptional regulatory networks from experimental microarray data have been published [3].

Macrophages play a key role in the development of atherosclerosis. In atherosclerosis, the accumulation of apolipoprotein B-lipoproteins in the matrix beneath the endothelial cell layer of blood vessels leads to the recruitment of monocytes, the cells of the immune system that give rise to macrophages and dendritic cells. Macrophages derived from these recruited monocytes participate in a maladaptive, nonresolving inflammatory response that expands the subendothelial layer due to the accumulation of cells, lipid, and matrix [4].The uptake of

oxidized low density lipoprotein (oxLDL) by macrophages leads to foam cell formation and fatty streaks, which represent early sites of potential atheroma development. In addition, the transcriptome experiments found an increased inflammatory response under conditions of both acute and chronic oxLDL exposure. Overall the combined functional, proteomic, and transcriptomic experiments show that macrophages respond to oxLDL by developing an oxidative stress resistance that increases and stabilizes with chronic exposure. Furthermore this protective response and the increased foam cell survival that it supports amplifies their proatherogenic role by promoting a continued inflammatory state [5].

The above study only provided the microarray data to the scientific community. This study aims to construct the functional network and prior the signaling programs involved in pathogenesis of oxLDL exposed macrophages. Moreover this study intended to compare the similar or resemblance gene expression events associated with other expression states based on comparative transcriptomic analysis.

EXPERIMENTAL SECTION

Microarray Data collection

In this analysis Microarray data was collected from the paper published by James P. Conway and Michael Kinter et al. [5]. The genes with significant fold change in J774 oxLDL treated versus J774 untreated were taken for this study.

Comparative transcriptomic analysis of gene expression signatures

The up and down regulated signature was submitted for the comparative transcriptomic analysis. MARQ available at http://marq.dacya.ucm.es offers an easy-to-use and integrated environment to mine GEO, in order to identify conditions that induce similar or opposite gene expression patterns to a given experimental condition. In this study, a high positive score indicates that the database signature shares a significant proportion of over-expressed and under-expressed genes with the query signature. The score was calculated for the up and down-regulated genes using a weighted Kolmogorov–Smirnov-like statistic [6].

Protein Interaction network construction

Network analysis of gene products which were up and down regulated were searched against the STRING database version 9 [http://string-db.org] for protein-protein interactions, using a STRING confidence score set to ≥ 0.4 - medium confidence [7].

Identification of miRNAs responsible for co-regulated gene expression

A possible involvement of miRNAs in the deregulation and miRNAs responsible for co-regulated patterns of gene expression can be computationally predicted. MiRVESTIGATOR [http://mips.helmholtz-muenchen.de/proj/gene2mir/], a web tool which can identify significant subsets of genes from the given gene list which are the targets of a single or several miRNAs [8]. The top 2 miRNA from the results were considered for analysis.

Gene Ontology analysis

To assign biological meaning to the group of genes with changed expression, the subset of genes which met the above criteria was analyzed with the Gene Ontology [GO] classification system, using DAVID software http://david.abcc.ncifcrf.gov. Over-representation of genes with altered expression within specific GO categories was determined and clustered with high stringency by choosing the 'GOTERM_BP_5', KEGG pathway options [9].

RESULTS

Figure 1. Protein-protein interaction (PPI) network of upregulated genes

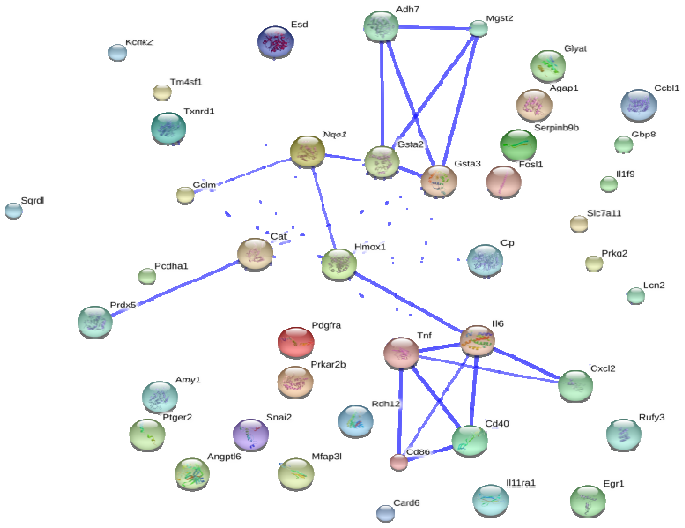

Figure 2. Protein-protein interaction (PPI) network of down regulated genes

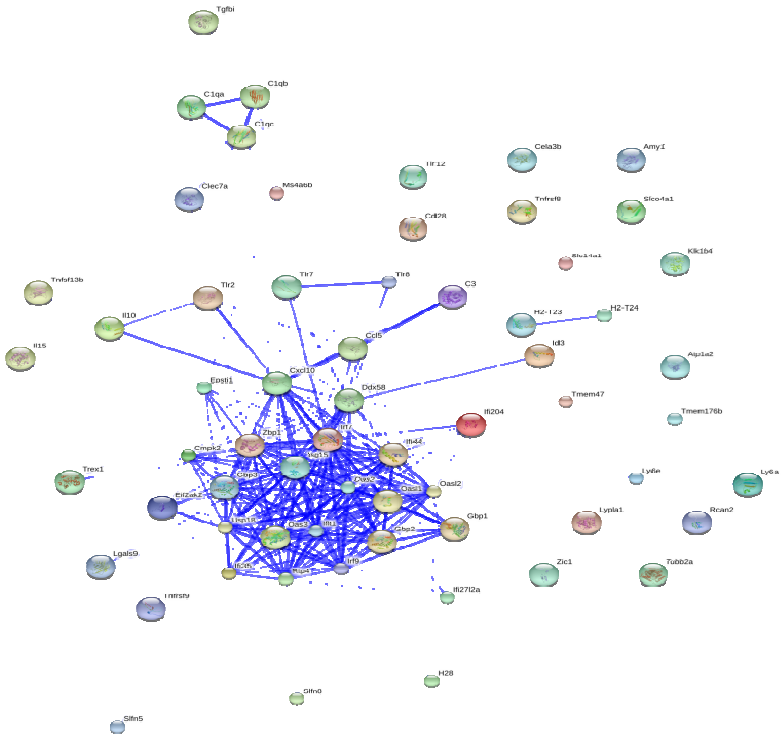

Table 1. Biological processes of upregulated genes - geneontology analysis (DAVID)

Category	Term	Count	%	P-Value	Benjamini
GOTERM_BP_5	hydrogen peroxide catabolic process	2	4.9	2.4E-2	1.0E0
GOTERM_BP_5	regulation of apoptosis	5	12.2	2.4E-2	9.7E-1
GOTERM_BP_5	regulation of programmed cell death	5	12.2	2.5E-2	9.1E-1
GOTERM_BP_5	regulation of secretion	3	7.3	2.7E-2	8.4E-1
GOTERM_BP_5	cellular response to reactive oxygen species	2	4.9	3.6E-2	8.7E-1

Table 2. Biological processes of down regulated genes - geneontology analysis (DAVID)

Category	Term	Count	%	P-Value	Benjamini
GOTERM_BP_5	positive regulation of immune response	10	16.1	8.0E-11	2.7E-8
GOTERM_BP_5	regulation of defense response	7	11.3	3.4E-7	5.6E-5
GOTERM_BP_5	activation of immune response	6	9.7	3.6E-6	4.0E-4
GOTERM_BP_5	positive regulation of defense response	5	8.1	1.7E-5	1.5E-3
GOTERM_BP_5	B cell mediated immunity	5	8.1	2.9E-5	2.0E-3

Table 3. KEGG map of upregulated genes

Category	Term	Count	%	P-Value	Benjamini
KEGG_PATHWAY	Glutathione metabolism	4	9.8	1.4E-3	6.8E-2
KEGG_PATHWAY	Metabolism of xenobiotics by cytochrome P450	4	9.8	2.8E-3	6.7E-2
KEGG_PATHWAY	Drug metabolism	4	9.8	4.0E-3	6.5E-2
KEGG_PATHWAY	Cytokine-cytokine receptor interaction	5	12.2	2.0E-2	2.2E-1
KEGG_PATHWAY	Intestinal immune network for IgA production	3	7.3	2.3E-2	2.1E-1

Table 4. KEGG map of down-regulated genes

Category	Term	Count	%	P-Value	Benjamini
KEGG_PATHWAY	Cytosolic DNA-sensing pathway	6	1.0	4.6E-6	1.3E-4
KEGG_PATHWAY	Toll-like receptor signalling pathway	6	1.0	8.3E-5	1.2E-3
KEGG_PATHWAY	Systemic lupus erythematosus	6	1.0	1.0E-4	9.3E-4
KEGG_PATHWAY	Prion diseases	4	0.7	5.5E-4	3.8E-3
KEGG_PATHWAY	Cytokine-cytokine receptor interaction	7	1.2	7.7E-4	4.3E-3

Table 5. Comparative transcriptomic signatures of oxLDL exposed macrophages

S. No	Experiment	Score
1.	MyD88 deficient macrophage response to zymosan .zymosan <=> control [agent]	1.43
2.	Mechanical stress effect on fibroblasts from various fetal tissues [MG-430A]. stimulated <=> control [stress]	1.35
3.	Lung response to carcinogens that test positive in two-year rodent bioassays . positive lung carcinogen <=> control [other]	1.3
4.	Palmitate effect on myoblast cell line . palmitate <=> control [agent]	1.28
5.	Megakaryocytes at successive stages of maturation . 6 days in culture <=> 3 days in culture [development stage]	1.25
6.	Obliterative bronchiolitis and tracheal allograft . untransplanted <=> allograft [protocol]	1.21
7.	Myocardial infarction time course . 24 hour <=> 1 week [time]	1.81
8.	Lung response to carcinogens that test positive in two-year rodent bioassays . 1,5-Naphthalenediamine <=> Feed control [agent]	1.17
9.	Signal transduction adaptor MyD88 deficient lung response to Chlamydia pneumoniae infection . mock <=> C. pneumoniae [infection]	1.16
10	Hexamethylene diisocyanate effect on lungs: time course 6 h <=> 0 h [ratio] [time]	1.13

Table 6. Predicted miRNA down-regulated in oxLDL exposed macrophages

miRNA Name	**miRNA Seed**	**Seed Model**	**Length of Complementarity**	**Complementary Base-Pairing**	**Complementarity P-Value**
mmu-miR-128	ACAGUG	6mer_1	6	**Motif**_5'_AUCACUGU_3' |||||| 3'_--GUGACA_5'_**miRNA Seed**	2.40E-04
mmu-miR-216a*	ACAGUG	6mer_2	6	**Motif**_5'_AUCACUGU_3' |||||| 3'_--GUGACA_5'_**miRNA Seed**	2.40E-04

DISCUSSION

In atherosclerosis, monocytes hone to focal area of the arterial subendothelium in response to matrix-retained apolipoprotein B-lipoproteins. Monocyte-derived macrophages then participate in a maladaptive, non-resolving inflammatory response that leads to subendothelial expansion with additional cells, lipid, and matrix. A few lesions undergo necrotic disruption, triggering acute thrombotic vascular disease, including myocardial infarction, stroke, and sudden cardiac death. In addition, pro-atherogenic lipids delivered to cells by lipoproteins, particularly modified lipoproteins, may have potent effects. An example includes apoptosis induced by oxysterols and oxidized phospholipids delivered through the uptake of oxidatively modified forms of lipoproteins [4].

Macrophages are dynamic cells integrating signals from their microenvironment to develop specific functional responses. Although, microarray-based transcriptional profiling has established transcriptional reprogramming as an

important mechanism for signal integration and cell function of macrophages, current knowledge on transcriptional regulation of human macrophages is far from complete.

In the gene ontology point of view, results illustrate the up and down regulated process associated with pathogenesis of macrophages under oxLDL associated condition.

The highest hits of DAVID bioprocess level 5 indicates, Hydrogen peroxide catabolic process, regulation of apoptosis, regulation of programmed cell death, regulation of secretion and cellular response to reactive oxygen species are the most common terms associated with up regulated genes of oxLDL exposed macrophages. This result indicates that, apoptosis is one of the major events associated with oxLDL exposure. A previous study indicates that, a key cellular event in the conversion of benign to vulnerable atherosclerotic plaques is endoplasmic reticulum (ER) stress-induced macrophage apoptosis [10].

The gene ontology terms of down regulated genes shown that, positive regulation of immune response, B-cell mediated immunity, lymphocyte mediated immunity, acute immunity response etc., this indicates that the normal function of macrophage in the immunity network gets down regulated during the oxLDL exposure.

The KEGG pathway analysis have shown that, cytosolic DNA sensing pathway and toll like receptor signaling pathway are associated with up regulated genes and glutathione metabolism related genes are down regulated. This indicates the oxidative stress related pathway have prominent role during oxLDL exposure.

In the comparative transcriptomic analysis, we query a signature database derived from GEO to find experiments that induce similar or opposite gene expression patterns of our experiment. The results found that, MyD88 deficient macrophage response to zymosan, mechanical stress effect on fibroblasts from various fetal tissue, lung response to carcinogens that test positive in two-year rodent bioassays, palmitate effect on myoblast cell line. Myocardial infarction time course analysis were become the top hits.

Based on the similar expression features, the study explains that, similarity of our experiment with MyD88 deficient macrophage response to zymosan indicates, NFAT activation regulated IL-2, IL-10 and IL-12 p70 production by zymosan is the major outcome of the signature [11].

Comparative signature of oxLDL exposed macrophages with signature of palmitate effect on myoblast cell line indicates that, palmitate reduced expression of tricarboxylic acid cycle and oxidative phosphorylation mitochondrial genes and reduced oxygen consumption. These effects were reversed by overexpression of PGC-1alpha or -beta, indicating PGC-1 dependence. Palmitate effects also required p38 MAPK, as demonstrated by palmitate-induced increase in p38 MAPK phosphorylation [12].

Myocardial infarction time course analysis indicates the involvement of apoptosis or programmed cell death. Apoptosis is characterized by shrinkage of the cell and the nucleus. The nuclear chromatin is condensed into sharply delineated masses, and eventually breaks up. The cell then detaches from the surrounding tissue. This indicates that oxLDL induced macrophage apoptosis also one of the prominent event with apoptosis related gene expression signature.

All the above important hits shown that oxLDL induced macrophages shows similar cell stress related gene expression programme. This will help us to compare the drug associated gene signature in future.

Network-level analysis has revealed detailed insight on metabolic regulation in type 2 diabetes and insulin resistance. Previous study has proposed that in the emerging systems-level view of molecular biology, diseases should be viewed as a function of network perturbation rather than as isolated local changes. Molecular networks may be classified in two categories: metabolic networks and protein interaction networks [13].

This study explores, TNF and IL-6 nodes have more attention due to its higher connectivity among the up regulated genes network. It has been increasingly apparent that cardiovascular disease (CVD) is associated with a persistent systemic inflammatory response. IL-6 and TNF-α, seem to have proinflammatory and proatherogenic properties. TNF, a proinflammatory cytokine (17 kD) originally associated with killing of tumor cells, has a pivotal role in

regulating both pro- and anti-inflammatory mediators. TNF-α has been regarded a "master regulator" of the cytokine cascade that provides a rapid form of host defence against infection but is fatal in excess [14].

The upstream causal signaling analysis shown that, IL-1, TLR, MAPK-PI3K pathways are highly activated during macrophage exposure. Previous study indicates that, IL-1, p38 MAPK plays an important role in the regulation of lipopolysaccharide-induced iNOS and COX-2 expression in J774 macrophages [15].

The miRNA prediction method found that, miRNA-128 has shown to be down regulated. Previous study indicates that, over expression of miR-128 specifically inhibits the truncated isoform of NTRK3 and up-regulates Bcl2 in SH-SY5Y neuroblastoma cells [16]. The down regulation of miRNA 216a indicates that, the expression of the pro-apoptotic gene *Bim*, a FoxO3a target, was inhibited by miR-192 and miR-216a/217 as well as TGF-β while inhibitors of these miRs reversed the effects of TGF-β. Moreover, miR-192, miR-216a/217 and TGF-β prevented serum-depletion-induced MMC apoptosis [17]. This results indicates that, oxLDL induced apoptosis of macrophages may be due to the mechanism of down regulation of miRNA-128 and miR-216a.

The overall study provides a view on contribution of multilevel regulation in macrophage foam cell formation upon oxLDL exposure. This study will led to find new drug targets for treating atherosclerosis in future.

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