# Identifying Stress-induced and LDL-induced Differentially Expressed Genes in Vascular Smooth Muscle Cells

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***Abstract*—These Cardiovascular diseases (CVDs) are the number one cause of death globally, more people die annually from CVDs than from any other cause. There are a number of risk factors for CVDs, and we focused on high blood pressure (hypertension) and high blood cholesterol. In this study, we examined and analyzed the differential expression of genes of VSMC separately subjected to mechanical stress and oxidized form of low-density lipoprotein cholesterol (ox-LDL). Time course microarray experiments were used to identify differentially expressed genes (DEGs) for both conditions. We found a group of common DEGs that are involved in these two conditions. Gene set enrichment analysis suggested that the enriched biological processes are cell-cycle-related processes. This group of common DEGs is putative candidates that might have a synergistic effect on VSMC proliferation. The results of the identified DEGs involved in the two conditions can be accessed at http://ppi.bioinfo.asia.edu.tw/vsmc/.**

***Index Terms*—cardiovascular disease, low-density lipoprotein cholesterol, mechanical stress, time course microarray, vascular smooth muscle cell**

## I. Introduction

Cardiovascular diseases (CVDs) is a series of diseases related to the circulatory system, such as coronary heart attacks, arrhythmia, and cerebrovascular diseases (strokes) etc. These diseases originated in vascular lesions, such as abnormal vascular tone and vasomotor etc., often have a similar cause and disease process, and they are the leading causes of death in most of the developed countries. Therefore, how to improve the diagnosis, treatment and prevention of CVDs is an urgent and important issue.

The vascular function and structure abnormalities will lead to its morphological and molecular changes in blood vessels, cause a series of responses, such as endothelial damage, inflammatory cascades initiate, migration and phenotype changes in vascular smooth muscle cells (VSMC), as well as extracellular matrix (ECM) imbalances [1].

The vessel wall is an initiative and integrative organ, which owns three main cell types, including endothelial cells (ECs) lining the tunica intima, VSMC in the tunica media and fibroblasts within the adventitia, may through producing locally active substances to self-modulate the structure and function of the blood vessel for responding to various stimuli. The hypertrophy and proliferation of VSMC contribute to the formation of atherosclerosis, hypertension, and restenosis [1-3].

Various local or systemic risk factors may initiate atherosclerosis (AT) by inducing endothelial dysfunction and vascular injury. It is known that upon the vascular wall exposed to high pressure (such as mechanical stretch) or oxidized form of low-density lipoprotein cholesterol (ox-LDL), both will lead to differential gene expression, then promote the growth and migration of VSMC, result in severe vascular lesions and sequelae which caused CVDs [2-7].

Mechanical force is a particularly important modulator in circulatory systems. Once mechanical forces (such as pressure-induced mechanical stretch) exert injurious forces on the vessel wall, where mechanical forces are largely disturbed, followed by the modulation of gene expression is observed in VSMC. As a result of up- and down-regulation of specific genes, key cellular processes may be modulated, such as cell proliferation, apoptosis, cell migration and the synthesis, degradation and reorganization of the ECM. These differential expressions of genes will encode relevant factors to counter the effects of mechanical forces exerted on the vessel wall and minimize its notable complications. Namely, mechanical forces may directly perturb or alter the manner of the genes in the cell, thereby initiating signalling pathways usually used by growth factors [1-4, 8].

On the other hand, many studies support that low-density lipoprotein cholesterol (LDL) plays a central role in the pathogenesis of atherosclerosis (AT), and the oxidized form (ox-LDL) activates both cell-mediated and immune

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responses, that perpetuate the chronic inflammatory reactions characteristic of AT, and also has been shown to induce VSMC from the contractile state transform to the migratory and proliferative state [8-12].

LDL is a major risk factor in AT development and formation. The lowering LDL cholesterol may reduce or prevent the occurrence of CVDs [13-15]. VSMC exposed to atherogenic stimuli, such as oxLDL, express high levels of a variety of lipid-binding membrane receptors for cholesterol uptake. When the binding occurs it may result in the accumulation of high levels of cholesterol and cholesteryl esters [11, 16, 17].

LDL is oxidated into the ox-LDL form, which will become more toxic and take part in inflammation response to contribute the plaque formation and development by multiple mechanisms, including promoting endothelial cell dysfunction, forming macrophage foam cell, and modulating VSMC phenotype state. Clearly, the ox-LDL effect is mediated by the transcriptional induction of proinflammatory cytokines and growth factors, initiated multiple signal transduction pathways that induced effector genes of cell proliferation, migration and ECM formation. Finally, these transcriptional alterations result in abnormal cell growth and apoptosis, and phenotypic alteration in VSMC [12].

Time course gene expression experiments have been widely used for studying a wide range of biological processes, due to their capability to capture the dynamical behavior of the systems. To analyze the differential expression of genes of VSMC separately subjected to mechanical stress and ox-LDL at the molecular level, we proposed to make use of the time course microarray experiment for identifying significantly abnormal expression gene (namely differentially expressed genes, DEGs), further understanding of the commonality among this two conditions.

In this work, DEGs are identified first. Then, we conduct gene set enrichment analysis to highlight the most relevant biological process terms associated with a given gene list.

## II. METHODS

### A. Datasets

To examine the correlation between stress and LDL contribute to the response of VSMC, we make use of the time course microarray experiment. For the stress-induced condition, E-MEXP-569 was downloaded from ArrayExpress database [18]. The experiment compared the gene expression profiles of the VSMC in response to a cyclical mechanical strain over a time course of 0, 2, 4 and 24 hours. Each sample consisted of two replicates prepared from human aortic VSMC purchased from Cambrex Bioscience.

For the ox-LDL condition, GSE13139 was downloaded from Gene Expression Omnibus [18]. This experiment measured the genes expression changes activated by OxLDL binding to LOX-1 of VSMCs at several different time course of 0, 2, 6, 12 and 24 hours. Each time point measurement was repeated twice to examine the temporal patterns of the gene expression in response to ox-LDL. Each sample consisted of three replicates prepared from HAECT cells.

### B. Differentially Expressed Genes Identification

Many statistical methods are available for microarray data analysis. The use of false discovery rate (FDR) is a common approach to discover significant genes [19]. Another approach is to use ANOVA to investigate the impact of microarray gene expression values within a single factor [20]. Among the many statistical methods, Significance Analysis of Microarray (SAM) [21, 22], Empirical Bayes Analysis of Microarrays (EBAM) [23], and empirical Bayes statistics (eBayes) [24] are three commonly employed approaches to identify DEGs.

SAM is a statistical method for identifying DEGs by comparing two or more groups of samples. It uses repeated permutations of the data to estimate FDR based on observed versus expected score, which is obtained from randomized data. A gene, which has an observed score that deviates significantly from the expected score, is considered as a DEG.

EBAM performs one and two class analyses using either a modified t-statistic or standardized Wilcoxon rank statistic, and a multiclass analysis using a modified F-statistic. Moreover, this function provides an EBAM procedure for categorical data such as SNP data and the possibility of employing a user-written score function.

The EBAYES algorithm computes moderated t-statistics, moderated F-statistics, and log-odds of differential expression by empirical Bayes shrinkage of the standard errors towards a common value.

In a previous study [25] our study suggested that, EBAYES, SAM, and EBAM, achieve a similar level of cancer gene prediction accuracy, i.e. around 20%, therefore, EBAYES is adopted in the present analysis.

The publicly available microarray data analysis package Bioconductor [24, 26] was adopted in the present study. In particular, we used the EBAYES algorithm, an intrinsic function of the limma package, to identify DEGs, assuming a FDR less than 1% [19].

### C. Gene Set Enrichment Analysis

Functional annotation of the DEGs is given by implementing the Database for Annotation, Visualization and Integrated Discovery, DAVID [27]. DAVID accepts batch annotation and conducts GO term enrichment analysis to highlight the most relevant GO terms associated with a given gene list.

## III. RESULTS

To determine the DEGs, we made use of the Linear Models for Microarray Data (*limma*) package, which is available from *Bioconductor*. Details are described in the following section.

### A. Differentially expressed gene identification

In this study, Robust Multi-array Average (RMA) was used for gene expression normalization. After that, a model matrix (use the function, model.matrix) was created with rows and columns denote the replicates and the time points respectively. Then, we seeked a linear model to describe each gene using the lmFit function provided by the limma package [28, 29].

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DEGs are determined by first constructing the contrast matrix (use the function, cont.matrix), which make pair-wise comparisons between the two replicates. EBAYES analysis was subsequently conducted on the previous results, and the DEGs were selected by setting a FDR threshold of 0.01.

A total of 473 stress-induced DEGs and 8217 LDL-induced DEGs were obtained. There are 268 genes involved in both conditions, in which at least 15 genes are relevant to VSMC phenotypic modification. The results of the identified DEGs involve in the two conditions can be accessed at http://ppi.bioinfo.asia.edu.tw/vsmc/ , which provide several important genetic information, such as, the chromosomal locations, GenBank, cytoband and pathway information.

### *B. The results of gene set enrichment analysis*

A total of 268 common DEGs were submitted to DAVID for clustering, thus, enriched biological processes (BPs) related gene groups were obtained.

Details of the top two clusters (with enrichment scores (ES) 8.18 and 3.47 respectively) enriched BPs information are presented in Table 1. The top five found enriched BPs are cell-cycle-related processes, such as, M phase, regulation of mitotic cell cycle and cytoskeleton organization. For more details about the results of enriched analysis, see our web site information.

Many studies have noted that haemodynamic factors and oxidized low-density lipoprotein regulating VSMC; for instance, (i) with increasing LDL concentration, shear stress increases LDL uptake by human ECs when co-cultured with VSMC [30], and (ii) trigger many cell-cycle-related molecules [31, 32]. In particular, the results of Ref. 31 indicated that ox-LDL and mechanical stretch have a synergistic effect on VSMC proliferation, which is induced through a stimulation of nuclear protein import via HSP60 and an activation of the MAPK pathway [31].

TABLE I
THE RESULTS OF GENE SET ENRICHMENT ANALYSIS

| **Enrichment Score: 8.18** | **Count** | **p-value** | **Benjamini** |
|---|---|---|---|
| cell cycle | 43 | 9.40E-14 | 1.60E-10 |
| cell cycle process | 32 | 1.80E-10 | 1.60E-07 |
| cell cycle phase | 27 | 3.50E-10 | 2.00E-07 |
| M phase | 24 | 4.90E-10 | 2.10E-07 |
| organelle organization | 50 | 8.20E-10 | 2.80E-07 |
| mitotic cell cycle | 24 | 4.80E-09 | 1.20E-06 |
| mitosis | 17 | 1.40E-07 | 3.10E-05 |
| nuclear division | 17 | 1.40E-07 | 3.10E-05 |
| M phase of mitotic cell cycle | 17 | 1.80E-07 | 3.20E-05 |
| organelle fission | 17 | 2.50E-07 | 3.90E-05 |
| cell division | 15 | 1.10E-04 | 9.60E-03 |
| **Enrichment Score: 3.47** | **Count** | **p-value** | **Benjamini** |
| cytoskeleton organization | 19 | 7.20E-05 | 7.30E-03 |
| microtubule cytoskeleton organization | 10 | 3.10E-04 | 1.90E-02 |
| spindle organization | 6 | 4.80E-04 | 2.20E-02 |
| microtubule-based process | 12 | 1.20E-03 | 4.10E-02 |

## IV. CONCLUSIONS

To examine how VSMC in response to mechanical stress and ox-LDL, we employed time course microarray experiment to identify the DEGs. Our results have suggested that mechanical stress or ox-LDL may induce a group of common DEGs. Analysis indicated that this group of DEGs could be clustered, in which cell-cycle-related events are the major enriched BPs. Furthermore, this group of common DEGs may be worth for further study, because they could have a synergistic effect on VSMC proliferation.

There are some tasks are undergoing to examine how VSMC react in response to mechanical stress. A three-phase study is proposed to examine this problem. The gene association network for VSMC can be inferred by using Gaussian graphical model. Graph theory and cluster analysis is employed to analyze the gene association network [33].

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