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Impaired Contractile Response of Human Peripheral Arterioles to Thromboxane A-2 after Cardiopulmonary Bypass

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Abstract

Background—We studied the contractile response of human peripheral microvasculature to thromboxane A-2 (TXA-2) before and after cardiopulmonary bypass (CPB), with and without the blockade of TXA-2 receptors, or the inhibition of phospholipase C (PLC), phospholipase A-2 (PLA-2) or protein kinase C- α (PKC- α). We also examined the protein/gene expression and localization of TXA-2 receptors, TXA-2 synthase, PLC and other TXA-2-related proteins.

Methods and Results—Skeletal muscle arterioles (90–180 μ m in diameter) were harvested pre- and post-CPB from patients (n = 28) undergoing cardiac surgery. The post-CPB contractile response of skeletal muscle arterioles to TXA-2 analog U-46619 was significantly impaired compared with pre-CPB (P < 0.05). The presence of TXA-2 receptor antagonist SQ-29548 (10^{−6}M) prevented the contractile response to U-46619 (P < 0.05). Pretreatment with the PLC inhibitor U-73122 (10^{−6}M) significantly inhibited the U-46619-induced contractile response (P < 0.01). Administration of the PLA-2 inhibitor quanaquine (10^{−6}M) or PKC- α inhibitor safinolol (2 × 10^{−5}M), however, failed to affect U-46619-induced contraction. Total protein levels and gene expression of TXA-2 receptors, and TXA-2 synthase of skeletal muscle, were not altered post-CPB. Confocal microscopy showed no significant differences in the expression of PLC β -3 in the microcirculation. PLC β -3 was localized to both smooth muscle and endothelium.

Conclusion—CPB decreases the contractile response of human peripheral arterioles to TXA-2 soon after cardiac surgery. This response may be in part responsible for the decrease in vascular tone, and accompanying hypotension sometimes observed after cardiac operations utilizing CPB.

Introduction

Cardiopulmonary bypass (CPB) is associated with a systemic inflammatory response, resulting in vasomotor dysfunction in multiple tissues and organs, such as, peripheral microvasculature.^{1–6} Previous studies have shown that systemic phospholipase A-2 (PLA-2), thromboxane A-2 (TXA-2), and TXA-2 synthase production are significantly

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increased following CPB.^{1,7-11} TXA-2 is thought to be responsible for a pro-thrombotic milieu, multi-organ dysfunction, following CPB due to its biological activities including vasoconstriction, and platelet activation.¹²⁻¹³ Administration of prostacyclin, prostaglandin E-1, TXA-2 receptor antagonist and TXA-2 synthase inhibitor have been reported to reduce thrombosis and preserve cardiovascular function during CPB.¹⁴⁻¹⁶

In smooth muscle cells, activation of G-protein-coupled TXA-2 receptors induces vascular smooth muscle contraction through the initial activation of phospholipase C (PLC) with production of 1,4,5-inositol phosphate, release of intracellular calcium stores, and phosphorylation of myosin light chain.^{11,12} However, the role of TXA-2 in CPB-related microvascular vasomotor dysfunction in human peripheral arterioles and the signaling pathways underlying TXA-2 induced vasomotor response after CPB remain to be elucidated. Thus, this study was designed to examine the effect of the TXA-2-pathway on peripheral microvasculature before and after CPB.

MATERIALS AND METHODS

Human subjects and tissue harvesting

Samples of skeletal muscle from the left internal mammary artery bed were harvested before and after CPB from 28 patients. The pre-CPB specimen was taken after cannulation and the post-CPB specimen was collected from a different location in the left internal mammary artery bed after removal of the aortic cross-clamp and weaning from CPB. Tissue samples for immunoblot and microarray analysis were immediately frozen in liquid nitrogen. Tissue for immunofluorescent staining was fixed in 10% formalin-buffered solution for 24 hours followed by paraffin mounting and sectioning into 5 μ m slices. Tissue for microvascular studies was placed in cold (5° to 10°C) Krebs-Henseleit buffer (KHB) solution. All procedures were approved by the Institutional Review Board of Rhode Island Hospital, Warren Alper Medical School of Brown University, and informed consent was obtained from all enrolled patients as required by the institutional review board.

Pressure Myography

Microvessel studies were performed by *in-vitro* organ bath video-microscopy as described previously.⁴⁻⁶ Skeletal muscle microvessels (90–180 μ m internal diameter) were dissected from harvested human tissue by the use of a dissecting microscope (Olympus Optical, Tokyo, Japan). The microvessels were placed in an organ chamber (University of Iowa Medical Instrumentation, Iowa City, IA) and cannulated with 10-0 nylon monofilament suture (Ethicon, Somerville, NJ). Oxygenated (95% O₂/5% CO₂) KHB solution warmed to 37°C was continuously circulated through the organ chamber. The microvessels were pressurized to 40 mm Hg in a non-flow state filled with KHB solution. With an inverted microscope (Olympus Optical) connected to a video camera, the vessel image was projected onto a television monitor. An electronic dimension analyzer (Living System Instrumentation, Burlington, VT) was used to measure the internal diameter, and measurement was recorded. After a 60 minute equilibration period, pre- and post-CPB microvessels were exposed to the TXA-2 analog U-46619 (10⁻⁹-10⁻⁵ mol/L). Selected vessels were pretreated with TXA-2 receptor antagonist SQ-29548 (10⁻⁶ mol/L; Sigma-Aldrich, St. Louis, MO) 5 min before U-46619 (10⁻⁹-10⁻⁵ mol/L) administration. Other microvessels were pre-incubated with either PLC inhibitor U-73122 or PLA-2 inhibitor quancrine (both 10⁻⁶ mmol/L, Sigma-Aldrich) or PKC- α inhibitor safinol (2 $\times$ 10⁻⁵ M, Avanti Polar Lipids, Alabaster, AL) 20 min before U-46619 administration. The baseline diameter was defined as the diameter measured after cannulation and equilibration in the aerated KHB solution. Internal diameter was measured after treatment with U-46619 and normalized to the baseline diameter (% contraction). The microvessels were washed with the

KHB solution and allowed to equilibrate 15–30 minutes between pharmacologic interventions.

Immunoblotting

Human skeletal muscle tissue (n = 6) collected from pre- and post-CPB was analyzed for levels of total TXA-2 receptor proteins, and TXA-2 synthase by immunoblotting. The total protein (40µg) was fractionated on an 4-20% sodium dodecyl sulfate polyacrylamide gel by electrophoresis then transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore Corporation, Bedford, MA) as described previously.^{2–4} The membranes were incubated overnight at 4°C with 1:500 dilutions of individual polyclonal rabbit primary antibodies to TXA-2 receptor (Cayman Chemical, Ann Arbor, MI), and TXA-2 synthase (abcam, Cambridge, MA). The membranes were then incubated for one hour with horseradish peroxidase-conjugated secondary anti-immunoglobulin, washed 3 times in Tris saline buffer, and processed for chemiluminescent detection (Pierce, Rockford, IL). The band intensity was measured by densitometric analysis of digitalized images (G:Box, Chemi HR16, Syngene, Frederick, MD) using NIH Image J 1.43.

Immunofluorescence Staining and Confocal Microscopy

Skeletal muscle tissue sections from patients (n = 6) were deparaffinized in xylene, rehydrated in graded ethanol and phosphate-buffered saline (PBS) solution, and antigen unmasked with sodium citrate (10 mmol/L, pH = 6.0), followed by PBS wash and blocking with 2% bovine serum albumin in PBS at room temperature for 2 hours. After PBS wash, overnight incubation with polyclonal mouse antibodies to PLCβ-3(1:50, Cell Signaling Technology, Inc.), were performed at 4°C. Anti-mouse, smooth muscle alpha-actin (1:200; Sigma-Aldrich) was used to detect microvascular smooth muscle. Sections were then washed in PBS and incubated with the appropriate Alexa fluor secondary antibody and mounted using fluorescent mounting medium (Vector Laboratories, Burlingame, CA). The tissue was visualized using a confocal microscope system Nikon C1SI (Nikon, Tokyo, Japan). Tissue labeling with secondary antibodies alone, served as negative controls.^{4–6}

RNA Isolation and Microarray Processing

Total RNA (n = 5) was isolated from 200 mg of skeletal-muscle tissue samples with a Trizol-based method, following the Gibco BRL protocol (Invitrogen, Carlsbad, CA). For microarray analysis, after the quantitative and qualitative assessment of extracted total RNA, single-stranded followed by double-stranded cDNA synthesis was performed. Biotin-labeled cRNA was obtained by *in vitro* transcription of double-stranded cDNA using AFFI kit (Affymetrix, Santa Clara, CA). cRNA was further purified, fragmented, hybridized overnight onto Affymetrix gene chips, washed in streptavidin followed by array scanning as previously described.^{1,6}

Microarray Analysis

Transcriptional profiling was performed on HG-U133 Plus 2.0 Affymetrix chips (Affymetrix, Fremont, CA) with matched pre-bypass samples serving as controls. Quality control, normalization, differential gene expression, and statistical analysis of microarray data were performed using JMP Genomics (SAS, Cary, NC). After quality control analysis, one outlier sample was eliminated from the analysis. Chips were then normalized using the Robust Multichip average (RMA), and gene expression in post CPB samples compared to pre CPB samples using one-way ANOVA. A post-hoc false detection rate (FDR) algorithm with alpha of 0.05 was applied to control for false positives. Fold changes >1 or <-1 were considered real changes, and -log₁₀ (P value) > 4.14 were considered significant. Gene

ontology and canonical pathway analyses were carried out using Ingenuity Pathway Analysis (Ingenuity Systems, Redwood City, CA).

Chemicals

U-46619 and U-73122 were obtained from Sigma. U-46619 was dissolved in ethanol and prepared on the day of the study. SQ-29548 was purchased from Cayman Chemical. Safingol was purchased from Avanti Polar Lipids. U-73122, SQ-29548 and quancrine were dissolved in dimethylsulfoxide to make a stock solution. All stock solutions were stored at -20°C . All dilutions were prepared fresh daily.

Data analysis

The data are presented as the mean and standard error of the mean. Repeated measures analysis of variance and the Student t test were used to compare variables among or between vessels. The treatment effects were statistically examined by paired or independent 2-tailed Student t tests. Statistical significance was taken at a probability value of <0.05 .

Results

Patient characteristics are listed in Table 1. All patients with pre-operative hypertension were receiving antihypertensive medication (β -blocker, aspirin, calcium channel blocker, or angiotensin-converting enzyme inhibitor). All patients with hypercholesterolemia were receiving statin treatment.

Microvascular Reactivity

There was no significant difference in the baseline diameter of the microvessels between the pre- and post-CPB groups (pre-CPB: $136 \pm 6.5 \mu\text{m}$; post-CPB: 165 ± 4.7 , $P>0.05$). The post-CPB contractile response of skeletal muscle arterioles to TXA-2 analog U-46619 was significantly decreased compared with pre-CPB responses ($P<0.05$, Figure 1A). Pre-treatment of pre- and post-CPB peripheral arterioles with TXA-2 receptor antagonist SQ-29548 (10^{-6}M) abolished the U-46619-induced vasoconstriction, respectively ($P<0.05$, Figure 1B, C). In addition, the presence of the PLC inhibitor U-73122 (10^{-6}M) reduced U-46619-induced contraction in both pre- and post-CPB arterioles ($P<0.05$, respectively, Figure 2A–B). In contrast, administration of either PLA-2 inhibitor, quancrine (10^{-6}M , Figure 3A–B) or PKC- α inhibitor safingol ($2 \times 10^{-5}\text{M}$, Figure 4A–B) to pre- or post-CPB arterioles failed to affect U-46619-induced vasoconstriction ($P>0.05$, respectively).

Effect of CPB on Levels of TXA-2 Receptor, and TXA-2 Synthase Polypeptides

Skeletal tissue levels of the TXA-2 receptor, and TXA-2 synthase were unchanged after post-CPB, as detected by immunoblot (Figure 5A, B).

The Effect of CPB on the Gene Expression

The post-CPB fold changes of gene expression of TXA-2 receptors and TXA-2 synthase were less than one fold, and $-\log(\text{pvalue})$ less than 4.14, respectively, suggesting there were no significant changes in TXA-2 receptors and TXA-2 synthase gene expression (Figure 6). The data of CPB-induced other gene expression obtained from microarray analysis are summarized in table 2. The genes of cytokines and chemokines, such as, interleukin 6, interleukin 8, nicotinamide phosphoribosyltransferase, chemokine (C-X-C motif) ligand 1 (CXCL-1), and chemokine (C-X-C motif) ligand 2 (CXCL-2), intercellular adhesion molecule 1 (ICAM1) were significantly up-regulated post-CPB, respectively (Table 2).

CPB Induced PLC Microvessel Localization

Confocal microscopy revealed PLC- β 3 (Figure 5B) localized to skeletal muscle microvessel (red). Immunofluorescent double staining demonstrated that PLC β -3 localized to both smooth muscle (Orange) and endothelium (Red). There are no significant differences in PLC β -3 signal in vessels between pre- and post-CPB samples. Negative controls documented a low level of background fluorescence (red) and a strong signal of α -actin staining on smooth muscle (green).

Discussion

Peripheral microcirculation contributes significantly to tissue perfusion. It has been shown that the microvascular response to neurohumoral substances is often different in magnitude or opposite to that observed in conduit or large vessels.¹⁻⁶ Peripheral arterioles with an internal diameter of less than 200 μ m, are the primary resistance vessels in the circulatory system and of paramount importance in governing tissue blood flow.¹⁻⁶ Thus, the present study of microvascular regulation is likely more physiologically relevant than studies of larger vessels. We and others have previously shown that CPB causes vasomotor dysfunction with altered contractile responses of the human peripheral microvasculature to phenylephrine, vasopressin, serotonin, endothelin-1 and other substances.¹⁻⁶ The present study demonstrates a similarly diminished contractile response of human peripheral microvessels to the TXA-2 agonist U-46619 after CPB.

The mechanism responsible for this alteration may be related in part to platelets, PLA-2, TXA-2 synthase, and TXA-2 receptor responses to CPB. The endogenous stress response to CPB results in enhanced release and activation of platelets, PLA-2, TXA-2 synthase, and vaso-active TXA-2. The sustained increase in circulation levels of PLA-2, TXA-2 synthase and TXA-2 *in vivo*,²⁻⁷ or prolonged exposure to exogenous TXA-2 *in vitro*, may cause subsequent loss of TXA-2-mediated vascular smooth muscle cell contraction. Furthermore, CPB induces a systemic inflammatory response, resulting in the activation or release of neutrophils, oxygen free radicals, endotoxin, other prostaglandins, nitric oxide, catecholamine, serotonin, endothelin-1, cytokines, chemokines and other detrimental factors. In addition, large doses of catecholamines and other vaso-active agents are frequently used soon after CPB and cardiac surgery to support systemic circulation. Collectively, these can lead to increased vascular permeability, worsened vascular injury, and finally reduced contractile response to TXA-2.

TXA-2 is generated from arachidonic acid and prostaglandin H-2 by cyclo-oxygenase and TXA-2 synthase.^{1,12} The human TXA-2 receptor is a typical Gq protein-coupled receptor (GPCR) with seven trans-membrane segments.¹² In the present study, U-46619-induced constriction was prevented by the TXA-2-receptor antagonist SQ-29548, proving that U-46619 selectively activates TXA-2 receptors. Previous studies have demonstrated that CPB altered several kinds of protein expression after CPB.^{1,3,17} However, our immunoblotting and microarray experiments demonstrated that CPB did not alter total protein and gene expression of TXA-2 receptors and TXA-2 synthase, suggesting that a period of intra-operative hypothermic CPB may modify the functional status of these proteins, rather than their steady-state levels.

Activation of PLC is an initial step for TXA-2-induced cell signaling. At least six families of PLCs have been identified: PLC β , PLC γ , PLC δ , PLC ϵ , PLC ζ , and PLC η .^{18,19} The PLC β subfamily includes four members: PLC β 1-4. The PLC γ subfamily includes four members as well: PLC γ 1-4. Our immune-histochemical studies demonstrated that PLC β -3 is presented in both skeletal muscle smooth muscle and endothelium, but there were no differences in localization after CPB. The responses of pre- and post-CPB vessels to U-46619 were

significantly reduced after the administration of PLC inhibitor U-73122, but, not PLA-2 inhibitor, suggesting PLC alone is involved in TXA-2-induced signaling in human peripheral microvasculature.

PKC- α has been found to be the predominant conventional PKC isoform present in human microcirculation.²⁻⁴ We recently found that phenylephrine, and endothelin-1-induced vasoconstriction in the human microvasculature is partially mediated by PKC- α , and that CPB results in reduced PKC- α activity in human peripheral microcirculation.²⁻⁴ However, in this study, blockade of PKC- α did not affect vasoconstriction of peripheral arterioles, indicating mechanisms other than PKC- α may be responsible for TXA-2-induced vasoconstriction in peripheral tissue. In addition, our recent studies also show no significant changes after CPB in other TXA-2 related gene expression, such as, PLC, and PKC, etc.⁵

In the present study, we also found that several genes of cytokines and chemokines were significantly up-regulated post-CPB. These inflammatory mediators are known to play a critical role in the regulation of vascular permeability. The profile of gene expression in patients could be associated with increased tissue edema after CPB and may be related to altered contractile responses of peripheral arterioles to vaso-active agents, such as TXA-2. Variety of inflammatory triggers during CPB, such as, activated neutrophils, surgical trauma, and mechanical trauma of low-pulsatile flow to the peripheral tissue may contribute to the regulated gene expression.

In conclusion, CPB decreases the contractile response of human peripheral arterioles to TXA-2 analog U-46619 soon after termination of cardiopulmonary bypass. This response may be in part responsible for the decrease in vascular tone, and accompanying hypotension sometimes observed after cardiac operations utilizing CPB. The contractile response to U-46619 requires activation of TXA-2 receptors and PLC, but neither PLA-2 nor PKC- α . CPB does not change the protein or gene expression of TXA-2 receptors and TXA-2 synthase, nor does not alter the localization of PLC β -3.

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Figure 1.

(A) **Peripheral** arteriolar vasoconstriction in response to TXA-2 analog U-46619 before vs. after cardiopulmonary bypass (pre-CPB vs. post-CPB); * $P < 0.05$ vs. pre-CPB;; (B) Administration of U-46619 with or without TXA-2 antagonist SQ-29548 to microvessels of pre-CPB [U-46619 (pre-CPB) or SQ-29548 + U-46619 (pre-CPB)]; * $P < 0.05$, vs. U-46619 (pre-CPB); (C) Infusion of U-46619 with or without TXA-2 antagonist SQ-29548 to post-CPB vessels [U46619(post-CPB) or. SQ-29548 + U-46619 (post-CPB)]; $P < 0.05$ vs. U-46619 (post-CPB); $n = 8-11$ /group.

Figure 2.

(A) Application of U-46619 to peripheral arterioles of pre-CPB with or without the presence of PLC inhibitor U-73122 [U-46619 (Pre-CPB) or U-73122 + U-46619 (pre-CPB), $*P < 0.05$ vs. U-46619 (pre-CPB); (B) Application of U-46619 to post-CPB peripheral arterioles with or without the presence of PLC inhibitor U-73122 [U-46619 (post-CPB) or U-73122 + U-46619 (post-CPB)], $*P < 0.05$ vs. U-46619 (post-CPB); $n = 8-11$ /group.

Figure 3.

(A) Exposure of U-46619 to peripheral arterioles of pre-CPB with or without the presence of PLA inhibitor, Quanacrine [U-46619 (Pre-CPB) or Quacrine + U-46619 (pre-CPB)]; (B) Exposure of U-46619 to peripheral arterioles of post-CPB with or without the presence of Quanacrine [U-46619 (Post-CPB) or Quacrine + U-46619 (post-CPB)] n = 7–11/group,

Figure 4.

(A) Administration of U-46619 to the vessels from pre-cardioplegia followed by reperfusion (pre-CPB) samples in the absence or presence of PKC inhibitor safingol [U-46619 (pre-CPB) or Safingol + U-46619 (pre-CPB)], (B) Administration of U-46619 to the vessels from post-CPB samples in the absence or presence of PKC safingol [U-46619(post-CPB) or Safingol + U-46619 (post-CPB)]; n= 7–11/group.

Figure 5. Representative immunoblot of skeletal muscle tissue (top). Lanes 1-12 loaded with 40 μ g protein were developed for TXA-2 receptor, and TXA-2 synthase. Immunoblot band intensity shows unaltered levels of TXA-2 receptor, and TXA-2 synthase after CPB (bottom); (n = 6/group).

Figure 6. Gene expression of TXA-2 receptor and TXA-2 synthase of the pre- and post-CPB skeletal-muscle samples (n = 5/group).

Figure 7.

Immuno-localization of TXA-2 receptor, and PLC β -3 polypeptides in human peripheral microvessels (n = 6). Vessels were co-stained for smooth muscle alpha-actin with PLC β -3 antibody. Matched negative controls are displayed below each row of primary antibody.

Table 1

Patient Characteristics

	Values
Number of patients	28
Age (years)	73 ± 5
Sex (male/female)	21/7
Hypertension (n)	20/8
Hypercholesterolemia (n)	22/6
Diabetes mellitus (n)	0
Duration of CPB (min)	97 ± 5
Cross-clamp time (min)	86 ± 6
Atrial fibrillation (n)	5
Number of grafts performed [†]	3(3,4)
Redo Case	0
Surgical procedure	CABG

CPB = cardiopulmonary bypass;

[†] Data expressed as median (minimum, maximum).

Table 2

CPB Induced Gene Expression

Symbol	Entrez Gene Name	Fold change	-log (P) (>4.14 is significant)	Location	Type
ADAMTS9	ADAM metalloproteinase with thrombospondin type 1 motif, 9	1.439	4.930	Extracellular Space	Peptidase
AKAP12	A kinase (PRKA) anchor protein 12	1.408	5.004	Cytoplasm	Transporter
APOLD1	apolipoprotein L domain containing 1	1.508	4.526	Unknown	Other
BCL3	B-cell CLL/lymphoma 3	1.080	4.352	Nucleus	Transcription regulator
CCNL1	cyclin L1 CCAAT/enhancer	1.523	4.749	Nucleus	Other
CEBPD	binding protein (C/EBP), delta	1.272	4.157	Nucleus	Transcription regulator
CHSY1	chondroitin sulfate synthase 1	1.094	4.485	Cytoplasm	Enzyme
CSRNP1	cysteine-serine-rich nuclear protein 1	1.761	5.809	Nucleus	Other
CXCL1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	1.776	4.725	Extracellular Space	Cytokine
CXCL2	chemokine (C-X-C motif) ligand 2	1.898	4.235	Extracellular Space	Cytokine
EMPI	epithelial membrane protein 1	1.203	5.631	Plasma Membrane	Other
GADD45A	growth arrest and DNA-damage- inducible, alpha	1.778	4.149	Nucleus	Other
GADD45B	growth arrest and DNA-damage- inducible, beta	1.984	4.958	Cytoplasm	Other
GCH1	GTP cyclohydrolase 1	2.017	5.983	Cytoplasm	Enzyme
ICAM1	intercellular adhesion molecule 1	1.055	4.176	Plasma Membrane	Transmembrane receptor
IL6	interleukin 6 (interferon, beta 2)	2.311	4.332	Extracellular Space	Cytokine
IL8	interleukin 8	2.940	4.305	Extracellular Space	Cytokine
INSIG1	insulin induced gene 1	1.360	5.194	Cytoplasm	Other
JMJD1C	jumonji domain containing 1C	1.500	4.565	Unknown	Other
KIAA0146	KIAA0146	1.447	6.702	Unknown	Other
LDLR	low density lipoprotein receptor	1.911	5.135	Plasma Membrane	Transporter
LOC38773	hypothetical protein LOC387763	1.812	5.005	Unknown	Other
MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F	2.757	5.193	Nucleus	Transcription regulator
MAP3K8	mitogen-activated protein kinase kinase kinase 8	1.434	4.924	Cytoplasm	Kinase
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	2.273	4.867	Nucleus	Transcription regulator
NAMPT	nicotinamide phosphoribosyltransferase	1.119	4.263	Extracellular Space	Cytokine

Symbol	Entrez Gene Name	Fold change	-log (P) (>4.14 is significant)	Location	Type
NFIL3	nuclear factor, interleukin3 regulated	1.424	4.228	Nucleus	Transcription regulator
NP	nucleoside phosphorylase	1.731	4.738	Nucleus	Enzyme
PIM1	pim-1 oncogene	1.202	4.953	Cytoplasm	Kinase
PLA2G4C	phospholipase A2, group IVC(cytosolic, calcium-independent)	-1.094	6.230	Cytoplasm	Enzyme
PPP1R3C	protein phosphatase 1, regulatory(inhibitor), subunit 3C	-1.784	6.095	Cytoplasm	Phosphatase
RASA4(includes EG:10156)	RAS p21 protein activator 4	-1.116	4.441	Cytoplasm	Other
RGS16	regulator of G-protein signaling 16	1.307	4.148	Cytoplasm	Other
SIK1	salt-inducible kinase 1	2.042	4.748	Cytoplasm	Kinase
SLC22A4(includes EG:6583)	solute carrier family 22 (organic cation/ergothioneine transporter), member 4	1.210	4.397	Plasma Membrane	Transporter
SLC2A3	solute carrier family 2(facilitated glucose transporter), member 3	2.288	4.790	Plasma Membrane	Transporter
SOC2	suppressor of cytokine signaling 2	1.820	4.222	Cytoplasm	Other
SOC3	suppressor of cytokine	2.071	4.748	Cytoplasm	Other
TGIF1	TGFB-induced factor homeobox 1	1.476	4.625	Nucleus	Transcription regulator
THBD	thrombomodulin	2.368	5.692	Plasma Membrane	Transmembrane receptor
UGCG	UDP-glucose ceramide glucosyltransferase	1.346	4.425	Cytoplasm	Enzyme