

*Full Length Research Paper*

## Gene expression profiles in adenosine-treated human mast cells

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Accepted 24 June, 2011

The role of mast cells in allergic diseases and innate immunity has been widely researched and much is known about the expression profiles of immune-related genes in mast cells after bacterial challenges. However, little is known about the gene expression profiles of mast cells in response to adenosine. Herein, we profiled the transcriptome changes of human mast cells treated with adenosine. To perform comparative transcriptome analysis between adenosine-untreated control mast cells (MN) and adenosine-treated mast cells (MT), two independent cDNA libraries were constructed using the 5'-oligo-capping method. Analysis of the 3,968 (MN, 1,994; MT, 1,974) expression sequence tags (ESTs) generated from these libraries identified 369 contigs (MN, 189; MT, 180) and 2,655 singletons (MN, 1,289; MT, 1,366) with average lengths of 668 and 655 bp, respectively. Furthermore, comparison of our EST sequences against the eukaryotic orthologous group (KOG) database showed that 2,134 (52.92%) out of 4,032 sequences could be grouped into three major functional categories. As a result of analysis at the individual level of the genes, we found that the expression of genes encoding Pdia (protein disulfide isomerase-associated), adaptor-related protein complex, ATP-dependent DNA helicase II, cyclin M4, reticulon 3 isoform, CD37 antigen isoform A, glutamine synthetase, WD repeat domain, programmed cell death and proliferating cell nuclear antigen increased by 4-fold. In contrast, the expression of genes encoding thymosin beta 4, ring finger protein, high-mobility group, calmodulin 2, RAN binding protein, solute carrier family 25, tubulin alpha and peroxiredoxin decreased by 4-fold. Information obtained from our study will enhance the understanding of defense mechanisms associated with innate immune responses by human mast cells, for which identification of immune regulators of those genes is required.

**Key words:** Mast cells, adenosine, expression sequence tags (EST).

## INTRODUCTION

Adenosine is a purine nucleoside which is formed in the extracellular space by two pathways. One is that adenosine is released into the extracellular space via nucleoside transporter from the cytoplasm of the adenosine-producing cell where ATP is converted to adenosine by 5'-nucleotidase. Adenosine can also be formed by conversion of ATP to AMP by CD39 (nucleoside triphosphate dephosphorylase), and conversion of AMP to adenosine by CD73 (5'-ectonucleotidase) in the extracellular space (Hasko and Cronstein, 2004). It is known that increased adenosine in the extracellular space binds to adenosine receptors (A1, A2a, A2b and A3), and act as an endogenous modulator of innate immunity in mast cells (Kumar and Sharma, 2009).

Mast cells play a critical role in host innate immune responses associated with either IgE or bacterial infection, and in the pathogenesis of arthritis and asthma (Lu et al., 2006). Many researchers have attempted to study comparative gene expression patterns of various immune-related genes from mast cells. For example, immune-related genes are up-regulated in response to *Escherichia coli* in mast cells (Kulka et al., 2006). In addition, the expression level of Fc epsilon RI was down-regulated by lipoteichoic acid (LTA) and peptidoglycan (PGN) in human mast cells (Yoshioka et al., 2007). Furthermore, gene expression patterns during Fc epsilon RI activation were analyzed in human mast cells (Wakahara et al., 2001). Finally, genome-wide analysis shows that inflammation-related genes (559 genes) are over-expressed in IgE-challenged mast cells (Jayapal et al., 2006).

Although, adenosine is known to play an important role as the regulator of innate immunity against bacteria in host immune cells, no report has been made on the expression profiles of mast cells challenged with adenosine (Hasko and Cronstein, 2004). In this context, we profiled the transcriptome changes of human mast cells sensitized with adenosine. We constructed a cDNA library for adenosine treated human mast cells and performed expression sequence tags (EST) analysis through control random sequence analysis. Our study will be helpful in screening immune-related genes and also in studying defense mechanisms associated with innate immune responses.

## MATERIALS AND METHODS

### Human mast cell culture and adenosine treatment

Human mast cells (HMC-1) was used for the entire study, and

maintained as previously reported (Butterfield et al., 1988). Briefly, HMC-1 was cultured in a total of 1000 ml of Iscove's modified maintained as previously reported (Butterfield et al., 1988). Briefly, HMC-1 was cultured in a total of 1000 ml of Iscove's modified Dulbecco's medium containing penicillin and streptomycin (100 units/ml), FBS 100 ml (10%), L-glutamine 10 ml (2mM) and mono-thioglycerol 0.947 µl. To examine differentially expressed genes between control and adenosine-treated mast cells, we treated HMC-1 mast cells ( $0.3 \times 10^6/150 \mu\text{l}$ ) with 80 µl of adenosine for three hour.

### cDNA library construction

Total RNA was isolated from both untreated and adenosine-treated mast cells using Trizol reagent. In addition, mRNA was further purified with mRNA purification kits Clontech. cDNA library was constructed using Stratagene cDNA Library Construction Kit. Both LD-PCR method and primer extension method were used to synthesize double-strand cDNA. After ligation to lambda TriplEx2 vector, Gigapack Gold Packaging System (Stratagene, La Jolla, Calif.) was used for packaging.

The cDNA library was constructed from cultured human mast cells using the ZAP Express cDNA Synthesis Kit [pBK-CMV vector]. Library titers were  $9.3 \times 10^6$  and  $8.7 \times 10^6$  in the experimental and the control group, respectively. In total, 2,016 of EST DNA were sequenced from the control (MN) and treatment (MT) group. Total schematic diagram of the EST analysis is shown in Figure 1. The cDNA size fractionation was performed for large-insert collection (>500 bp). Insert size was estimated by screening cDNA library using PCR. Following titers and insert size estimation, 33 out of 48 (69%) were screened in the experimental group (MT) and 34 out of 48 (71%) were screened in the control group (MN). We also confirmed the insert size by gel electrophoresis after plasmid DNA preparation.

### Sequencing

Lambda TriplEx2 (linear form) was converted to pTriplEx2 TriplEx2 (circular form) using *E. coli* strain BM25.8. After plating on LB agar plates with carbenicillin and kanamycin used for each of the vector system. it was cultured overnight at 37°C. Plasmid DNAs were isolated using NucleoGen plasmid purification kit and sequenced using ABI-3730XL (Applied Biosystems).

### Sequence analysis

For comparative analysis of transcripts between control and adenosine treated cells, we constructed 2 types of full-length enriched cDNA libraries by using the 5'-oligo-capping method, and determined 4,032 clones by 5' end-single path sequencing. The chromatogram files obtained from the automatic sequencer were initially submitted to Phred program for base calling and quality assignment (Ewing and Green, 1998a; Ewing et al., 1998b). The trace files were trimmed with trim-alt 0.05 (P-score>20).

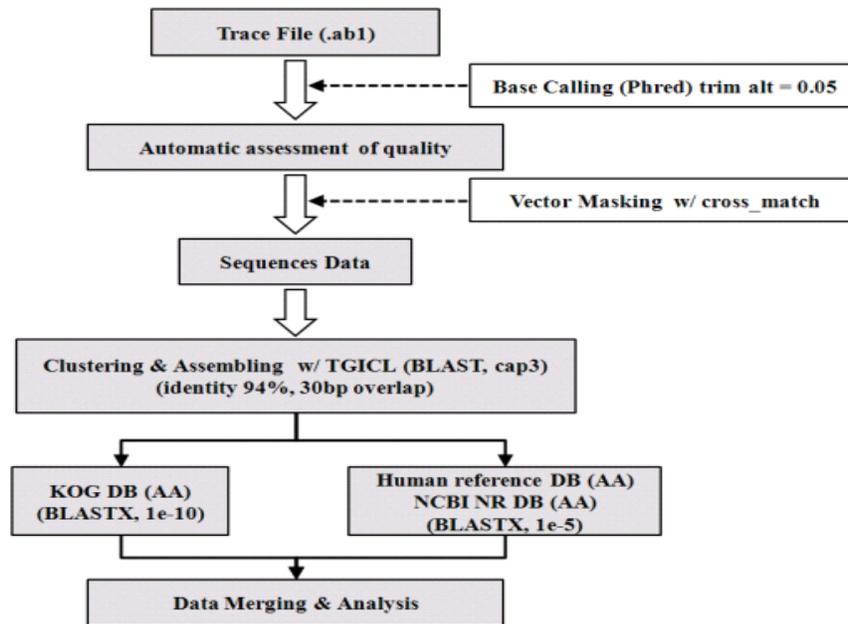
In addition, vector trimming was conducted with cross-match software. Each gene expression pattern was analyzed by clustering (30 bp or more 94% homology) and assembly. The cleaned data were finally analyzed with NCBI local BLAST. Sequences were searched against the NCBI human reference database (release 4).

### EST annotation

Each sequence of control and experimental group was prepared as

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**Figure 1.** Schematic diagrams of EST analysis in human mast cells.

a multi-fasta format for sequence analysis and the annotation was performed through local BLAST search (Altschul et al., 1990). Local BLAST search using the NCBI nr database was performed with data not retrieved from the human reference sequence database. The function of genes was analyzed by KOG (Clusters of orthologous groups for eukaryotic complete genomes) (Tatusov et al., 2003).

#### Genbank registration

The genes were registered at the NCBI Genbank by Sequin programs and Perl script after changing format for gene registration (Genbank accession number: BY993651 - BY997618).

## RESULTS

### Sequence analysis and annotation

The chromatogram files (4,032 clones) obtained from the automatic sequencer were pre-processed to determine the sequence quality and cloning vector sequences were removed from the reads using Phred and Cross-match software (<http://www.phrap.org>) (Ewing and Green, 1998a; Ewing et al., 1998b).

The trace files were trimmed with trim-alt 0.05 (P-score>20) condition by Phred software. Furthermore, analysis of the 3,968 (MN, 1,994; MT, 1,974) ESTs generated from these libraries identified 369 contigs (MN, 189; MT, 180) and 2,655 singletons (MN, 1,289; MT, 1,366) with average lengths of 668 and 655 bp,

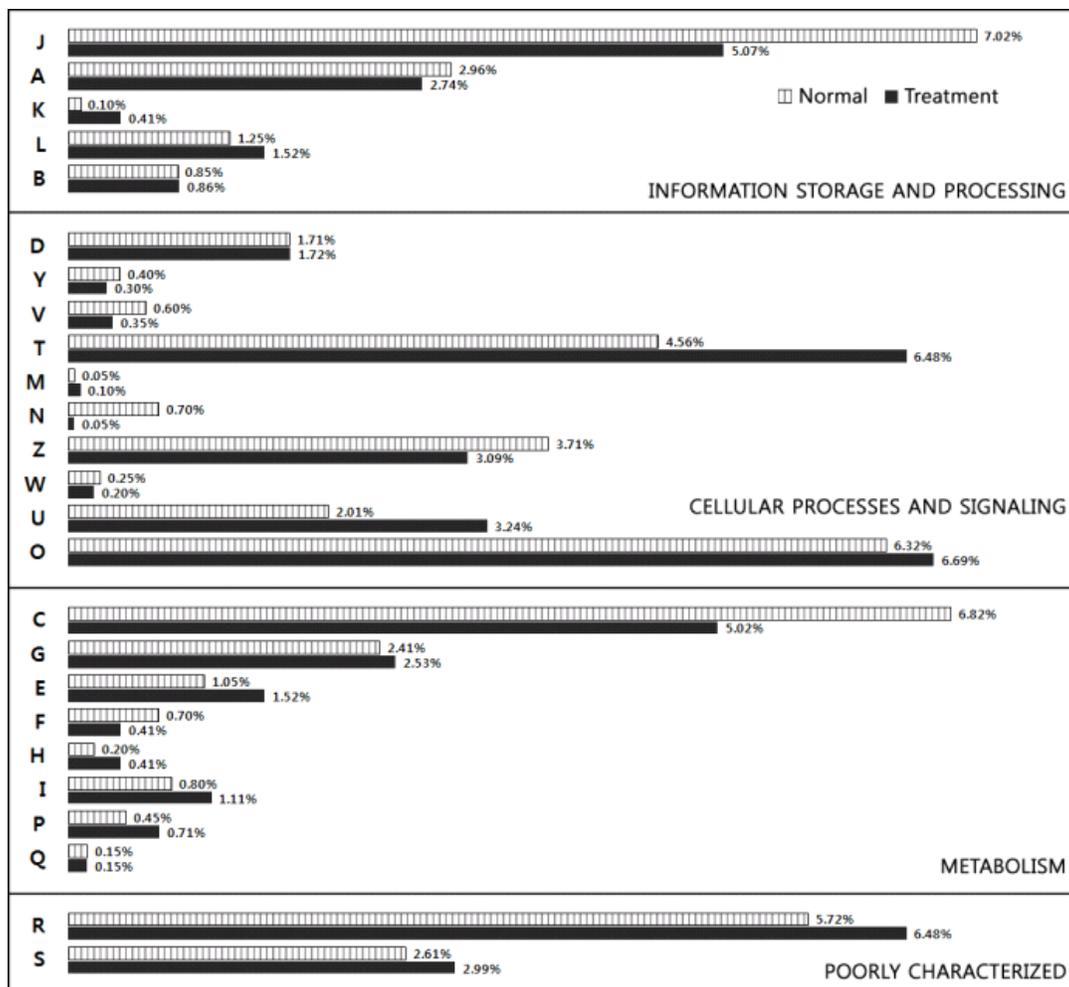
respectively.

All the sequences derived from adenosine-untreated group and adenosine-treated group were compared against the NCBI human reference sequence database using the BLASTX algorithm with a cut-off E-value of <math>e-10</math>. As a result, 1,317 of sequences in the MN group and 1,320 of sequences in the MT group had homologous sequences in the database. In addition, sequences lacking any significant sequence matches were further analyzed using the "nr" database as described earlier, resulting in 81 and 85 additional sequence matches in the control group and the treated group, respectively.

### KOG analysis

In order to predict the gene function analysis was performed (Figure 2). The gene function of two group's sequences were predicted through local BLAST (blastx, E <math>e-10</math>) search against (the) KOG database. In total, 1,069 of sequences were annotated in the MT group, whereas 1,065 of sequences were annotated in the MN group (Table 1).

KOG analysis showed that specific changes in gene expression occurred (Table 2). Genes that belong to the J code (translation, ribosomal structure and biogenesis) and C code (energy production and conversion) appear to be decreased in adenosine treated mast cells. On the other hand, genes belonging to T code (signal transduction mechanisms) and U code (intracellular



**Figure 2.** KOG analysis results of EST sequences in human mast cells. Code descriptions of KOG: J (Translation, ribosomal structure and biogenesis), A (RNA processing and modification), K (transcription), L (replication, recombination and repair), B (chromatin structure and dynamics), D (cell cycle control, cell division, chromosome partitioning), Y (nuclear structure), V (defense mechanisms), T (signal transduction mechanisms), M (cell wall/membrane/envelope biogenesis), N (cell motility), Z (cytoskeleton), W (extracellular structures), U (intracellular trafficking, secretion, and vesicular transport), O (posttranslational modification, protein turnover, chaperones), C (energy production and conversion), G (carbohydrate transport and metabolism), E (amino acid transport and metabolism), F (nucleotide transport and metabolism), H (coenzyme transport and metabolism), I (lipid transport and metabolism), P (inorganic ion transport and metabolism), Q (secondary metabolites biosynthesis, transport and catabolism), R (general function prediction only), S (function unknown).

trafficking, secretion and vesicular transport) appear to be significantly increased (Table 2). The up-regulated genes are listed in Table 3.

### Gene registration

Both the available sequences (1,974) retrieved from adenosine treatment group and the other sequences (1,994) retrieved from control group were submitted to NCBI Genbank site (Genbank accession number:

BY993651 - BY997618).

### DISCUSSION

In this study, we constructed two independent cDNA libraries from adenosine-treated human mast cells and untreated human mast cells. EST analysis was then conducted through control random sequence analysis to observe the changes of gene expression patterns by adenosine treatment. The success rate of library con-

**Table 1.** Results of sequence analysis and annotation.

Parameter	MN	MT
<b>Sequence analysis</b>		
The number of clones sequenced	2016	2016
The number of clones used for sequence analysis after sequencing (base calling (Phred $\geq$ 20) / vector masking / ESTs $\geq$ 100 bp)	1994	1974
<b>Clustering and assembling</b>		
The number of clusters	185	180
The number of contigs	189	180
The number of singletons	1289	1366
<b>Annotation</b>		
Against human reference database	1320	1317
Against NCBI 'nr' database	1398	1389

**Table 2.** Gene expression fluctuation after adenosine treatment by KOG analysis.

KOG code	MN		MT		Fluctuation
	Clone	%	Clone	%	
J	140	7.02	100	5.07	↓
A	59	2.96	54	2.74	↓
K	2	0.10	8	0.41	↑
L	25	1.25	30	1.52	↑
B	17	0.85	17	0.86	=
D	34	1.71	34	1.72	=
Y	8	0.40	6	0.30	↓
V	12	0.60	7	0.35	↓
T	91	4.56	128	6.48	↑
M	1	0.05	2	0.10	↑
N	14	0.70	1	0.05	↓
Z	74	3.71	61	3.09	↓
W	5	0.25	4	0.20	↓
U	40	2.01	64	3.24	↑
O	126	6.32	132	6.69	↑
C	136	6.82	99	5.02	↓
G	48	2.41	50	2.53	↑
E	21	1.05	30	1.52	↑
F	14	0.70	8	0.41	↓
H	4	0.20	8	0.41	↑
I	16	0.80	22	1.11	↑
P	9	0.45	14	0.71	↑
Q	3	0.15	3	0.15	=
R	114	5.72	128	6.48	↑
S	52	2.61	59	2.99	↑
other	929	46.59	905	45.85	
total	1994	100	1974	100	

**Table 3.** The list of up-regulated genes (KOG (Eukaryotic clusters of orthologous groups) code and the number of treatment and control group).

Gene	KOG	MN	MT
Protein disulfide isomerase-associated	O	1	7
Adaptor-related protein complex	U	1	6
ATP-dependent DNA helicase II	L	0	5
Cyclin M4	S	0	5
Reticulon 3 isoform	U	0	5
CD37 antigen isoform A	R	0	4
Glutamine synthetase	E	0	4
WD repeat domain	R, T	0	4
Programmed cell death	T	1	4
Proliferating cell nuclear antigen	L	1	4
Nodal modulator	O	3	9
B-cell receptor-associated protein 31	V	0	3
BUB3 budding uninhibited by benzimidazoles 3 isoform	D	0	3
CD63 antigen isoform A	R	0	3
Family with sequence similarity	R	0	3
Methylosome protein 50	A	0	3
Resistance to inhibitors of cholinesterase 8 homolog A	T	0	3
Tryptophanyl-tRNA synthetase isoform a	J	0	3
ADP-ribosylation factor	U	2	5
Heat shock protein 90kDa alpha	O	2	5
Histidine decarboxylase	E	5	12
Triosephosphate isomerase 1	G	4	8
Adenylyl cyclase-associated protein	Z	3	6
Rho GDP dissociation inhibitor (GDI) beta	T	4	7
NADH dehydrogenase subunit	C	20	26
Hypothetical protein LOC55652	R, T	4	9
PREDICTED: similar to piwi-like 2	R, T	4	8

struction was 98.9% in the control group and 97.9% in the treatment group with the average insert size of 657 bp. These results indicate that cDNA library construction of both adenosine-untreated and treated human mast cells were very successful.

As shown in Figure 2, there are four groups such as "information storage and processing", "cellular process and signaling", "metabolism" and "poorly characterized". In the group of "information storage and processing", the genes involved in transcription (K category) tend to be increased by approximately 4-fold. In addition, in the group of cellular processes and signaling, the genes that play a critical role in M category (cell wall/membrane/envelope biogenesis) were increased by 2-fold. At the level of individual genes, genes induced by more than 4-fold were identified as follows: protein disulfide isomerase-associated (Pdia), adaptor-related

protein complex, ATP-dependent DNA helicase II, cyclin M4, reticulon 3 isoform, CD37 antigen isoform A, glutamine synthetase, WD repeat domain, programmed cell death and proliferating cell nuclear antigen.

Of these genes, Pdi is known to act as a chaperone and also plays an important role in catalyzing disulfide bond rearrangement (Gruber et al., 2006; Wilkinson and Gilbert, 2004). In particular, Pdi is essential for facilitating bacterial (Conant et al., 2007) and viral infection (Abell and Brown, 1993). Furthermore, Pdi is involved in phagocytosis of *Leishmaniachagasi* (Santos et al., 2009). In this context, it would be very interesting to further study the precise role of protein disulfide isomerase (Pdi) in mast cells in conjunction with pathogen-mast cell interactions.

In contrast, category analysis shows that the F category (nucleotide transport and metabolism) in group of

**Table 4.** The list of down-regulated genes (KOG code and the number of treatment and control group).

Gene	KOG	MN	MT
Thymosin, beta 4	N	14	1
Ring finger protein	O	5	1
High-mobility group	R	9	2
Calmodulin 2	T	4	1
RAN binding protein	Y	4	1
Solute carrier family 25	C, F	4	1
Tubulin, alph	Z	4	1
Peroxiredoxin	O	6	2
Cleavage stimulation factor subunit	A	3	0
Decay accelerating factor for complement	T	3	0
Nucleoporin 98kD	D, Y	3	0
Oxidase (cytochrome c) assembly 1-like	O	3	0
Ribosomal protein S4, X-linked X isoform	J	3	0
Serine/arginine repetitive matrix 1	A	3	0
Ubiquitin C	O	3	0
Tubulin, beta	Z	8	3
Splicing factor, arginine/serine-rich	A, R	5	2
Aldolase A	G	7	3
ATP synthase F0 subunit 6	C	6	3
Cytochrome b	C	13	8
Cytochrome c	C	71	44
Ribosomal protein L6	J	11	2
Ribosomal protein L14	J	8	1
Hypothetical protein LOC65996	O, T	3	0
Ribosomal protein S7	J	3	0
Ribosomal protein SA	J	3	0
Ribosomal protein P0	J	15	10

KOG, Eukaryotic clusters of orthologous groups.

“metabolism” was slightly decreased by 1.8-fold, whereas N category (cell motility) was dramatically decreased by 14-fold. At the level of individual genes, many genes that decreased by more than 4-fold were identified. They include thymosin beta 4, ring finger protein, high-mobility group, calmodulin 2, RAN binding protein, solute carrier family 25, tubulin alpha and peroxiredoxin. Of these genes, thymosin beta-4 was significantly reduced by 14-fold in response to adenosine treatment (Table 4). Thymosin beta-4 is largely responsible for sequestration of G-actin protein in motile and in proliferating cells (Yu et al., 1993, 1994). Many studies have implicated thymosin beta-4 in a number of cellular events, such as angiogenesis, wound healing, hair growth, apoptosis and inflammatory responses (Iguchi et al., 1999; Niu and Nachmias, 2000; Philp et al., 2004, 2007; Smart et al.,

2007; Young et al., 1999). Among the various functions of thymosin beta-4, involvement of inflammatory responses may be related to down-regulation of thymosin beta-4 by the treatment with adenosine in mast cells. Thymosin beta-4 shows an anti-inflammatory effect and is highly expressed in immune cells including monocyte and mast cells (Young et al., 1999). It also suggests that the down-regulation of adenosine-mediated thymosin beta-4 is related to the immune response in host to parasites.

In conclusion, we have conducted and identified the genes that are differentially regulated in mast cells in response to adenosine, the endogenous immune elicitor. This study will be helpful in screening for new and potential immune response target regulators based on gene expression regulation mechanism and also in studying mast cells associated with innate immune responses.

## ACKNOWLEDGEMENT

This work was supported by the 2005 Inje University research grant.

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