Original Article

Gene expression profile of THP-1 cells treated with heat-killed *Candida albicans*

Zhi-De Hu[1,2]*, Ting-Ting Wei[1]*, Qing-Qin Tang[1], Ning Ma[1], Li-Li Wang[1], Bao-Dong Qin[1], Jian-Rong Yin[1], Lin Zhou[1], Ren-Qian Zhong[1]

[1]Department of Laboratory Medicine, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China; [2]Department of Laboratory Medicine, General Hospital, Ji'nan Military Region of PLA, Ji'nan 250031, China

Contributions: (I) Conception and design: RQ Zhong, L Zhou, ZD Hu; (II) Administrative support: RQ Zhong; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*These authors contributed equally to this work.

Correspondence to: Ren-Qian Zhong, MD, PhD. Department of Laboratory Medicine, Changzheng Hospital, the Second Military Medical University, Shanghai 200003, China. Email: 13901628473@163.com; Lin Zhou, MD, PhD. Department of Laboratory Medicine, Changzheng Hospital, the Second Military Medical University, Shanghai 200003, China. Email: lynnzhou36@163.com.

Background: Mechanisms under immune response against *Candida albicans* (*C. albicans*) remain largely unknown. To better understand the mechanisms of innate immune response against *C. albicans*, we analyzed the gene expression profile of THP-1 cells stimulated with heat-killed *C. albicans*.

Methods: THP-1 cells were stimulated with heat-killed *C. albicans* for 9 hours at a ratio of 1:1, and gene expression profile of the cells was analyzed using Whole Human Genome Oligo Microarray. Differentially expressed genes were defined as change folds more than 2 and with statistical significance. Gene ontology (GO) and pathway analysis were used to systematically identify biological connections of differentially expressed genes, as well as the pathways associated with the immune response against *C. albicans*.

Results: A total of 355 genes were up-regulated and 715 genes were down-regulated significantly. The up-regulated genes were particularly involved in biological process of RNA processing and pathway of the spliceosome. In case of down-regulated genes, the particularly involved immune-related pathways were G-protein coupled receptor signaling pathway, calcium signaling pathway, MAPK signaling pathway and Ras pathway.

Conclusions: We depict the gene expression profile of heat-killed *C. albicans* stimulated THP-1 cells, and identify the major pathways involved in immune response against *C. albicans*. These pathways are potential candidate targets for developing anti-*C. albicans* agent.

Keywords: Gene expression profile; microarray; THP-1 cells; *Candida albicans* (*C. albicans*)

Submitted Dec 14, 2015. Accepted for publication Mar 08, 2016.
doi: 10.21037/atm.2016.05.03
View this article at: http://dx.doi.org/10.21037/atm.2016.05.03

Introduction

In healthy individuals, *Candida albicans* (*C. albicans*) is harmless and acts as a part of the normal flora in alimentary tract and mucocutaneous membranes. However, in immune comprised individuals, *C. albicans* can cause opportunistic infections named candidiasis, with symptoms ranging from superficial lesions to fatal systemic disease (1).

Innate immunity is the first line of immune system to defense against *C. albicans*. After sensing the pathogen-associated molecular patterns (PAMPs) on surface of *C. albicans*, the innate immune cells (e.g., monocyte, macrophage, and dendritic cell) are immediately activated and the inflammatory response is initiated. In the infection site, activated innate immune cells release inflammatory factors, such interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α), which can promote the clearance of

© Annals of Translational Medicine. All rights reserved.

C. albicans in early phase (2,3). In addition, the antigens of *C. albicans* are processed and presented to T cells by antigen presenting cells (APCs), and then the adapt immune response against *C. albicans* is initiated. The type and strength of adapt immune response are shaped and programmed by APCs released cytokines, such as IL-12, IL-23, transforming growth factor (TGF)-β (4). Actually, some of the innate immune cells (e.g., monocytes, macrophages, and dendritic cells) are also acted as APCs in the immune response. Taken together, the activation of innate immune cells is a crucial step in anti-*C. albicans* innate and adapt immune responses.

Currently, the molecular mechanisms under the production of inflammatory factors and cytokines are not fully understood. Two studies have investigated the gene expression profile of monocyte treated with *C. albicans*, but biological information analysis was not performed. To better understand the mechanisms of innate immune cell activation, we analyzed the gene expression profile of heat-killed *C. albicans* stimulated THP-1 cell, a widely used monocytic human cell line.

Methods

Cell culture and stimulation

THP-1 cells were obtained from the American Type Culture Collection (Manassas, VA). Log-phase cells were cultured in RPMI 1640 medium (HyClone, Logan, UT) containing 10% FBS (v/v) (Gibicol, Carlsbad, CA, USA), 100 U/mL penicillin-streptomycin (Mediatech). The cells were cultured at the concentration of 10^6 in a 6-well plate.

C. albicans was suspended in a PBS solution, washed three times and heat killed at 100 degrees for 30 minutes. Heat-killed *C. albicans* was added to THP-1 cells at a ratio of 1:1. PBS solution was used as a control. Nine hours later, cells were harvest and RNA was extracted.

Microarray analysis

The RNA of heat-killed *C. albicans* stimulated THP-1 cells was extracted by Trizol (Invitrogen, Carlsbad, CA) following manufacturer's protocols. RNA quantity and quality were measured using NanoDrop ND-1000. RNA integrity was assessed by a standard denaturing agarose gel electrophoresis.

The Whole Human Genome Oligo Microarray was a broad view that represents all known genes and transcripts in the human genome. Sequences were compiled from a broad source survey, and then verified and optimized by alignment to the assembled human genome. The microarray analysis was performed and analyzed as previously described (5). Briefly, RNA from each sample was linearly amplified and labeled with Cy3-UTP. An RNeasy Mini Kit (Qiagen) was used to purify the labeled cRNAs. The specific activity and concentration of the labeled cRNAs (pmol Cy3/μg cRNA) were measured by NanoDrop ND-1000. A total of 1 μg of each labeled cRNA was fragmented by adding 11 μL 10× Blocking Agent and 2.2 μL of 25× Fragmentation Buffer, then the mixture was heated at 60 ℃ for 30 min, and 55 μL 2× GE Hybridization buffer was then added to dilute the labeled cRNA. A total of 100 μL of hybridization solution was dispensed into the gasket slide and assembled to the gene expression microarray slide. The slides were put in an Agilent Hybridization Oven incubated for 17 hours at 65 ℃. The hybridized arrays were washed, fixed and scanned with using the Agilent DNA Microarray Scanner (part number G2505C).

Data analysis

We analyzed the acquired array images using Agilent Feature Extraction software (version 11.0.1.1). Quantile normalization and subsequent data analysis were performed using the GeneSpring GX v12.1 software package (Agilent Technologies). Genes that at least 3 out of 6 samples have flags in detected were used for further analysis. Differentially expressed genes were defined as change folds more than 2 and with statistical significance ($P<0.05$). Scatter plot and heatmap were used to depict the differentially expressed genes. GO analysis and Pathway analysis were performed in the standard enrichment computation method.

Results

RNA quantity and quality

As shown in *Figure 1*, RNA extracted from THP-1 cells was intact. The concentration of RNA ranged from 617 to 1,177 ng/μL.

Gene expression microarrays

We found 1,070 differentially expressed genes, of which 355 were up-regulated and 715 were down-regulated [for more details, please contact the corresponding authors or Dr. Zhi-De Hu (hzdlj81@163.com)]. Scatter plot of detected genes is showed in *Figure 2A*, and a heatmap of differentially expressed genes is showed in *Figure 2B*. *Table 1*

© Annals of Translational Medicine. All rights reserved.

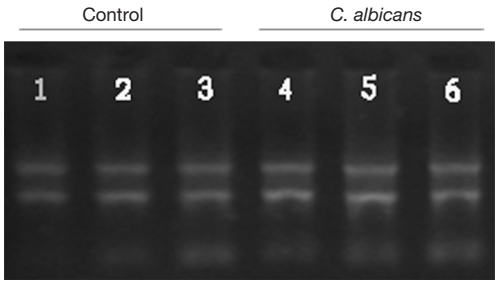

Figure 1 RNA quantity and quality.

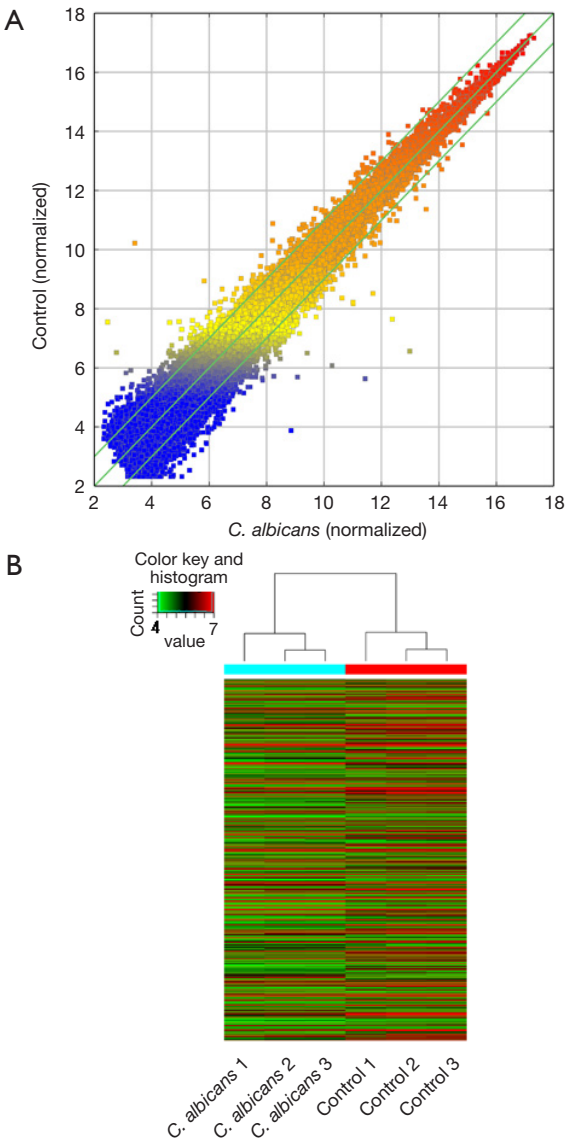

Figure 2 Scatter plot (A) and heatmap (B) depicting the differentially expressed genes.

Table 1 Top 10 differentially expressed genes

Gene symbol	GenBank accession	Fold change*	P value
CD86	NM_006889	+112.5	<0.01
SPEN	NM_015001	+34.2	0.01
PPP6R1	NM_014931	+13.5	<0.01
EYA2	NM_005244	−6.9	0.01
LRRC2	NM_024512	+6.7	0.01
GOLT1A	NM_198447	−6.6	<0.01
SCAF4	NM_020706	+6.3	0.01
FCRL2	NM_030764	−5.7	0.02
ALDOB	NM_000035	−5.3	0.02
RNU105B	NR_004386	−5.3	<0.01

*, – and + indicate down- and up-regulation, respectively.

lists top ten differentially expressed genes.

Gene ontology (GO) and pathway analysis

GO analysis showed that up-regulated genes were particularly involved in biological process of RNA processing (*Figure 3A*). Consistent with the GO results, pathway analysis also indicated that the up-regulated genes were critically involved in pathway of spliceosome and carbon metabolism (*Figure 3B*). In the case of down-regulated genes, the most significant involved immune-related biological process was G-protein coupled receptor signaling pathway (*Figure 3C*), followed by some terms of sensory perception of taste. Among the top ten down-regulated gene involved pathways, three were closely related to immune response (calcium signaling pathway, MAPK signaling pathway and Ras pathway, *Figure 3D*).

Discussion

Response against *C. albicans* is initiated by innate immune cells. The strength, duration, and type of the immune response against *C. albicans* are largely affected by these innate immune cells. Therefore, it is interesting and valuable to investigate the mechanisms under the activation of innate immune cells. In present study, we depict the gene expression profile of heat-killed *C. albicans* stimulated

© Annals of Translational Medicine. All rights reserved.

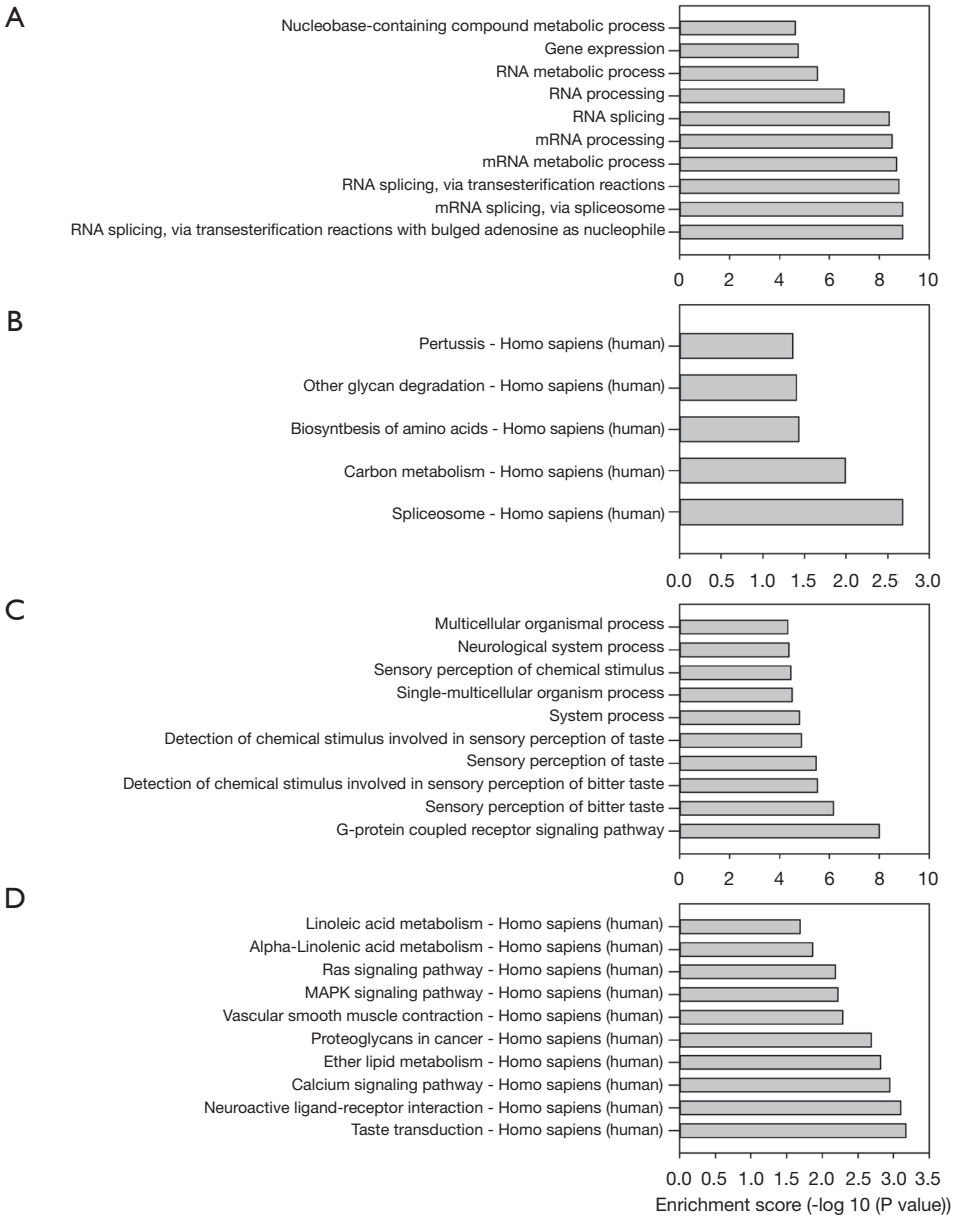

Figure 3 GO and pathway analysis of differentially expressed genes. GO (A) and pathway (B) analysis for up-regulated genes. GO (C) and pathway (D) analysis for down-regulated genes. GO, gene ontology.

THP-1 cell, a widely used monocytic human cell line. A total of 355 genes were up-regulated and 715 genes were down-regulated.

Compared with previous study that investigated the gene expression profile of THP-1 cells stimulated with *C. albicans* (6), one strength of present study is that we performed GO and pathway analysis to systematically identify biological connections of differentially expressed genes, as well as the pathways associated with the immune response against *C. albicans*. We found that the differentially expressed genes were involved in many pathways related to immune response. For example, we found that the down-regulated genes were involved in mitogen-activated protein kinases (MAPKs) signaling pathway. MAPK is a family of serine/threonine kinases that participates in pathogen-derived signal transduction

© Annals of Translational Medicine. All rights reserved.

events. Three members of this family have been identified: p38 MAPK, extracellular signal-regulated kinases (ERK, also termed classic MAPK pathway), and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK). Previous studies have shown that *C. albicans* could activate classic MAPK signaling pathway in J744 cell line (7) and human monocytes (8), which could promote the phagocytosis of *C. albicans* and inhibit IL-12 production. In addition, Dectin-1 has been reported to be one of the pattern recognition receptor in innate immune sensing of *C. albicans* (9). Jia *et al.* (10) found that *C. albicans* could activate ERK through Dectn-1 and H-Ras, which is involved in the releasing of inflammatory factors. We found that the genes downregulated in MAPK pathway contain MAP kinase-specific phosphatase (MKP), protein tyrosine phosphatases (PTPs) and protein kinase C (PKC) et al. It has been reported that MKP and PTP were critically involved in the regulation of MAPK pathway (11). Therefore, we speculate that the down-regulation of MKP and PTP may be attributed to the activation of MAPK pathway, which may promote the production of inflammatory factors.

One weakness of the present study is that we only depict the gene expression profile of heat killed *C. albicans* treated THP-1 cells using microarray. Reverse transcription PCR (RT-PCR) and western blot were not used to validate the microarray results. Because false positive or negative results are not avoidable in microarray analysis, further studies are needed to validate the results of the microarray.

In summary, present study depicted the gene expression profile of heat killed *C. albicans* treated THP-1 cells and identified major pathways involved in the immune response against *C. albicans*. These pathways are potential targets for developing anti-*C. albicans* agent.

Acknowledgements

The authors would like to thank Kangchen Bio-tech Inc. (Shanghai, China) for technical assistance.
Funding: This work was supported by a grant from the National Natural Science Foundation of China (Grant Number 81302541; 81471608).

Cite this article as: Hu ZD, Wei TT, Tang QQ, Ma N, Wang LL, Qin BD, Yin JR, Zhou L, Zhong RQ. Gene expression profile of THP-1 cells treated with heat-killed *Candida albicans*. Ann Transl Med 2016;4(9):170. doi: 10.21037/atm.2016.05.03

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the institutional ethic review board and informed consent was obtained from all patients.

References

1. Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. Virulence 2013;4:119-28.
2. Gil ML, Gozalbo D. Role of Toll-like receptors in systemic Candida albicans infections. Front Biosci (Landmark Ed) 2009;14:570-82.
3. Gozalbo D, Gil ML. IFN-gamma in Candida albicans infections. Front Biosci (Landmark Ed) 2009;14:1970-8.
4. Romani L. Immunity to fungal infections. Nat Rev Immunol 2011;11:275-88.
5. Zhou J, Gao J, Liu Y, et al. Human atrium transcript analysis of permanent atrial fibrillation. Int Heart J 2014;55:71-7.
6. Barker KS, Liu T, Rogers PD. Coculture of THP-1 human mononuclear cells with Candida albicans results in pronounced changes in host gene expression. J Infect Dis 2005;192:901-12.
7. Ibata-Ombetta S, Jouault T, Trinel PA, et al. Role of extracellular signal-regulated protein kinase cascade in macrophage killing of Candida albicans. J Leukoc Biol 2001;70:149-54.
8. Tang N, Liu L, Kang K, et al. Inhibition of monocytic interleukin-12 production by Candida albicans via selective activation of ERK mitogen-activated protein kinase. Infect Immun 2004;72:2513-20.
9. Netea MG, Brown GD, Kullberg BJ, et al. An integrated model of the recognition of Candida albicans by the innate immune system. Nat Rev Microbiol 2008;6:67-78.
10. Jia XM, Tang B, Zhu LL, et al. CARD9 mediates Dectin-1-induced ERK activation by linking Ras-GRF1 to H-Ras for antifungal immunity. J Exp Med 2014;211:2307-21.
11. Keyse SM. Protein phosphatases and the regulation of mitogen-activated protein kinase signalling. Curr Opin Cell Biol 2000;12:186-92.

© Annals of Translational Medicine. All rights reserved.

Supplementary

Proteotypic	*P-value and FDR	*Fold change and Regulation	Uniprot accession	Genbank accession	Gene symbol	Description	GO	Interactome	Pathway annotation	Uniform TF	Cofactor	Enzyme ID	Gene name	PMID	Sequence	Group - Low intensity Median	Group - Low intensity Quartile 1	Group - Low intensity Quartile 3	Group - High intensity Median	Group - High intensity Quartile 1	Group - High intensity Quartile 3	Normalized intensities								
P-value	FDR	Fold change	Regulation																			1N1	1N2	1N3	2N1	2N2	2N3	3N1	3N2	3N3
0.JTK_P00170	0.00000847	0.00000000	1.49E08E	Up	NM_207310	AK1	CAR1 NM001146051	ENSG00000	Homo sapiens SLC16A8 (Rho-GTPase-activating protein 26 (GRAF26), mRNA (cDNA) [DQ127475]							0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
0.JTK_P00170	0.00000847	0.00000000	1.49E08E	Up	NM_207310	AK1	CAR1 NM001146051	ENSG00000	Homo sapiens cDNA FLJ41800 fis, clone TESTI2000239, weakly similar to ADENYLATE KINASE 1 [AK1]							0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
0.JTK_P00170	0.00000847	0.00000000	1.49E08E	Up	NM_207310	AK1	CAR1 NM001146051	ENSG00000	Homo sapiens mRNA (cDNA clone MGC:110928 IMAGE:5270311), complete cds [BC130466]							0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
0.JTK_P00170	0.00000847	0.00000000	1.49E08E	Up	NM_207310	AK1	CAR1 NM001146051	ENSG00000	Homo sapiens cDNA: FLJ21839 fis, clone HEP03893, weakly similar to ADENYLATE KINASE 1 [AK1]							0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
0.JTK_P00170	0.00000847	0.00000000	1.49E08E	Up	NM_207310	AK1	CAR1 NM001146051	ENSG00000	Homo sapiens mRNA for protease-activated receptor 1 (PAR1), complete cds [AJ000176]							0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
0.JTK_P00170	0.00000847	0.00000000	1.49E08E	Up	NM_207310	AK1	CAR1 NM001146051	ENSG00000	Homo sapiens mRNA for protease-activated receptor 1 (PAR1), complete cds [AJ000176]							0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
0.JTK_P00170	0.00000847	0.00000000	1.49E08E	Up	NM_207310	AK1	CAR1 NM001146051	ENSG00000	Homo sapiens cDNA FLJ45444 fis, clone TESTI4000175, weakly similar to ADENYLATE KINASE 1 [AK1

Probe name	T value and SNR	Fold change and regulation	Genome coordinates	Gene symbol	Gene name	Description	GO	Annotations	Refseq transcripts	Uniprot ID	Ensembl ID	Gene name	TSS	Sequence	Group 1 Max intensity	Group 1 Min intensity	Group 1 Average intensity	Group 1 Median intensity	Group 1 Standard deviation	Group 2 Max intensity	Group 2 Min intensity	Group 2 Average intensity	Group 2 Median intensity	Group 2 Standard deviation
2_10_P62259	0.00000007	1.12420548	1.49005696	hs	19q13.43	protein kinase C, eta	GO:0007205: protein kinase C-activating G-protein receptor signaling pathway	NM_006255	NP_006246	P24723	ENST00000000233	ARF5	5'-ACTTTTTTGCTTTCGCTTAGCCTCCCACACATTCCACCACCACTGTGCTGCTAAGATGGGGCTTACTATCTTTGCTAACCTCTTCTCCTTCCTCATCGGCATGATTGTGCTCATCATCATTGGCAACATCCTCAACTCCTTCATCAAGGCCATCATCTTCTTGATCGACGACGTGCTCAGCACCCCCCAGCCCGACGTGCCCAACATCCTGTCCAACATCAACGCCTACCTCATCCTCACCCCCGAGGAGAAGAACAAGCAGCTGGCCCAGCACGTGCCCAAGACCAAGCTGCTCATCTACGCCTACAAGGTGGCCCAGGGCGACAAGGGCATCGACAAGAACAAGTTCCTCAAGAAGGTGAACAAGGCCTTCGAGTACGCCGACGTGCTCGAGTACCTCAAGGACAAGGGCAAGGCCGTGGACAAGACCAAGCTGCTCAAGGTGGTGGACTACCTCGTGGAGCAGCAGAAGGAGGGCAAGGTGGACGCCGCCGTGCTCATCAAGGAGCTCAACAAGCTCAAGGAGGAGG