Quick gene expression profiling of promyelocytic cell line HL-60

Syed S. Hussain, Fil Lic, PhD, Hala S. Khalil, BSc, MSc, Tala M. Bakheet, BSc, MSc, Suad M. Bin Amer, MSc, PhD.

ABSTRACT

Objective: A number of techniques have been developed to perform gene expression profiling. We report preliminary results from our exploratory study, using sequential analysis of gene expression (SAGE) technique, to profile the undifferentiated and differentiated HL-60 cells in line with our interest to characterize the cancer phenotype. The aim of the study is to evaluate the technique and to understand the molecular bases of these 2 states of cells.

Methods: HL-60 cells were differentiated after treatment with dimethyl sulfoxide. Tag libraries were prepared from the messenger RNAs of the undifferentiated and differentiated cells according to the SAGE protocol. The search for genes corresponding to the tags was carried out using SAGE software. The tags and the genes from the 2 libraries were compared for their levels of expression. The study was carried out at the King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia during the year 2001.

Results: A comparison of tags from the 2 libraries revealed that 151 tags corresponding to 57 genes expressed differentially: 60 tags were elevated and 59 were repressed in the undifferentiated cells. Thirty- two tags were equally expressed in both types of cells. Of the corresponding genes, 25 were expressed at higher, 17 at lower, while 15 were expressed at comparable levels in both cell types. In the profile of undifferentiated cells, the genes involved in mitochondrial function and protein synthesis were prominent, while in the differentiated cells, the genes coding for proteins associated with cell membranes, signal transduction and for cell specific functions were prominent. The genes, expressed equally in both the cell types, were concerned with the maintenance of the living state.

Conclusion: Sequential analysis of gene expression is a useful technique for gene expression profiling. As previously indicated by others, a dedicated team can generate useful data within reasonable time limits.

Saudi Med J 2003; Vol. 24 (11): 1199-1204

A phenotype is the result of the integrated action of a number of gene products; hence, the detection of expression of a single gene reveals only part of the picture. As an accompaniment of the sequencing of the human genome, some high throughput techniques have evolved and made it possible to develop profiles of the entire functional equivalent of the genome at any particular point of time. The knowledge of the context of other genes provides the proper background to understand the significance of the expression of a single gene, moreover, comprehensive gene expression profiling of human genome promises to reveal the molecular basis of human diseases. Among the newly developed techniques, sequential analysis of gene expression (SAGE) is an open-system approach[1-2] capable of detecting the level of expression of known and unknown genes. It is based on 2 empirically derived principles: 1) a tag of 9-10 bases from a conserved domain is enough to identify a gene, and 2) if these tags are concatenated and sequenced, the frequency of the occurrence of these tags reflects the level of expression of the corresponding genes. By

From the Department of Biological and Medical Research (Hussain, Khalil, Bin Amer) and the Department of Biostatistics, Epidemiology and Scientific Computing (Bioinformatics Section) (Bakheet), King Faisal Specialist Hospital and Research Centre, Riyadh, *Kingdom of Saudi Arabia*.

Address correspondence and reprint request to: Dr. Syed S. Hussain, Department of Biological and Medical Research, King Faisal Specialist Hospital and Research Centre, MBC 03, PO Box 3354, Riyadh 11211, *Kingdom of Saudi Arabia*. Tel. +966 (1) 4427865. Fax. +966 (1) 4427858. E-mail: hussain@kfshrc.edu.sa

now this technique has been employed to display the transcriptomes (functional equivalent of the genome in the cell) of a variety of phenotypes and has provided valuable insights in the involvement of different pathways and their corresponding genes.[3-7] Thus, data from >100 libraries, constructed from different tissues in the normal and disease states, has been generated by others using SAGE. Initial studies on global profiling reveal that the expression of most genes is comparable in the normal and cancer cells and that the differences between the 2 states are based on a few hundred genes:[8] the normal state displays a prominent representation of genes with roles in differentiation, while the malignant state exhibits the activity of genes that are instrumental in proliferation. Another aspect of the carcinogenesis process, which has become evident from identical studies in breast cancer is that the transition from the normal to in situ carcinoma involves the suppression of the action of genes encoding secreted and cell autonomous factors implicating autocrine and paracrine signaling mechanisms in tumorigenesis.[9] Obviously, these data, resulting from comprehensive analysis of gene expression, will affect the strategies for prevention as well as the future therapeutic modalities as they provide clues leading to the target genes with key roles underlying the disease phenotype. We have used SAGE to partially describe the transcriptomes of differentiated and undifferentiated HL-60 cells within the framework of our project to characterize the cancer phenotype. HL-60 cell line has a promyelocytic origin and the cells are in an undifferentiated state. This cell line can be differentiated into granulocytoid phenotype to serve as a model system for granulocytes. For our purpose the undifferentiated state served as a model system for cancer phenotype and the differentiated state served as a model system for the normal phenotype. In this report, we present our preliminary results in an attempt to partially describe the differentiated and undifferentiated cells with the aim to evaluate the technique.

Methods. ***Cell line.*** HL-60 cell line was obtained from American Type Culture Collection and cultured in RPMI-1640 medium (Gibco, Grand Island, New York) supplemented with 10% heat-inactivated fetal bovine serum (Sigma Chemicals, St. Louis, MO), penicillin (100 U/ml), streptomycin (25ug/ml) and L-glutamine (1 mmole/L). The cells were split and cultured in 2% DMSO to induce differentiation. The cells were judged to be differentiated by morphology and by detecting the production of reactive oxygen metabolites by luminol-dependent chemiluminescence in response to the chemotactic peptide (f-Met-Leu-Phe).

Generation of SAGE libraries. The total RNA was isolated from undifferentiated and differentiated HL-60 cells by direct lysis using TRI-reagent (Sigma, St Louis, USA). The poly (A) RNA was isolated using Oligotex kit of Qiagen (Valencia, USA). Complimentary DNA was synthesized using a biotinylated oligo (dT) primer along with a Stratagene kit reagents (La Jolla, USA). The cDNA was digested with the anchoring enzyme NlaIII. The 3'restriction fragments were separated after mixing the digestion products with streptavidin coated magnetic beads using a magnet. After dividing into 2 parts, the fragments were ligated to separate linkers (sequences of primers and linkers are given in the SAGE protocol). Each of the linkers contained a recognition sequence for a type II restriction enzyme, BsmF1, which cuts at a distance of 20 base pair away from its recognition site. In this way short tags of equal length were generated for each transcript. The tags were ligated and amplified by polymerase chain reaction, then released from the linkers and ligated to form concatenates of multi-tag chains. The concatenated tags were then cloned into pZERO plasmids (Invitrogen, Carlsbad, USA) and sequenced using M13 primers.

The study was carried out at the King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia during

Results. Sequencing of the inserts yielded 1150 and 1082 tags from the clones of undifferentiated and differentiated cells. A comparison of the 2 lists showed 151 differentially expressed tags. Out of these, 60 tags were elevated in their expression and 59 tags reflected a low level expression in undifferentiated cells compared to differentiated HL-60 cells. The number of tags equally expressed in both cell types was 32. Identification of genes corresponding to these tags showed that whereas some tags identified multiple genes others identified none. Some tags identified 2 genes. We identified 59 genes (**Table 1**), among which 25 were expressed at higher levels, 15 were equal in both the cell types and 17 were expressed at lower levels in undifferentiated cells compared to differentiated cells. The genes showing elevated expression were involved in mitochondrial function and protein synthesis while the genes with a low level expression were known to be involved in signal transduction, cytoskeleton and membrane maintenance.

Discussion. In a previous study, Ito et al[10] displayed the expression profile of undifferentiated and differentiated HL-60 cells adopting a 3'cDNA library construction methodology. In that study, the gene signature of the granulocytes comprised the genes for cell surface membrane components, nuclear DNA binding proteins, secretory proteins and components for signal transduction. It is interesting to note that the genes for energy production, lysosomal proteins, protein synthesis and cytoskeleton were not prominently represented. Granulocytoids, obtained after differentiation of HL-60 cells with DMSO,

Table 1 - Transcripts increased in undifferentiated HL-60 cells.

Tag sequence	Undifferentiated/ differentiated*	SAGE tag	Description
AAAACATTCT	10	AAAACATTCT	Tag matches mitochondrial sequence
GCCGAGGAAG	10	GCCGAGGAAG	Multiple match
TCAAATTAAG	10	TCAAATTAAG	Expressed sequence tags
GTGAAACCCC	9	GTGAAACCCC	Multiple match
CCCATCGTCC	8	CCCATCGTCC	Tag matches mitochondrial sequence
GGCAAGAAGA	8	GGCAAGAAGA	Ribosomal protein L27
AGCCCTACAA	7	AGCCCTACAA	Tag matches mitochondrial sequence
GGTTGAAAAA	6	GGTTGAAAAA	Melanoma cell adhesion molecule
TACCTATTAA	6		No match
TCCTATTACG	6		No match
AATAAAGCAA	4	AATAAAGCAA	Multiple match
ACAGGTGACC	4		No match
AGAATCGCTT	4	AGAATCGCTT	Multiple match
AGGTGGCAAG	4	AGGTGGCAAG	Tag matches mitochondrial sequence
ATGAAACTTC	4	ATGAAACTTC	EST, Weakly similar to MUC2 HUMAN MUCIN 2 PRECURSOR [H.sapiens]
CACTTGCCCT	4	CACTTGCCCT	Multiple match
CCCCCGTACA	4		No match
CCCCTATTAA	4	CCCCTATTAA	Hypothetical protein FLJ10618
CTAGCTTTTA	4		No match
CTGGCTGCAA	4	CTGGCTGCAA	Cytochrome c oxidase subunit Vb
GATGTAGTAT	4	GATGTAGTAT	H4 histone family, member G
GTGGCAGGCA	4	GTGGCAGGCA	Multiple matches
GTGGTGGGTG	4	GTGGTGGGTG	Multiple matches
TCCAAATTAA	4	TCCAAATTAA	Thymopoietin
TCCAATATTA	4		No match
TCCCCAGTAC	4		No match
TCCCCGTACG	4		No match
TCCCGTACAC	4		No match
TCTCTATTAA	4	TCTCTATTAA	Hypothetical protein FLJ22875
TCTGGGGACG	4	TCTGGGGACG	Eucaryotic translation initiation factor 2, subunit 2 (beta, 38kD)
TGAAACCCCA	4	TGAAACCCCA	Hypothetical protein
TGCTGAATGG	4		No match
TTGGAGATCT	4	TTGGAGATCT	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4 (9kD, MLRQ)
TTTATTCCTC	4	TTTATTCCTC	Multiple match
ACTAACACCC	4	ACTAACACCC	Tag matches mitochondrial sequence
TCCACTATTA	3		No match
CAAGCATCCC	3	CAAGCATCCC	Tag matches mitochondrial sequence
CTCATAAGGA	3	CTCATAAGGA	Tag matches mitochondrial sequence
TCCCCTATTA	3		No match
TCCCTATTTA	3		No match
TGTGTTGAGA	3	TGTGTTGAGA	Multiple match
TCCTATTAAG	3		No match
CCTGTAATCC	2	CCTGTAATCC	Multiple match
TCCCGTACAT	2		No match
CACACTGGGC	2		No match
CCATCGTCCT	2		No match
CCTGTAATCT	2	CCTGTAATCT	Multiple match
CTGGGTTAAT	2	CTGGGTTAAT	Ribosomal protein S19
GCGACGAGGC	2	GCGACGAGGC	Ribosomal protein L38
TCCCCGTAAT	2		N o match
TCCCTATTAG	2		No match
TCCCTATTAT	2		No match
TGATTTCACT	2	TGATTTCACT	Tag matches mitochondrial sequence
TTCATACACC	2	TTCATACACC	Tag matches mitochondrial sequence
ATTCAGAGCT	2	ATTCAGAGCT	Hemoglobin, gamma A
CACCTAATTG	2	CACCTAATTG	Tag matches mitochondrial sequence
CTAAGACTTC	2		No match
TCCCCGTACA	1.346		No match
CGCCCAGTAT	1.333		No match
TCCCTATTAA	1.248		No match

*Ratio of transcripts accumulation between undifferentiated and differentiated HL-60 cells.
FLJ - full length Japanese cDNA, Oxidase subunit Vb - oxidase subunit 5b, SAGE - sequential analysis of gene expression

Table 2 - Transcripts increased in differentiated HL-60 cells.

Tag sequence	Differentiated/ undifferentiated*	SAGE tag	Description
TCCCTTTAAG	40		No match
TCCCTAGTTA	10		No match
TCCCTTTTAA	10		No match
ACGTTTAAGG	6	ACGTTTAAGG	Growth Factor Receptor-Bound Protein 2
CCAAACGTGT	6	CCAAACGTGT	Multiple match
GTGGCTCACA	6	GTGGCTCACA	Multiple match
TCCCCTTACA	6		No match
TCCCTATAAA	6		No match
TCCGCTATTA	6		No match
AAAGAAGCCT	4	AAAGAAGCCT	Multiple match
AAGAGTAAAA	4	AAGAGTAAAA	Expressed sequence tags
AAGTTGCTAT	4	AAGTTGCTAT	Prosaposin (Variant Gaucher disease and variant metachromatic leukodystrophy)
ACCCCTAACA	4		No match
ACCGGGAGGT	4	ACCGGGAGGT	Hypothetical protein
CAATACTGCA	4	CAATACTGCA	Pro1073 protein
CCCTGGGTTC	4	CCCTGGGTTC	Ferritin, light polypeptide
CCGTCACAAG	4		No match
CCGTCCAAGG	4	CCGTCCAAGG	Ribosomal protein S16
CGCCGGAACA	4	CGCCGGAACA	Multiple match
CGCCTATATC	4		No match
CGTGTAATCC	4	CGTGTAATCC	Multiple match
CTGTGCCCAG	4	CTGTGCCCAG	Multiple match
GAAATGAAGA	4	GAAATGAAGA	Multiple match
GTGAAACCCA	4	GTGAAACCCA	Multiple match
GTGATGGATG	4	GTGATGGATG	Dual Specificity Phosphatase 3 (vaccinia virus phosphatase Vh1-related)
GTGCAAAATG	4	GTGCAAAATG	Multiple match
TACCCGTACA	4		No match
TACTTGGTCC	4		No match
TCCCCATTAA	4		No match
TCCCCCGTAC	4		No match
TCCCCGTAAA	4		No match
TCCCTACTTA	4		No match
TCCCTATAGC	4		No match
TCCCTCTTAA	4		No match
TCCCTTCTTA	4		No match
TCCCTTTTTA	4	TCCCTTTTTA	Tak1-binding protein 2
TCCGTATTAA	4		No match
TCCTATAAGG	4		No match
TGTCAGGCCC	4		No match
TGTGATGGAT	4		No match
TTAAAGATTT	4	TTAAAGATTT	Tropomyosin 1 (Alpha)
TTAATTCCAG	4	TTAATTCCAG	Multiple match
TTCCCCGTAC	4		No match
AAGACAGTGG	3	AAGACAGTGG	Ribosomal protein L37a
CCACTGCACT	3	CCACTGCACT	Multiple match
TGCACGTTTT	3	TGCACGTTTT	Ribosomal protein L32
AACTAAAAAA	2	AACTAAAAAA	Multiple match
ATAAATTGGG	2	ATAAATTGGG	Atp Synthase, H+ Transporting, mitochondrial F0 complex, subunit B, isoform 1
CATTTGTAAT	2		No match
CCAGAACAGA	2	CCAGAACAGA	Multiple match
CTCGCAGCGG	2		No match
CTGCTATACG	2	CTGCTATACG	Ribosomal protein L5
CTGTTAGTGT	2	CTGTTAGTGT	Malate dehydrogenase 1, Nad (soluble)
GTGAAACCCT	2	GTGAAACCCT	Multiple match
TGGTGTTGAG	2	TGGTGTTGAG	Ribosomal protein S18
TTGGGGTTTC	2	TTGGGGTTTC	Ferritin, heavy polypeptide 1
TTGGTGAAGG	2	TTGGTGAAGG	Multiple match
TCCCTATAAG	1.666666627		No match
TCCATATTAA	1.5		No match

*Ratio of transcripts accumulation between differentiated and undifferentiated HL-60 cells. SAGE - sequential analysis of gene expression

reflected to some extent (not fully) the expression profile of granulocytes. Though the present study does not include the expression profile of granulocytes, it presents a comparison between the undifferentiated HL-60 cells and the granulocytoids (**Table 1**). The gene signatures of undifferentiated cells include the genes for mitochondria and protein synthesis (ribosomal proteins, eukaryotic translation initiation factor 2) and an expressed sequence tags similar to a mucin 2 precursor. Mucin 2 is associated with the type of gastric tumor and location[11] and its expression is inversely related to the Ki-67 labeling index.[12] The granulocytoids expression profile consists of genes active in cell membranes (prosaposin, sterol-C5-desaturase, dual specificity phosphatase,[3] growth factor receptor-bound protein 2), signal transduction (TAK1-binding protein2 involved in the activation of MAPKKK in the IL-1 signal transduction pathway, and in the induction of NF-kB1) and for the cell specific function (gene for ferritin, which is used to store iron in a readily available form to enable the transport of electrons for the production of superoxide radicals; **Table 2**). Among these genes detected in the granulocytoids, 2 proteins appear to be peculiar to these cells: ferritin and prosaposin besides these 2 proteins, TAK1-binding protein 2, an adaptor for the association of TAK1 with TRAF6 in the IL-1 signaling pathway,[13] appears to be prominent. Only ferritin has been detected by Ito et al[10] in these cells. Among the genes common to undifferentiated and differentiated cells, detected in the present study, are those involved in protein synthesis (ribosomal proteins) mitochondrial functions, cytoskeleton and maintenance (actin, actinin, prefoldin 2, flotillin 1 and integrin beta[1]). Actinin has been reported only in the granulocytes by Ito et al.[10] Notably a growth arrest protein is expressed equally in the 2 cell types (**Table 3**).

With this number of tags we were unable to detect the expression of gene for nicotinamide adenine dinucleotide phosphate (reduced form) oxidase, which is prominent during the maturation of the neutrophils.[14] Even Ito et al[10] did not detect the transcript of this enzyme. However, an elevated expression of the gene for nicotinamide adenine dinucleotide (reduced form) oxidase was noted in the present study in the undifferentiated cells (**Table 1**). The number of tags considered for comparison is low, but these numbers at least represent the transcripts expressed at high levels.[1]

Table 3 - Transcripts equally expressed in differentiated and undifferentiated HL-60 cells.

Tag sequence	Undifferentiated/ Differentiated*	SAGE tag	Description
AAAGTGAAGA	1	AAAGTGAAGA	Multiple match
AAGGAGATGG	1	AAGGAGATGG	Multiple match
ACTTTCCAAA	1	ACTTTCCAAA	Tag matches mitochondrial sequence
AGACTTTTCA	1		No match
AGGAAAGCTG	1	AGGAAAGCTG	Ribosomal protein L36
AGGTCAGGAG	1	AGGTCAGGAG	Multiple match
AGTATGTATG	1	AGTATGTATG	Protein kinase domains containing protein similar to phosphoprotein
ATCAAGGGTG	1	ATCAAGGGTG	Ribosomal protein L9
ATGCAGAGCT	1	ATGCAGAGCT	Multiple match
CACACTGGCG	1		No match
CCAAAAAAAA	1	CCAAAAAAAA	Multiple match
CCTCGGAAAA	1	CCTCGGAAAA	Ribosomal protein L38
CGAGGGGCCA	1	CGAGGGGCCA	Actinin, alpha 4
GAAAAATGGT	1	GAAAAATGGT	Multiple match
GAAATCAGTG	1	GAAATCAGTG	Sjogren syndrome antigen B (autoantigen La)
GAGAGCTCCC	1	GAGAGCTCCC	Multiple match
GAGGGAGTTT	1	GAGGGAGTTT	Ribosomal protein L27a
GCACAAGAAG	1	GCACAAGAAG	Growth arrest-specific 5
GCGAAACCCT	1	GCGAAACCCT	Multiple match
GCTTTTAAGG	1	GCTTTTAAGG	Ribosomal protein S20
GGAGAAGATG	1	GGAGAAGATG	Prefoldin 2
GTAAGTGTAC	1		No match
GTCCCCGTAC	1		No match
TATCTGTCTA	1	TATCTGTCTA	SET translocation (myeloid leukemia-associated)
TCACCCACAC	1	TCACCCACAC	Multiple match
TCAGCCTTCT	1	TCAGCCTTCT	Fotillin 1
TCCCGCGTAC	1		No match
TCCCTACTAA	1		No match
TCCTATTAGT	1		No match
TGAAGTTATA	1	TGAAGTTATA	Integrin, beta 1 Ribosomal protein L39
TTACCATATC	1	TTACCATATC	No match
TTCCTATTAA	1		

*Ratio of transcripts accumulation between undifferentiated and differentiated HL-60 cells. SAGE - sequential analysis of gene expression

Although we are able to identify some key genes, with a doubling of the tag number, we could have picked up some more salient features of gene signatures for both types of cells. However, for a quick look into the cell expression profile, the identification of more than 50 genes in one run is of importance. The microarray and macroarray techniques are faster, but when the target genes are not known, SAGE will be the technique of choice. This technique has now been modified to describe the transcriptome of single cells.[15] This raises the possibility to describe the phenotypes of interest more precisely using the least amount of starting material thereby allowing subdivision of disease phenotypes into subtypes based on molecular differences.

Some difficulties remain, regarding the interpretation of SAGE data. For example, some tags identify multiple genes and some tags identify none. Similarly, some genes are identified by 2 tags. In the multiple identification of genes, probably the long SAGE method will be more appropriate as it will ensure an increased specificity. The tags with no corresponding genes may have been derived from transcripts so far not associated with any known genes. Despite some of these difficulties, this technique for gene expression profiling is most useful and with a dedicated laboratory and team it is possible to define the molecular basis of a phenotype in a short time period thereby paving the way for effective treatment strategies.

Acknowledgment. Software for SAGE and the protocol for the construction of libraries were kindly provided by Dr. K. Kinzler, Johns Hopkins University, USA. Thanks are due to Dr. Brian Meyer under whose supervision primers and linkers were synthesized by Mr. Abdul I. Butt and sequencing of templates was performed by other staff. Dr. Ranjit Parhar propagated the cells. This work was carried out under the King Faisal Specialist Hospital and Research Centre Project # 2010011.

References

1. Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. Serial analysis of gene expression. ***Science*** 1995; 270: 484-487.
2. Velculescu VE. Tantalizing transcriptomes - SAGE and its use in global gene expression analysis. ***Science*** 1999; 286: 1491-1492.
3. Velculescu VE, Madden SL, Zhang L, Lash AE, Yu J, Rago C et al. Analysis of human transcriptomes. ***Nat Genet*** 1999; 23: 387-388.
4. Madden SL, Galella EA, Zhu J, Bertelsen AH, Beaudry GA. SAGE transcript profiles for p53-dependent growth regulation. ***Oncogene*** 1997; 15: 1079-1085.
5. Nacht M, Ferguson AT, Zhang W, Petroziello JM, Cook BP, Gao YH et al. Combining serial analysis of gene expression and array technologies to identify genes differentially expressed in breast cancer. ***Cancer Res*** 1999; 59: 5464-5470.
6. Madden SL, Wang CJ, Landes G. Serial analysis of gene expression from gene discovery to target identification. ***Drug Discov Today*** 2000; 5: 415-425.
7. Hough CD, Sherman-Baust CA, Pizer ES, Montz FJ, Im DD, Rosenshein NB et al. Large-scale serial analysis of gene expression reveals genes differentially expressed in ovarian cancer. ***Cancer Res*** 2000; 60: 6281-6287.
8. Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR et al. Gene expression profiles in normal and cancer cells. ***Science*** 1997; 276: 1268-1272.
9. Porter D, Krop IE, Nasser S, Sgroi D, Kaelin CM, Marks JR et al. SAGE (serial analysis of gene expression) view of breast tumor progression. ***Cancer Res*** 2001; 61: 5697-5702.
10. Ito K, Okubo K, Utiyama H, Hirano T, Yoshi J, Matsubara K, Expression profile of active genes in granulocytes. ***Blood*** 1998; 92: 1432-1443.
11. Pinto-de-Sousa J, David L, Reis CA, Gomes R, Silva L, Pimenta A. Mucins MUC1, MUC2, MUC5AC and MUC6 expression in the evaluation of differentiation and clinic-biological behaviour of gastric carcinoma. ***Virchows Arch*** 2002; 440: 304-310.
12. Li A, Goto M, Horinouchi M, Tanaka S, Imai K, Kim YS et al. Expression of MUC1 and MUC2 mucins and relationship with cell proliferative activity in human colorectal neoplasia. ***Pathol Int*** 2000; 51: 853-860.
13. Takaesu G, Kishida S, Hiyama A, Yamaguchi K, Shibuya H, Irie K et al. TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. ***Mol Cell*** 2000; 5: 649-658.
14. Hua J, Hasebe T, Someya A, Nakamura S, Sugimoto K, Nagaoka I. Evaluation of the expression of NADPH oxidase components during maturation of HL-60 cells to neutrophil lineage. ***J Leukoc Biol*** 2000; 68: 216-224.
15. Chen YO. Single-cell SAGE (scSAGE) will facilitate expression analysis in samples of limited quantity. In: Riggins GJ, Velculescu VE, Baas F, Elaloof JM, Madden S, Strausberg RL. SAGE 2001: Frontiers in transcriptome exploration. San Diego (CA): Genzyme Therapeutics Corporation; 2001. p. 65.