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PRINCIPAL INVESTIGATOR: Shixia Huang, Ph.D.
Doctor Harold Varmus

CONTRACTING ORGANIZATION: Memorial Sloan-Kettering Cancer Center
New York, New York 10021

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6. AUTHOR(S)
Shixia Huang, Ph.D.
Doctor Harold Varmus

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Memorial Sloan-Kettering Cancer Center
New York, New York 10021

E-Mail: huangs@mskcc.org

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13. ABSTRACT *(Maximum 200 Words)*

To understand the multi-step process of mammary tumorigenesis, we have compared gene expression profiles in different stages of mammary oncogenesis in MMTV-Wnt-1 transgenic mice. Specifically, we have collected tissue samples from virgin mammary glands, hyperplastic mammary glands, Wnt-1 mammary tumors, and tumors metastasized to the lung, and compared their gene expression patterns. Hierarchical clustering analysis and multidimensional scaling analysis showed that gene expression profiles are associated with tumor progression. Each sample group from the same stage cluster separately from samples at other stages. Using the statistical analysis permutation t-test, I identified groups of genes differentially expressed in each of the stages. I also identified a group of genes as potential direct or indirect target genes of Wnt signaling since they are only regulated in mammary tumors induced by the MMTV-Wnt-1 transgene in comparison with tumors induced by other transgenes such as (MMTV)-c-myc, MMTV-Ha-ras, MMTV-neu, MMTV-polyoma middle T antigen, C3(1)/simian virus 40 T/t antigen, and WAP-SV40 T/t antigen (Desai et al., 2002). In addition, I established gene expression profiles for mammary tumors induced by Wnt1, and by Wnt1 in cooperation with loss of tumor suppressor genes such as Pten or P53.

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Introduction

Cancer is a multi-step process, and the molecular events responsible for specific steps in the initiation and progression of breast cancer are not yet clear. Gene expression profiling of human breast cancers has increased our understanding of breast cancer development (Perou et al., 2000; Sorlie et al., 2001), however, since most breast cancers develop sporadically, and the genetic variation between individuals is large, it is difficult to identify genes responsible for each of the steps in tumor development or progression. Transgenic mouse models provide an advantage for studying breast cancer tumor progression due to the use of a defined genetic background. The MMTV-Wnt-1 model (Tsukamoto et al., 1988) develops mammary hyperplasia in utero, tumors are detected at a median age of 6 months, and the tumors metastasize to the lung with low frequency. The extent of hyperplasia throughout the whole gland and the ability to induce gross metastasis surgically in this model offer great benefits for studying gene expression during tumor progression, since we can obtain adequate amounts of RNA from the different types of samples for the preparation of fluorescent-tagged cDNA for microarray analysis. Other models such as (MMTV)-c-myc, MMTV-Ha-ras, MMTV-neu, only develop focal hyperplasia, which makes it harder to obtain enough RNA for the study. In addition, other genetic lesions, including p53 and Pten null mutations, and a MMTV-Fgf3 transgene, have been shown to accelerate tumorigenesis in Wnt-1 transgenic mice. In the present report, I have compared gene expression profiles between each of the stages of mammary tumorigenesis in Wnt-1 transgenic mice, and between mammary tumors collected from Wnt-1 transgenic mice and Wnt-1 in cooperation with loss of tumor suppressor genes such as Pten or p53, to ask whether changes in gene expression are associated with tumor progression, to determine whether the expression phenotype can be correlated significantly with the genotype of the cancers, and to identify genes responsible for each of the steps during tumor progression.

Body

Technical objective 1. Construct a cDNA microarray to be used in studying gene expression in Wnt-1 induced mouse mammary tumors
Status: Completed.

cDNA microarray slides: The mouse 8.7k and 15k array slides were prepared at the National Cancer Institute Applied Technology Center. The 8.7k slides are prepared from the 8.7k Incyte GEM1 clone sets, while the 15k slides combine the 8.7k Incyte GEM1 clone sets and the mammary 6k clone sets. The gene lists are available at http://nciarray.nci.nih.gov. For the 15k slides, approximately 15200 clones were mapped to 10062 unique genes as defined by a Unigene cluster ID. Among the 10062 Unigene clusters, there are 3750 named genes, 3922 expressed sequence tags (EST), and 2390 Riken cDNAs. The 8.7k slides include 6877 Unigene clusters, with 2953 named genes, 1628 ESTs, and 2296 Riken cDNAs. Figures 1-4, and 8 are from data generated with the 15k slides, while figures 5-7 are from data generated with the 8.7k slides.

Experimental quality control: To establish confidence in our microarray data, I have performed rigorous quality controls to ensure accurate comparison between the samples and have achieved a

high level of reproducibility. For example, high quality RNA samples were used and labeling and hybridization conditions were optimized to ensure both clean images and high reproducibility. I have used a common reference RNA for all the hybridizations reported in this study to allow cross sample comparisons, and I used multiple datasets for each group of samples to enable statistical analysis between different groups. The reference RNA was extracted from multiple mouse organs from normal virgin FVBN mice.

Technical objective 2. Analyze differential gene expression patterns in various mammary tumor samples
Status: Completed

2. 1 We initially proposed to compare gene expression patterns in mammary tumor samples induced by Wnt-1, c-myc, -ras, -neu, etc., and to identify common set of genes whose expression are altered in all mammary tumor samples. Because Jeff Green's group in the NCI had undertaken array studies on tumors induced by myc, neu, ras, and other oncogenic transgenes (Desai et al., 2002), we decided to change the direction of this project to identify genes differentially expressed between different stages of mammary tumorigenesis in Wnt-1 transgenic mice. The results are summarized below.

Expression microarrays were performed with RNA extracted from tissues collected from virgin mammary glands (5 samples), Wnt-1 hyperplastic mammary glands (7 samples), Wnt-1 mammary tumors (10 samples), and tumors metastasized to the lung from Wnt-1 transgenic mice (5 samples). Since excessive stroma (mostly adipocytes) is present in virgin non-transgenic (non-TG) mammary glands and MMTV-Wnt-1-induced mammary glands, a direct comparison of expression profiles between mammary tumors and benign mammary tissues may be skewed. Therefore, as a control, I included mammary glands from day 1 lactating non-TG mice (3 samples), which contain much less stroma. Although caution was exercised in the dissection of pulmonary metastases, there is a possibility of contamination of pulmonary cells both surrounding and within the pulmonary metastatic nodules. For this reason, and to assess the influence of the microenvironment in which the metastatic cells were growing, normal lung tissues (3 samples) from MMTV-Wnt-1 transgenic mice without metastasis were included.

MDS analysis (Fig 1a) with the 33 samples showed that samples from different stages of tumorigenesis in MMTV-Wnt-1 TG mice segregated from each other and from non-TG mammary glands or normal lungs, indicating tumorigenesis is accompanied by global expression changes. Non-TG virgins and non-TG day-1 lactating mammary glands also segregated from each other.

Hierarchical clustering analysis (Fig 1b) separates all the samples into two main clusters. All of the tumor samples fell into one cluster (designated as "tumor cluster"), whereas all benign tissues, including normal virgin mammary glands, lactating mammary glands, hyperplastic mammary glands, and lung, were placed into another cluster (designated as "benign tissue cluster"). The benign cluster was further sub-grouped into lung and mammary tissues, and the latter was further sub-grouped into lactating day-1 mammary glands, MMTV-Wnt-1 hyperplastic mammary glands, and non-TG virgin mammary tissues.

The tumor cluster was separated into "primary tumor" and "lung metastasis" clusters. We included two primary tumors (MG tumors A and B) with their corresponding pulmonary metastases in this analysis (Mets A and B). The metastases did not cluster with their corresponding primary tumors, which differs from previous reports for human cancers and their paired metastases (Perou et al., 2000). The probable explanation for this observation is that Wnt-1 tumors are very similar to each other between individuals (correlation coefficiency between 0.86-0.92), while the human tumors are much more different (correlation coefficiency 0.7 or smaller) (Bittner, et al., 2000).

Although lung is dissimilar to breast, it clusters together with benign mammary tissues, including virgin and lactating mammary glands and MMTV-Wnt-1-induced mammary glands, suggesting that mammary tumors induced by the MMTV-Wnt-1 transgene are more different from lung and benign mammary tissues, than lung and benign mammary glands are from each other.

To identify genes differentially expressed between each of the individual tumorigenesis stages, a permutation t test was performed. With statistic significance ($p<0.001$), I identified 584 genes differentially expressed between virgin mammary glands and hyperplastic mammary glands, 1027 genes differentially expressed between hyperplastic mammary glands and mammary tumors, and 299 genes differentially expressed between mammary tumors and tumors metastasized to the lung.

From the hierarchical clustering analysis with the 584 genes differentially expressed between virgin mammary glands and hyperplastic mammary glands (figure 2), we observed that there are gene clusters whose expression is maintained at a similar level between mammary tumors and hyperplastic mammary glands (figure 2, cluster A and C), and other gene clusters regulated progressively in Wnt-1 hyperplastic mammary glands and Wnt-1 tumors compared with normal virgin mammary glands (figure 2, cluster B, D, and E).

Genes regulated similarly in Wnt-1 tumors and Wnt-1 hyperplasia include genes implicated as Wnt-1 target genes, such as Myc, CD44, and several frizzled genes, and those in the retinoic acid pathways, such as stimulated by retinoic acid gene 6 (Stra6), Kruppel-like factor 5 (figure 2, cluster A). This is consistent with the findings that the retinoic acid pathway can be positively regulated by Wnt-1, and that it acts synergistically with the Wnt signaling pathway to enhance the induction of the expression of some of the target genes of the Wnt signaling pathway (Szeto et al., 2001; Tice et al, 2002). In addition, a number of metabolism-regulated genes, including those encoding ribosomal proteins and eukaryotic translational elongation factors, were upregulated in MMTV-Wnt-1-induced hyperplastic mammary glands (Figure 3, cluster C).

Some genes that are associated with proliferation or growth were progressively up-regulated in hyperplastic mammary glands and mammary tumors from MMTV-Wnt-1 TG mice when compared to normal virgin mammary glands (cluster B of Fig 2). Among them are tripartite motif protein 29, transcription factor AP-2 gamma, kit oncogene, RAB 25, and the high mobility group gene 2.

A few apoptosis-inducing genes (figure 2, cluster D, E) were progressively down-regulated in Wnt-1 tumors and hyperplastic mammary glands, including cell death-inducing DNA fragmentation factor,

alpha subunit-like factor A (CIDEA) (figure 3, cluster D) and Bcl2/adenovirus EIB 19kD-interacting protein 3 (BNIP3) (figure 3, cluster E).

There is a group of genes, related to G protein signaling, whose expression is down-regulated in hyperplastic compared to normal virgin mammary glands, but up-regulated in primary tumors compared to hyperplasia. This group of genes includes RGS5 (regulator of G protein signaling 5, a negative regulator of G protein signaling), RGS3, RAB5C (member of Rab subfamily), and DRG2 (developmentally regulated GTP binding protein 2).

Among the 1027 genes differentially expressed between Wnt-1 mammary tumors and hyperplastic mammary glands, 363 genes have a 3-fold or greater average expression difference. From the clustering analysis of these 363 genes with samples from different stages of tumorigenesis, we observed that many genes and clusters are clearly genes involved in oncogenesis and/or proliferation (Figure 6, cluster A and B). However, some of the genes/clusters may have been due to the significant differences in cellular composition between the tumor and virgin mammary glands. The majority of cells in mammary tumors are ductal cells, whereas adipocyte cells comprise the majority of the mass in benign mammary tissues or virgin mammary glands. For this reason, some of the clusters that were found to be different between tumors and benign virgin mammary glands might be adipocyte-specific; indeed, some of the genes in these clusters appear to be fat-related (figure 6, cluster C and D). To control for this, expression arrays from lactating mammary glands, which contain few adipocytes, were also used in the hierarchical analysis; this allowed us to emphasize those clusters showing similar expression patterns in virgin and lactating mammary glands. Among the highly up-regulated genes, several important cell cycle regulators, including Cyclin D1, D2, and B1, and CDC2, CDC42, and CDC25 were more highly expressed in mammary tumors from MMTV-Wnt-1 TG mice than in glands from non-TG virgins. This data is consistent with the observation that mammary tumors in MMTV-Wnt-1 TG mice grow rapidly, usually reaching 2 cm in size within one month after the tumors become palpable. Over 50% of the cells in mammary tumors from MMTV-Wnt-1 TG mice are positive for the proliferation marker Ki67 and more than 10% of the cells are in S phase as determined by BrdU staining.

A hierarchical clustering analysis was performed on the 299 genes differentially expressed between Wnt-1 lung tumor metastasis and primary mammary tumors. Genes in clusters B, C, and D (figure 4) are genes whose expression in lung metastasis is different from that of the normal lung control tissues. Those clusters may represent candidate genes that are unique to pulmonary metastases. Notable genes include up-regulation of protein phosphatase 1, regulatory subunit 7 along with many Riken cDNAs in cluster C, P21, cyclin G, and tumor associated calcium signal transducer in cluster D, and down-regulation of chemokine (C-C) receptor 2, Dock2 (dedicator of cytokinesis 2), pro-platelet basic protein (a member of the intercrine family, small cytokine c-x-c, chemokine c-x-c).

Several gene clusters (cluster E, Figure 4) were expressed at higher levels in both lung and pulmonary metastases from MMTV-Wnt-1 TG mice. Among them were known genes involved in lung functions, including uteroglobin and related genes and surfactant-associated protein C. Other commonly regulated genes include cdc2-related kinase, cell division cycle 37, (figure 9, cluster E) and procollagens, thrombospondin2, and tenascin C (figure 9, cluster A). The increased expression

of these genes may be attributable to several causes. The pulmonary environment may have altered the gene expression in the metastatic cells; genes that are important in establishing or maintaining pulmonary metastases may also be expressed in the lung; and contamination of lung tissues may have also played a role although great caution was taken to dissect away the lung tissues from the focal metastases.

2. 2 Our initial proposal was to compare gene expression profiles between tumors from MMTV-Wnt-1 and MMTV-Wnt-1 in a p53 null background or in double transgenic mice that were MMTV-Wnt-1 and MMTV-Fgf3. We had shown, in the interim, that Pten deficiency could accelerate MMTV-Wnt-1 tumorigenesis (Li et al., 2001). We therefore compared samples between MMTV-Wnt-1 tumors in an otherwise wildtype background, or in a p53 null or Pten null background. Because the Pten null mutations were of greater interest, with respect to human cancer, we discontinued analyzing the double transgenes of MMTV-Wnt-1 and MMTV-Fgf3.

I have performed microarray analysis using 8.7k slides with samples collected from normal lactating mammary glands (3 samples), and mammary tumors of MMTV-Wnt-1 transgenic mice in an otherwise wild type background (10 samples), a Pten+/- background (5 samples, 3 with known LOH at the Pten locus), or a p53 null background (6 samples). Hierarchical clustering and an MDS plot showed that the expression patterns of the tumors were quite similar to each other and very different from normal lactating mammary glands (Figure 5). Both analyses clearly separated all the samples into two main clusters—the three normal lactating mammary glands as one cluster and the 21 tumor samples as another cluster.

To test whether Wnt-1 tumors in a Pten wild type background and tumors from Wnt-1/Pten+/- mice with Pten LOH could be differentiated, we compared the profiles of 10 Wnt-1 tumors with profiles of 3 tumors from Wnt-1/Pten+/- mice with Pten LOH (Figure 7). When all genes were included in the analysis, an MDS plot could not differentiate these two groups (Figure 6, left panel of panel B), suggesting that they are as similar to each other in their expression profiles as they are in their histopathological features. However, using a weighted gene analysis ($p \leq 0.002$), 51 genes appeared to differentiate the Wnt-1/Pten-/- tumors from Wnt-1/Pten+/+ tumors. Furthermore, using this selected set of genes, the two groups of tumors can clearly be differentiated by both MDS Plot and hierarchical clustering (Figure 6, A, and right panel of panel B). The potentially interesting features of this selected sub-group include up-regulation of a hypoxia-induced gene and placental growth factor. Since these genes have been shown to be involved in angiogenesis, it is possible that our analysis may have implicated the angiogenic pathway in Wnt-1/Pten-/- tumors. In fact, recent reports suggest that Pten can regulate angiogenesis (Wen, et al., 2001).

To test whether Wnt-1 tumors in a p53 wild type background and tumors arising in Wnt-1/$p53^{-/-}$ mice could be differentiated, we compared the profiles of 10 Wnt-1 tumors with profiles of 6 tumors from Wnt-1/$p53^{-/-}$ mice (Figure 7). When all genes were included in the analysis, an MDS plot could not persuasively differentiate these two groups (Figure 6, left panel of panel B). However, a significant difference was observed when hierarchical clustering and an MDS plot were performed on a subset of 114 genes selected by weighted gene analysis ($p \leq 0.001$). This difference is greater than that observed between tumors arising in Pten proficient and deficient backgrounds. Among the

genes that differentiate tumors with and without p53, Cyclin G, a p53 target gene, is down-regulated in 5 out of 5 Wnt-1/p53 null tumors, along with CD59a, down-regulated in 5 out of 6 Wnt-1/p53 null tumors, and cyclin D2, which is up-regulated in 5 of 6 Wnt-1/p53 null tumors.

Technical objective 3. Identify and characterize potential Wnt-1 responsive genes
Status: Completed.
The original proposal intended to identify Wnt-1 responsive genes using cultured cells lines. In the course of this grant, several cell lines have been constructed to express Wnt-1 using either viral systems or inducible methods. These cell lines are being used by others in the Varmus lab to identify genes regulated by Wnt-1.

I have taken a different approach to address the question of Wnt-1 responsive genes. This new approach takes advantage of the large set of array data I have generated from comparing samples from different stages of mammary tumorigenesis in Wnt-1 transgenic mice and from comparing tumors samples from Wnt-1 transgenic mice and tumors induced by other transgenes. Genes that are differentially expressed between Wnt-1-postive and –negative mammary glands or tumors include Wnt-1 response genes that are more relevant in breast tumorigenesis. The results are summarized below.

To identify genes that are specifically associated with Wnt-1 expression or the best candidates of Wnt-1 target genes, we selected genes that were either over- or under-expressed in both MMTV-Wnt-1-induced hyperplastic mammary glands and tumors at a similar level with respect to normal virgin mammary glands (with a ratio equal or greater than 2-fold between the expression in tumors than that in virgin mammary glands, and 1.5-fold between hyperplastic mammary glands and virgin mammary glands). A total of 445 genes were selected with this criteria.

Among the 445 genes, 185 were differentially expressed between Wnt-1-induced mammary tumors and those induced by other oncogenic transgenes (MMTV-c-Myc, MMTV-neu, MMTV-Polyoma middle T antigen, C3(1)-simian virus 40 T/t antigen, and WAP- simian virus 40 T/t antigen). (205 of the 445 genes are not present in the chip used by Desai et al. (ref); therefore it is impossible to determine if some of them are uniquely regulated by Wnt-1 in the mammary gland.) This subset of genes are important candidates for being Wnt-1 responsive. Among them are known transcriptional targets of Wnt-1 (all the 185 genes are listed in table 1). Examples are Stra6 (stimulated by retinoic acid 6) (Figure 8) and Wnt-5b. Interestingly, a number of other members of the retinoic acid pathway, including RBP1, are also over-expressed by the stimulation of Wnt-1, highlighting the interaction of these two pathways.

To identify the signature for tumors induced by the Wnt-1 transgene, I have compared genes regulated in Wnt-1 tumors with genes regulated in other tumors, such as (MMTV)-c-myc, MMTV-Ha-ras, MMTV-neu, MMTV-polyoma middle T antigen, C3(1)/simian virus 40 T/t antigen, and WAP-SV40 T/t antigen (Desai et al., 2002). From the comparison between Wnt-1 tumors and virgin mammary glands, I have identified 1539 genes which have a 2-fold average ratio. Among them, there are 938 genes present in the 8.7k slides used by Desai et al. (2002). Comparing these 938 clones with the 627 gene list (which has a mean ratio of 2-fold between the tumors and virgin

mammary glands), there are 192 genes in common and 735 clones that are unique in Wnt-1 tumors (after excluding the few genes that were regulated in the 2.7k slides). Figure 8 shows the clustering analysis of these 735 genes for Wnt-1 transgenic mice samples at different stages of mammary tumorigenesis.

Key research accomplishments

I have generated high quality control protocols and achieved a high degree of reproducibility for the microarray analysis

I have obtained training and become proficient in image analysis, bioinformatics, data analysis, and data annotation.

I have correlated gene expression with tumor progression and with tumor genotypes.

I have identified groups of genes that are differentially expressed in each of the tumorigenesis steps, genes that are candidates for Wnt-regulated genes, and genes that differentiate genotypes in mammary tumors.

Reportable outcomes

Expression profiles for each of the samples from tissues of normal virgin mammary glands, Wnt-1 hyperplastic mammary glands, Wnt-1 primary mammary tumors, and tumors metastasized to the lung..

Conclusions

Gene expression profiles can be correlated with tumor progression and with genotypes.

Groups of genes have been identified that are responsible for each of the steps during tumorigenesis.

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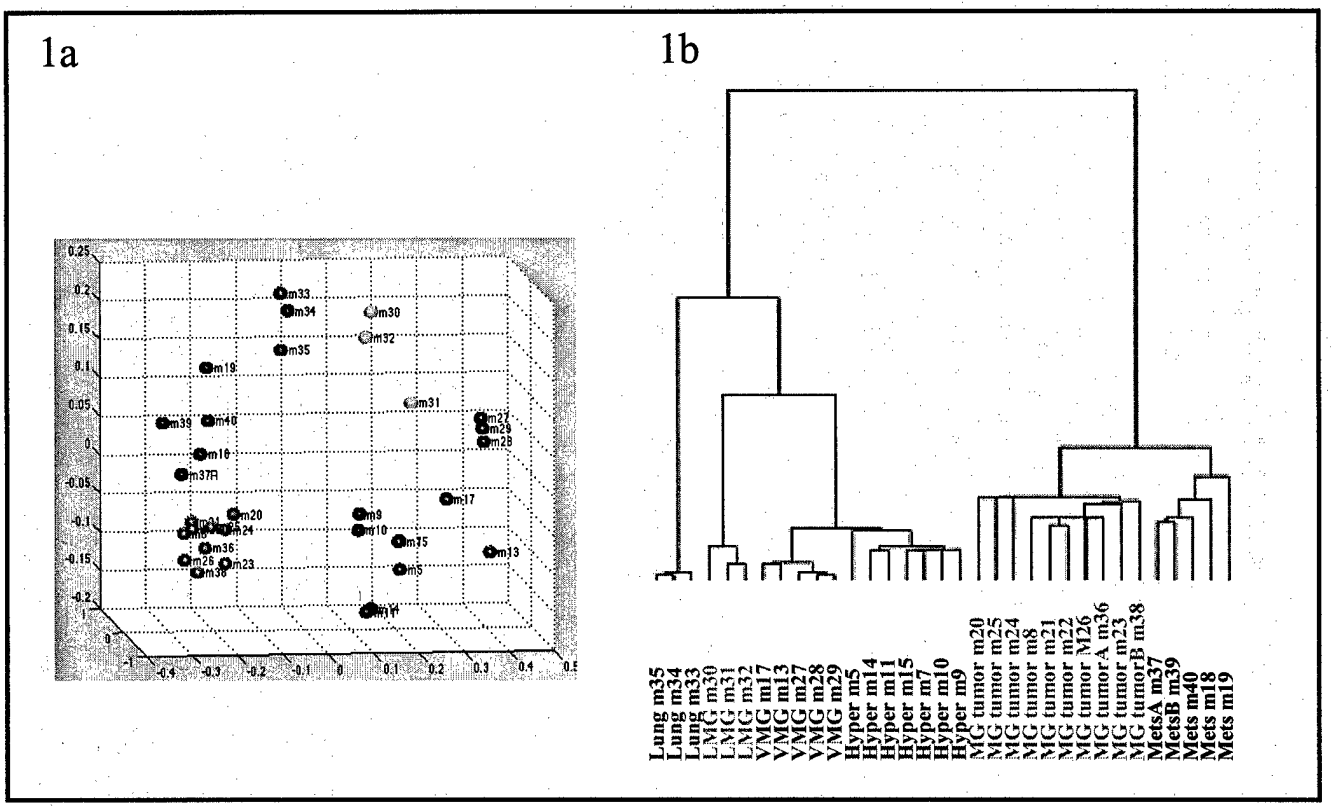


Figure 1 Comparison of samples from different stages of tumor development. (a) Multidimentional scaling analysis. The relationship of all the samples is portraited in the form of a multidimensional scaling plot. The expression of all the cDNA clones are considered in this analysis, and data are represented in three dimension. (b) A dendrogram depicting the degree of similarity between the different stages of samples by average linkage hierarchical clulstering analysis. The 15203 cDNA clones in the slides were filtered according to the following criteria: quality >0.6 in 80% the samples, which are 13815 out of 15203 cDNA clones, and ratios of genes were filtered as 3 observations with the ratio>2 across all the samples. 2434 genes were selected with such criteria and represented in the clustering analysis. Virgin mammary gland (VMG, red), Lactating mammary gland (LMG, yellow), Wnt-1 hyperplasitic mammary glands (Hyper, blue), Wnt-1 Primary tumors (MG tumors, green), Tumors metastasis to the lung (Mets, black), lung (Lung, pink).

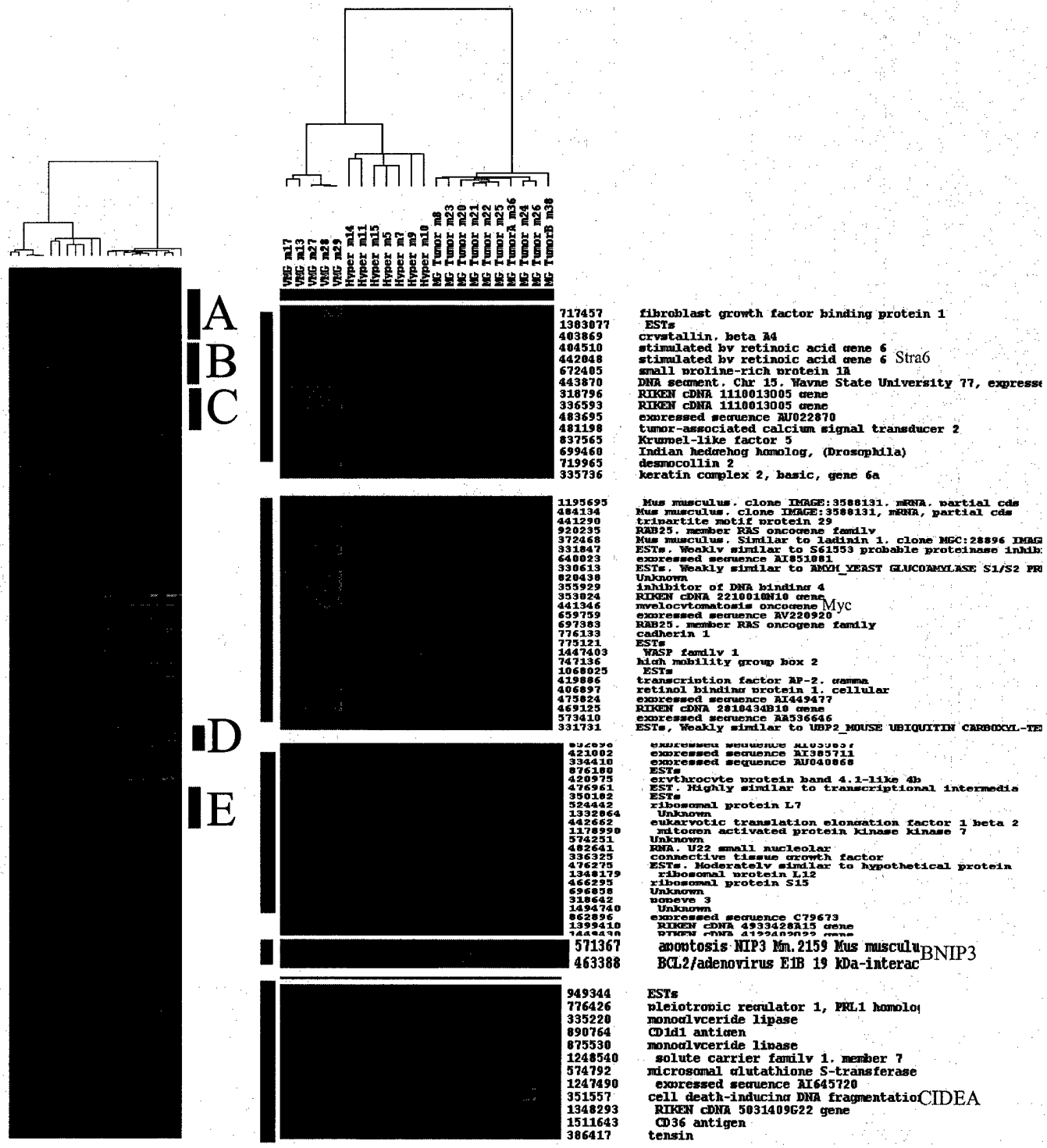


Figure 2. Clustering analysis of 584 genes selected from comparison between VMG and hyperplastic MG for samples from virgin, Wnt-1 hyeprplastic MG, and Wnt-1 mammary tumors.

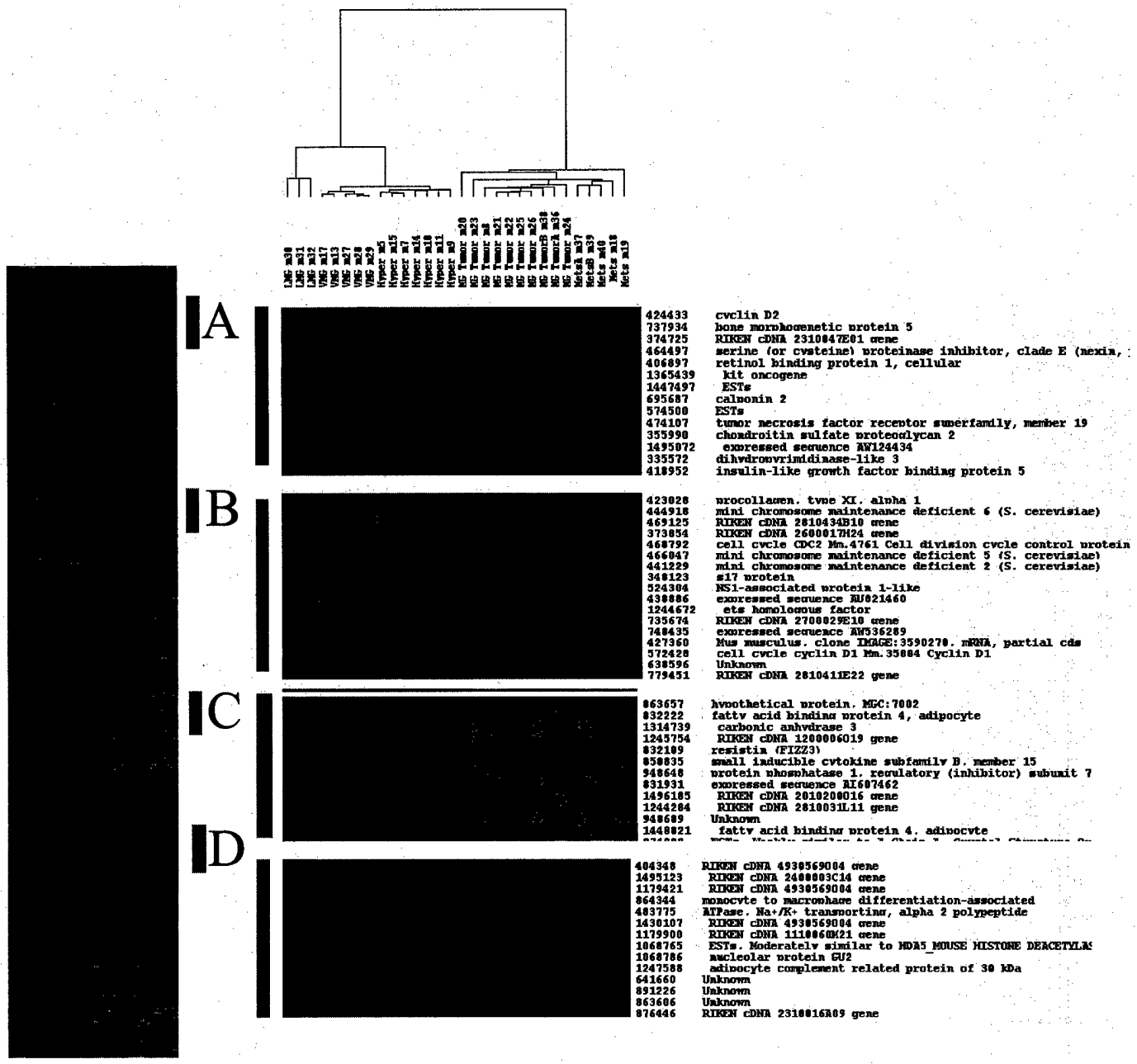


Figure 3. Average linkage hierarchical clulstering analysis of 363 genes with 3 fold average difference between Wnt-1 hyperplastic MG and mammary tumors, selected from the 1027 genes differentially expressed between hyperplastic MG and mammary tumors.

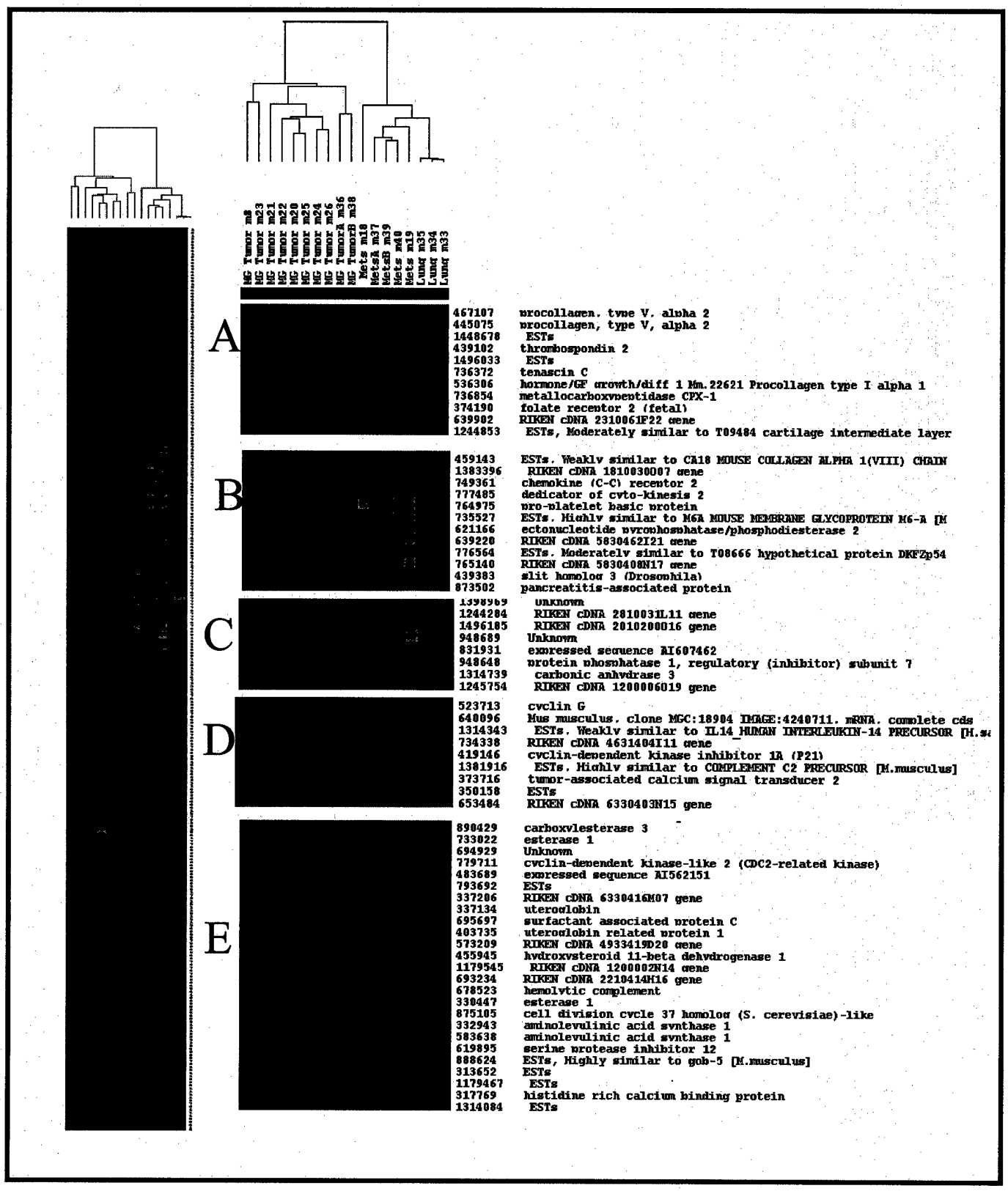


Figure 4. Average linkage hierarchical clulstering analysis with 299 differentially expressed genes between Wnt-1 primary tumors and lung metastases selected by permutation t test, cluster sample groups of primary tumors, metastases, and lung tissues.

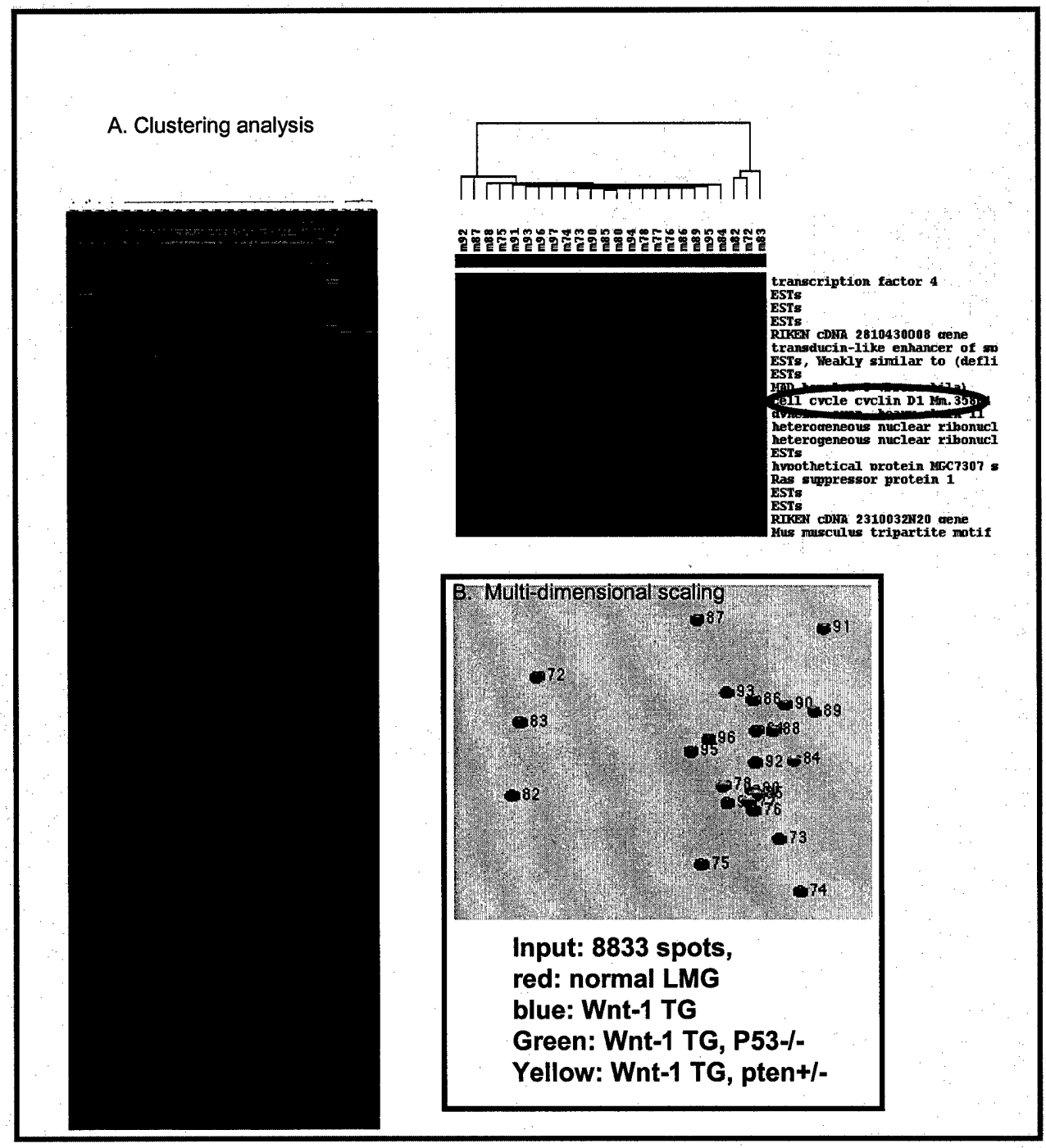
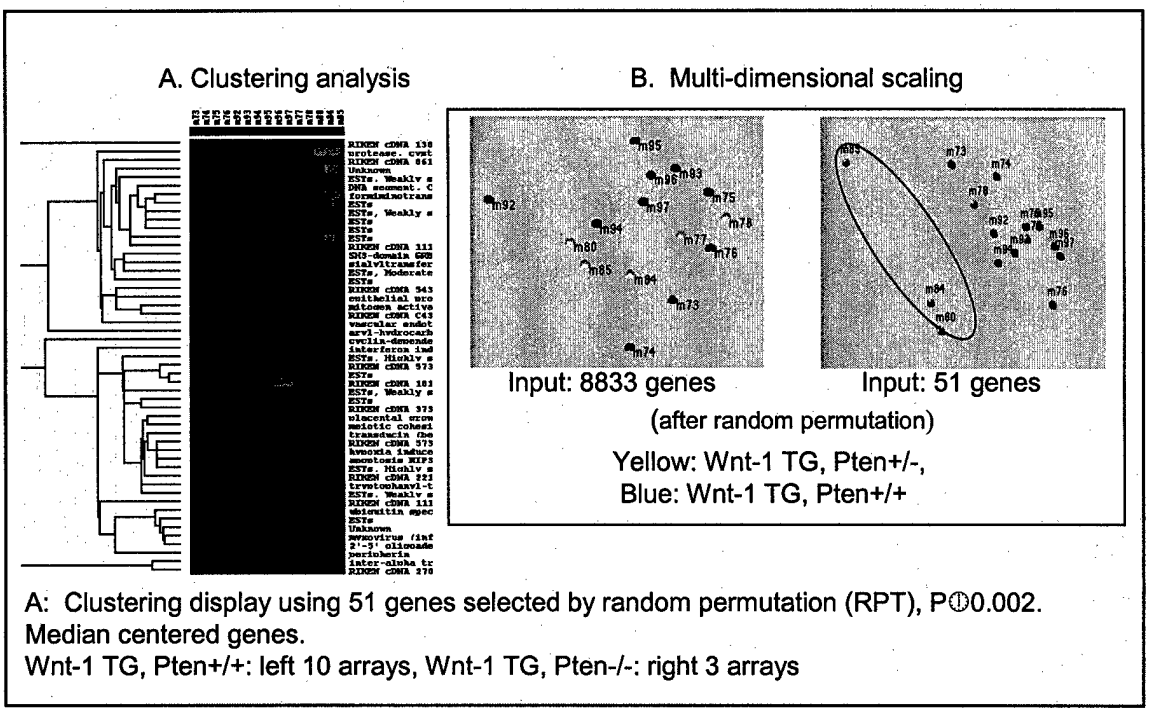


Figure 5 Comparison of samples from tumors with different genotypes, and normal lactating mammary glands. A, Clustering display of 1166 genes selected from all 8833 spots, cDNA clones in the slides were filtered according to the following criteria: 3 arrays with ratio of sample/reference ⊙2x.. Left 21 arrays: Wnt tumors; right 3 arrays: mammary glands collected at 1 day after parturition: B. multidimensional scaling analysis.



A: Clustering display using 51 genes selected by random permutation (RPT), P≤0.002.
Median centered genes.
Wnt-1 TG, Pten+/+: left 10 arrays, Wnt-1 TG, Pten-/-: right 3 arrays

Figure 6 Comparison of tumor samples from Wnt-1 and Wnt-1/pten+/-. A, Clustering display of 51 genes differentially expressed between Wnt-1 and Wnt-1/pten-/- tumors, selected by weighted gene analysis, Left 10 arrays: Wnt tumors; right 3 arrays: Wnt-1/pten-/-; middle 2 arrays: Wnt-1/pten+/-, unknown LOH status. B, multidimensional scaling analysis.

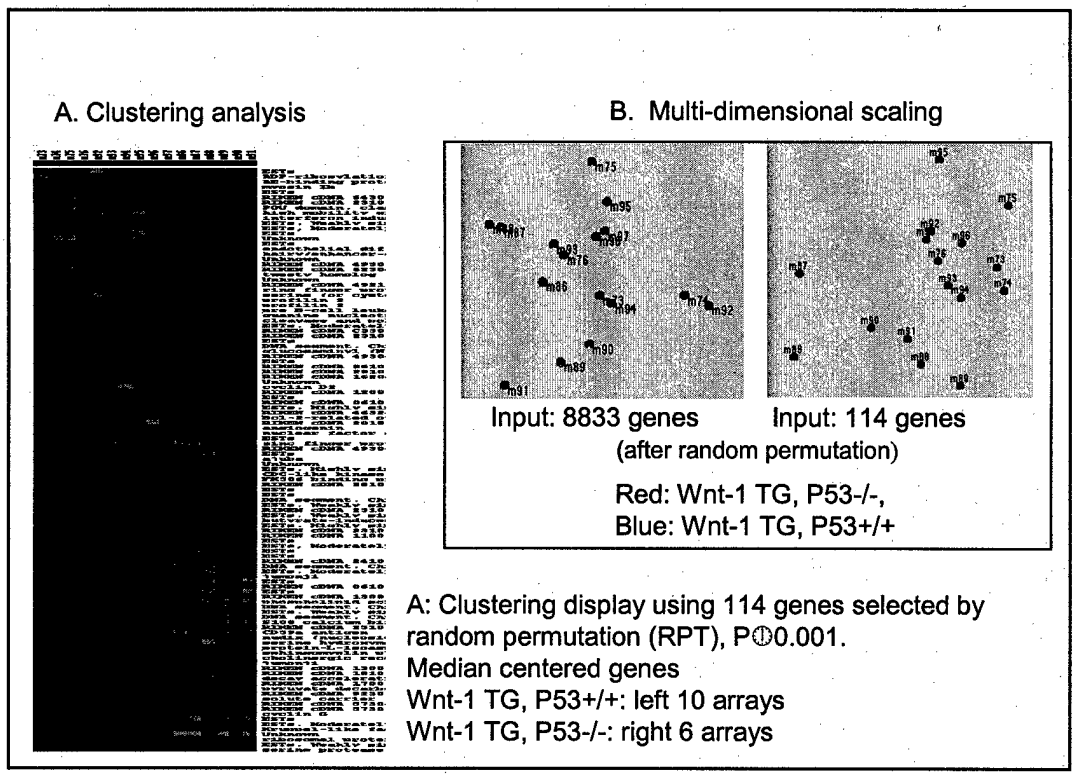


Figure 7 Comparison of tumor samples from Wnt-1 and Wnt-1/p53-/-. A, Clustering display of 114 genes differentially expressed between Wnt-1 and Wnt-1/p53-/- tumors, selected by weighted gene analysis, Left 10 arrays: Wnt tumors; right 6 arrays: Wnt-1/p53-/-. B. multidimensional scaling analysis.

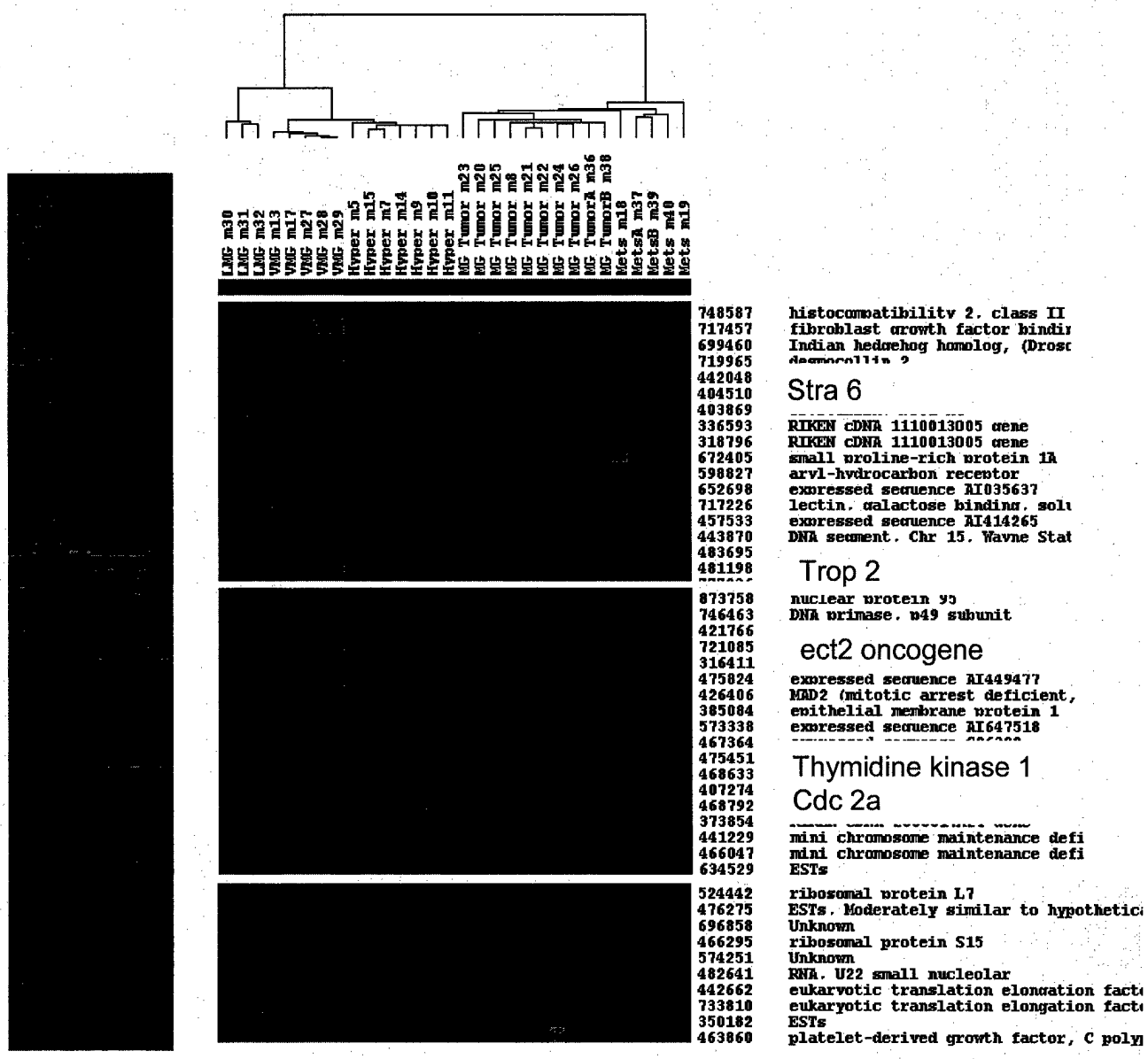


Figure 8. Clustering analysis of 735 Wnt-1 tumor specific genes for samples from: (left to right order) Lactating mammary glands (3 samples), virgin mammary glands (5 samples), Wnt-1 hyperplastic mammary glands (7 samples), and mammary tumors from Wnt-1 mice (10 samples), and tumors metastasis to the lung from Wnt-1 transgenic mice (5 samples). The dendrogram on the top was generated by hierarchical clustering analysis. These genes cluster samples according to their tumorigenesis stages.

Table 1. List of the 185 genes as the best candidates of Wnt targets

Gene Name	Mean ratio of Wnt-1 mamamry tumors to virgin mammary glands	Mean ratio of Wnt-1 hyperplastic mamamry glands to virgin mammary glands
expressed sequence AI851081	14.03	6.71
inhibitor of DNA binding 4	12.16	6.69
"tumor necrosis factor receptor superfamily, member 19"	9.6	2.56
RIKEN cDNA 2210010N10 gene	9.53	5.21
"retinol binding protein 1, cellular"	8.97	2.93
RIKEN cDNA 1010001N11 gene	8.54	2.58
RIKEN cDNA 1110013O05 gene	8	6.15
RIKEN cDNA 1110013O05 gene	7.48	6.92
fibroblast growth factor binding protein 1	7.18	12.34
"transcription factor AP-2, gamma"	6.94	2.77
"ESTs, Weakly similar to UBP2_MOUSE UBIQUITIN CARBOXYL-TERMINAL HYDROLASE 2 (UBIQUITIN THIOLESTERASE 2) (UBIQUITIN-SPECIFIC PROCESSING PROTEASE 2) (DEUBIQUITINATING ENZYME 2) (41 KDA UBIQUITIN-SPECIFIC PROTEASE)"	6.13	2.22
high mobility group box 2	6	3.36
stimulated by retinoic acid gene 6	5.95	4.65
small proline-rich protein 1A	5.94	5.43
stimulated by retinoic acid gene 6	5.57	5.9
desmocollin 2	5.41	7.5
expressed sequence AI449477	5.08	2.19
"lectin, galactose binding, soluble 3"	4.91	3.83
myelocytomatosis oncogene	4.89	3.56
tripartite motif protein 29	4.89	3.35
tripartite motif protein 29	4.39	2.73
"Mus musculus, Similar to ladinin 1, clone MGC:28896 IMAGE:4912735, mRNA, complete	4.37	2.98
"crystallin, beta A4"	4.2	6.19
"Mus musculus, clone IMAGE:3588131, mRNA, partial cds"	4.2	2.47
"DNA segment, Chr 15, Wayne State University 77, expressed"	4.14	3.62
tumor-associated calcium signal transducer 2	4.05	3.46
cadherin 1	3.94	3.64
expressed sequence AW123102	3.93	2.5
expressed sequence AA536646	3.77	2.07
"Indian hedgehog homolog, (Drosophila)"	3.48	5.07
expressed sequence AI461653	3.44	2.49
"RAB25, member RAS oncogene family"	3.32	2.28
cartilage derived retinoic acid sensitive protein	3.27	2.47
expressed sequence AU022870	3.18	2.96
wingless-related MMTV integration site 5B	3.11	1.83
expressed sequence AU040868	3.07	2.82
expressed sequence AU021460	3.07	1.67
expressed sequence AI035637	2.85	3.25
"potassium channel, subfamily K, member 5"	2.78	2.14
"ESTs, Weakly similar to ARL4 MOUSE ADP-RIBOSYLATION FACTOR-LIKE PROTEIN 4 [M.musculus]"	2.75	2
RIKEN cDNA 2310020A21 gene	2.74	2.08
aryl-hydrocarbon receptor	2.73	2.73
pituitary tumor-transforming 1	2.68	2.04
"keratin complex 1, acidic, gene 13"	2.57	2.99
"RNA, U22 small nucleolar"	2.55	2.46
"RAB25, member RAS oncogene family"	2.37	2.63

ESTs	2.34	2.32
erythrocyte protein band 4.1-like 4b	2.34	2.6
ribosomal protein L7	2.26	2.19
eukaryotic translation elongation factor 1 beta 2	2.22	2.17
"Mus musculus, clone MGC:8197 IMAGE:3590741, mRNA, complete cds"	2.17	3.48
Unknown	2.13	1.96
"ESTs, Moderately similar to hypothetical protein [H.sapiens]"	2.1	2.32
"platelet-derived growth factor, C polypeptide"	2.08	2.67
RIKEN cDNA 9130430L19 gene	2.07	2.06
ribosomal protein S15	2.07	2.27
Unknown	2.02	2.15
ESTs	0.49	0.49
"acetyl-Coenzyme A dehydrogenase, medium chain"	0.47	0.52
kininogen	0.45	0.52
"solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 10"	0.43	0.33
receptor TRK E-R Mm.4999 Neurotrophic tyrosine kinase receptor type 3	0.43	0.44
mitochondrial carrier homolog 2	0.43	0.46
"branched chain aminotransferase 2, mitochondrial"	0.43	0.42
carbonic anhydrase 4	0.41	0.41
expressed sequence AI316797	0.41	0.4
RIKEN cDNA 2410091N08 gene	0.41	0.45
RIKEN cDNA 1110025J15 gene	0.41	0.44
"histocompatibility 2, complement component factor B"	0.4	0.51
"malate dehydrogenase, mitochondrial"	0.4	0.46
expressed sequence AI047524	0.4	0.24
retinoid X receptor gamma	0.39	0.36
ferritin heavy chain	0.39	0.5
"ESTs, Moderately similar to ANG3 MOUSE ANGIOGENIN-3 PRECURSOR [M.musculus]"	0.39	0.44
RIKEN cDNA 1500004O06 gene	0.38	0.44
"benzodiazepine receptor, peripheral"	0.37	0.45
ectonucleotide pyrophosphatase/phosphodiesterase 5	0.37	0.4
"aminolevulinate, delta-, dehydratase"	0.37	0.43
RIKEN cDNA 2310034D06 gene	0.37	0.26
expressed sequence AA408225	0.36	0.43
sphingosine kinase 1	0.36	0.41
"ESTs, Weakly similar to T46271 hypothetical protein DKFZp564P1263.1 [H.sapiens]"	0.36	0.5
RIKEN cDNA 1700037H04 gene	0.35	0.23
phenylalanine hydroxylase	0.35	0.37
RIKEN cDNA 1810044O22 gene	0.35	0.5
CD59a antigen	0.35	0.34
stromal cell derived factor 1	0.35	0.48
expressed sequence AA409584	0.34	0.3
citrate synthase	0.34	0.25
RIKEN cDNA 2010015A21 gene	0.34	0.4
RIKEN cDNA 1300019P08 gene	0.34	0.25
pyruvate dehydrogenase E1 alpha 1	0.34	0.33
peroxisome biogenesis factor 16	0.33	0.38
RIKEN cDNA 1110014O20 gene	0.33	0.36
expressed sequence AW547365	0.33	0.52
expressed sequence AI593524	0.33	0.4
erythrocyte protein band 7.2	0.32	0.37
RIKEN cDNA 1700021K19 gene	0.32	0.38
N-myc downstream regulated 1	0.32	0.5

"protein tyrosine phosphatase, non-receptor type 16"	0.31	0.51
"selenoprotein P, plasma, 1"	0.31	0.38
ectonucleotide pyrophosphatase/phosphodiesterase 5	0.31	0.39
RIKEN cDNA 2900010I05 gene	0.3	0.42
isocitrate dehydrogenase 3 (NAD+) alpha	0.3	0.27
"Mus musculus N14A tumor-related protein mRNA, complete cds"	0.3	0.33
"Mus musculus hypertension related protein 1 mRNA, complete cds"	0.3	0.36
diacylglycerol acyltransferase 1	0.29	0.27
"solute carrier family 25 (mitochondrial carnitine/acylcarnitine translocase), member 20"	0.29	0.36
integrin alpha 7	0.29	0.55
A kinase (PRKA) anchor protein (gravin) 12	0.29	0.51
"propionyl Coenzyme A carboxylase, beta polypeptide"	0.28	0.38
nidogen 1	0.28	0.29
ectonucleotide pyrophosphatase/phosphodiesterase 2	0.28	0.45
aquaporin 1	0.28	0.42
expressed sequence AA589586	0.28	0.39
"solute carrier family 27 (fatty acid transporter), member 2"	0.27	0.33
microsomal glutathione S-transferase 3	0.27	0.44
expressed sequence AI666536	0.27	0.37
RIKEN cDNA 1110029F20 gene	0.27	0.31
"Mus musculus, Similar to staufen (Drosophila, RNA-binding protein) homolog 2, clone IMAGE:3156316, mRNA, partial cds"	0.27	0.48
expressed sequence AI465319	0.27	0.48
"solute carrier family 25 (mitochondrial deoxynucleotide carrier), member 19"	0.27	0.33
expressed sequence AI593524	0.26	0.32
X-linked nuclear protein	0.26	0.4
"Mus musculus, clone IMAGE:3586777, mRNA, partial cds"	0.26	0.36
Unknown	0.26	0.34
"solute carrier family 1, member 7"	0.26	0.4
expressed sequence AU041323	0.26	0.44
RIKEN cDNA 5530600A18 gene	0.26	0.37
"Mus musculus cytosolic aminopeptidase P mRNA, complete cds"	0.26	0.47
apoptosis apopt.inhib ILP Mm.6299 Apoptosis inhibitor 3	0.26	0.47
zipcode binding protein 1	0.26	0.47
isocitrate dehydrogenase 3 (NAD+) alpha	0.25	0.38
apolipoprotein A-V	0.25	0.36
"ESTs, Weakly similar to T00267 hypothetical protein KIAA0599 [H.sapiens]"	0.25	0.4
"DNA segment, Chr 9, ERATO Doi 394, expressed"	0.25	0.42
carbonic anhydrase 2	0.25	0.41
G protein gamma 3 linked gene	0.25	0.41
"engulfment and cell motility 2, ced-12 homolog (C. elegans)"	0.25	0.47
RIKEN cDNA 2810409H07 gene	0.25	0.46
expressed sequence C86169	0.25	0.46
inner mitochondrial membrane peptidase 2-like (S. cerevisiae)	0.25	0.42
"ESTs, Highly similar to T46500 hypothetical protein DKFZp434D098.1 [H.sapiens]"	0.24	0.45

RIKEN cDNA 0710008N11 gene	0.24	0.39
"synuclein, alpha"	0.23	0.43
"ESTs, Weakly similar to T33424 hypothetical protein M01G5.4 - Caenorhabditis elegans	0.23	0.23
ESTs	0.23	0.34
RIKEN cDNA 2310022B03 gene	0.23	0.24
diacylglycerol acyltransferase 1	0.22	0.25
"Mus musculus, Similar to nuclear receptor binding protein, clone MGC:6961 IMAGE:3154089, mRNA, complete cds"	0.22	0.47
"Mus musculus transcription repressor p66 mRNA, complete cds"	0.22	0.35
nuclear receptor binding factor 1	0.22	0.49
expressed sequence AA408484	0.21	0.21
"aldolase 1, A isoform"	0.21	0.32
expressed sequence AU043077	0.2	0.4
RIKEN cDNA 2410004M09 gene	0.2	0.39
Williams-Beuren syndrome chromosome region 14 homolog (human)	0.2	0.46
"enoyl coenzyme A hydratase 1, peroxisomal"	0.18	0.32
early B-cell factor	0.18	0.45
RIKEN cDNA 1190005K07 gene	0.18	0.39
RIKEN cDNA 5730469M10 gene	0.17	0.38
tensin	0.17	0.34
RIKEN cDNA 2310008J22 gene	0.17	0.31
RIKEN cDNA 2210414H16 gene	0.17	0.4
RIKEN cDNA 2010012F07 gene	0.17	0.46
RIKEN cDNA 1110013J02 gene	0.16	0.32
"acyl-Coenzyme A oxidase 1, palmitoyl"	0.16	0.25
"2,4-dienoyl CoA reductase 1, mitochondrial"	0.15	0.3
early B-cell factor	0.15	0.41
apoptosis NIP3 Mm.2159 Mus musculus E1B 19K/Bcl-2-binding protein homolog (Nip3) mRNA nuclear gene encoding mitochondrial protein	0.14	0.37
glutathione transferase zeta 1 (maleylacetoacetate isomerase)	0.14	0.42
RIKEN cDNA 4930458D05 gene	0.13	0.36
"fatty acid binding protein 7, brain"	0.13	0.33
"BCL2/adenovirus E1B 19 kDa-interacting protein 1, NIP3"	0.13	0.38
CD1d1 antigen	0.13	0.36
"ESTs, Weakly similar to BGAL MOUSE BETA-GALACTOSIDASE PRECURSOR [M.musculus]"	0.11	0.37
angiopoietin-like 4	0.1	0.28
RIKEN cDNA 2310016A09 gene	0.09	0.35
"ESTs, Moderately similar to UGS3 MOUSE GLYCOGEN [M.musculus]"	0.09	0.23
"Mus musculus, clone MGC:29420 IMAGE:5052893, mRNA, complete cds"	0.09	0.24
Unknown	0.07	0.4
RIKEN cDNA 1110025G12 gene	0.07	0.33
RIKEN cDNA 6330565B14 gene	0.06	0.3
"epoxide hydrolase 2, cytoplasmic"	0.05	0.18
neutrophil cytosolic factor 4	0.04	0.29