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(please add chapter "breast cancer")

Table 1 The breast cancer microarray classification by Sorlie is based on a intrinsic set of 457 genes.

Correlation of microarray classification with overall survival prognosis (Sorlie 2001) (n=49; p<0,01)	
Subtype	Prognosis
ER+/luminal like Typ A	good
ER+/luminal like Typ B	intermediate
ER+/luminal like Typ C	intermediate
Basal like	poor
ERB-B2	poor
Normal like	intermediate

The estrogen receptor positive ER+/luminal like group is subdivided into three subtypes. Correlation with overall survival reveals a poor prognosis for the Basal like and ERB-B2 group. Interestingly different prognosis for patients was found within the three estrogen receptor positive (ER+) groups.

(please ad chapter "breast cancer")

Table 2 Class prediction studies regarding ER-Status in Breast Cancer

Author	Patients	Techniuque	Statistical method	Number of genes of predictor	Training s set	Test set	correct predict (%)
West (2001)	48	cDNA microarray	Bayesianregression	100	38	9	100
Gruvberger (2001)	58	cDNA microarray	Artificial Neural Network	100	47	11	100

"Predictors" for estrogen receptor status based on microarray data were established by to different groups in 2001. Both "predictors"include 100 genes. After develop the "predictor" in a set of samples and corresponding clinical data (Trainigs-set) both groups could validate their "predictor" in independent set of samples and clinical data (Test set) with high accuracy.

(please add chapter "breast cancer")

Table 3 Top 5 ranked genes for prediction ER-Status

Rank	West 2001	Gruvberger 2001
1	Trefoil factor 1 (ps2)	Estrogen Receptor 1
2	Estrogen receptor	Trefoil factor 3
3	Cytochrom P450	GATA Bindind protein 3
4	Trefoil factor 3	ESTs
5	Estrogen like growth factor	Calgranulin A

West and Gruvberger established in 2001 independently "predictors" for estrogen-receptor status in breast cancer based on microarray data. The five genes with strongest correlation of expression and ER-status of the 100 gene"predictors" by West and Gruvberger are listed in this table. Both "predictors" show similarities. Beside the estrogen receptor itself the trefoil factor 3 is find within the five top ranked genes in both studies.

(please add chapter "breast cancer")

Table 4 Overall survival and distant metastasis free survival probability according the prognosis signature (vant Vijver 2002)

Group	No. of patients	Overall survival(%)		free of distant metastasis (%)	
		5YR	10YR	5YR	10YR
Poor prognosis signature	180	74.1	54.6	60.5	50.6
Good prognosis signature	115	97.4	94.5	94.7	85.2

A 70 gene prognostic marker ("predictor") was tested by van t Vijver in a series of 295 consecutive patients with stage I and II breast cancer who underwent surgery. They good distinguish 180 patients with poor prognosis (Poor prognosis signature) from 115 patients with good prognosis (Good prognosis signature) regarding to overall survival and distant metastasis free survival.

(please add chapter "lung cancer")

Table 5 Selected examples of the 50 gene risk index of Beer (2002)

Gene name	P (normal versus tumor t-test)	Coefficient β	Comment
Caspase 4	0,56	0,0022	apoptosis-related cysteine protease
LAMB 1	0,14	0,0027	Laminin β 1
BMP 2	0,54	0,0044	Bone morhogenetic protein 2
CDC 6	1,31E-05	0,0124	cell division cycle 6
Serpine 1	2,89E-03	0,0008	Serine (or cysteine) proteinase inhibitor (clade E)
ERBB2	0,04	0,0013	v-erb -b2 (Receptor)
PDE7A	0,12	- 0,0187	Phosphodiesterase 7a
PLGL	0,04	- 0,0011	Plasminogen like

The 50-gene-risk index was validated in an independent set of 84 tumor samples and corresponding A positive coefficient β is associated with poorer outcome. A 50 gene risk index ("predictor") for lung adenocarcinomas was established in a microarray based correlation study (Beer 2002). Selected examples for interesting genes of this risk index were shown in this table. The coefficient β shows the relation of gene expression and outcome. A positive coefficient β is associated with poorer outcome. This 50 survival data. Among the 62 stage I tumors including this set they could identify a high and a low risk group which differ significant in survival.

(please add to chapter " gastric cancer")

Table 6 Five genes for predicting risk of lymphnode metastasis in intestinal gastric cancer (Hasegawa 2002)

Title		Discriminant coefficient
DDOST	dolichyl-diphosphooligosaccharide-protein glycosyltransferase	1.87
GNS	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	1.26
NEDD8	neural precursor cell expressed, developmentally down-regulated 8	1.29
LOC51096	CGI-48 protein	1.36
AIM2	absent in melanoma 2	-1.54

Five genes were selected based on microarray data for predicting risk of lymph-node metastasis in intestinal gastric cancer (Hasegawa 2002).This "predictor" was validated in 9 additional independent cases. All cases were (four node positive and five node negative) were assigned to each classes.

(please add to chapter " lymphoma")

Table 7 Model of 13 genes predicting outcome in DLBCL Patients (Shipp 2002)

Genes associated with good outcome	Genes associated with poor outcome
-Dystrophin related protein 2	-H731
-3UTR of unknown protein	-Transduction like enhancer protein 1
-uncharacterised	-PDE 4 B
-Protein Kinase C gamma	-uncharacterised
-Minor / NOR 1	-Protein kinase C beta 1
-Hydroxitryptamine 2B Receptor	-Oviductal glycoprotein
-Zinc finger protein C2H2-150	

A 13-gene based "predictor" for outcome in DLBCL patients was developed based on microarray data by a supervised learning method (Shipp 2002). The expression of seven genes were associated with good and the expression of six genes was associated with poor outcome. This "predictor" was superior to "hierarchical clustering" based classification of Alizedah in predicting outcome of DLBCL patients.

Table 8 Analysis of microarray based correlation studies (1999-2003) by Nitzani and Ioannidis (Nitzani 2003) (please add chapter "Relability and.. "

Characteristic	Studies of major clinical outcomes (n=30)	Other studies (n=54)	Total (n=84)
Year of publication			
1999	1(3%)	2(4%)	3(4%)
2000	2(7%)	1(2%)	3(4%)
2001	6(20%)	18(33%)	24(29%)
2002	18(60%)	28(52%)	46(55%)
2003	3(10%)	5(9%)	8(10%)
Malignant disorder			
Haematological	9(30%)	9(17%)	18(21%)
Solid tumor	21(70%)	45(83%)	66(79%)
Median (IQR) number of samples			
Total	62(29-96)	30(18-44)	37(20-57)
Specific cancer	43(24-69)	20(13-36)	25(15-45)
Microarray type			
cDNA	19(63%)	31(57%)	50(60%)
Oligonucleotide	11(37%)	23(43%)	34(40%)
Median (IQR) number of probes	8683 (6817-18 624)	6936 (4569-12 600)	7014 (5534-12 600)
Training			
Independent	9(30%)	17(32%)	26(31%)
Dependent	8(27%)	20(37%)	28(33%)
Both	13(43%)	17(32%)	30(36%)
Validation			
Independent	3(10%)	1(2%)	4(5%)
Cross-validation	6(20%)	4(7%)	10(12%)
Both	3(10%)	5(9%)	8(10%)
None	18(60%)	44(82%)	62(74%)
Outcomes/correlates assessed			
One	9(30%)	35(65%)	44(52%)
Two to four	12(40%)	11(20%)	23(27%)
Five or more	9(30%)	8(15%)	17(20%)
Significant associations reported			
Yes	21(70%)	20(37%)	41(49%)
No	9(30%)	34(63%)	43(51%)

Microarray correlation studies focused on prediction outcome or other impotent clinico-pathological features were systematically analysed by Nitzani and Ioannidis in 2003. This table shows the results of their investigations. In 70% of the studies correlating major clinical outcome with gene expression significant associations were reported. However, in only 30 percent of the major outcome focused studies cross-validation or independent validation was performed. These findings underline the need for consequent quality control and validation in microarray based clinical studies.

Figure legends

Fig. 1. Clustering of gene expressions of tissues 3 from lung cancer patients (Ohira 2002). Tumor tissue and normal lung tissue was collected while surgery after neoadjuvant chemotherapy. Tumor tissue and normal tissue from the same patient show more similarities and clustered nearer than normal

Fig. 2. Histogram of gene expression profile of lung cancer tissue. Expression profile of cancer tissues as compared with normal tissues.

Case B; increased expression of the genes related to cell cycle regulator, intermediate filaments, adhesion motility and angiogenesis in the tumor tissues. Expression of the other gene group were decreased in tumor tissue. Case C; increased expression of genes related with cell cycle, adhesion were observed in the tumor tissue. Decreased expression of growth factor and cytokine related genes were also observed in tumor of Case C. Taken together, the expression profile of lung carcinoma could be characterized by the increased expression of the genes related with adhesion motility and angiogenesis.

Fig. 3(A). Average-linkage hierarchical clustering analysis of ten colorectal tumor samples on histological diagnosis. Right cluster shows the group of the well-differentiated and left shows the group of the other differentiations. (B) Principal component analysis on histological diagnosis. The numbers in blue indicate the patients with well-differentiated adenocarcinoma and the numbers in red indicate the patients with the other differentiations. The c-myc binding protein gene and the c-jun proto oncogene were identified as possible markers for histological differentiation.

Fig. 4. Macroarray analysing of the 21 samples including PCNSCL, Glioblastoma, Oligodendroglioma and normal tissue. The phylogenetic tree obtained by application of the " clustering algorithm shows separation of the PCNSL.

Fig. 5 . Re-clustering was performed using selected genes related with to response to chemo-radiotherapy.The responders (described as GOOD) and non-responders (described as POOR) were clearly separated clearly by the re-clustering method.

Fig. 6. Gene amplification by T7-based RNA amplification method. In a 2 step approach first cDNA was synthesized (RNA→DNA) followed by c.a.RNA synthesis (DNA→RNA) we could purify 10-100µg RNA of amplified cRNA from small amount of total RNA (1µg or less).

Fig. 7. Is differential expression conserved even after amplification? In order to analyze the reproducibility of the clinical samples, the gene expression profile of non-amplified and amplified samples were compared in scattered plot. Upper: gene expression data of duplicate samples of peripheral blood mononuclear cells were compared in scattered blot. High reproducibility (R=0.93) was obtained. These reproducible profiling was also observed in the amplified samples (R= 0.91). Lower: In a second experiment we compared the differential gene expression of the PC 14 cell line and of peripheral blood mononuclear cells using mRNA and after amplification a.cRNA (amplified cRNA). Also the reproducible profiling was lower after amplification (R=0.50) than in non-amplified samples (R=0.83) we could conserve significant differences in gene expression after amplification.

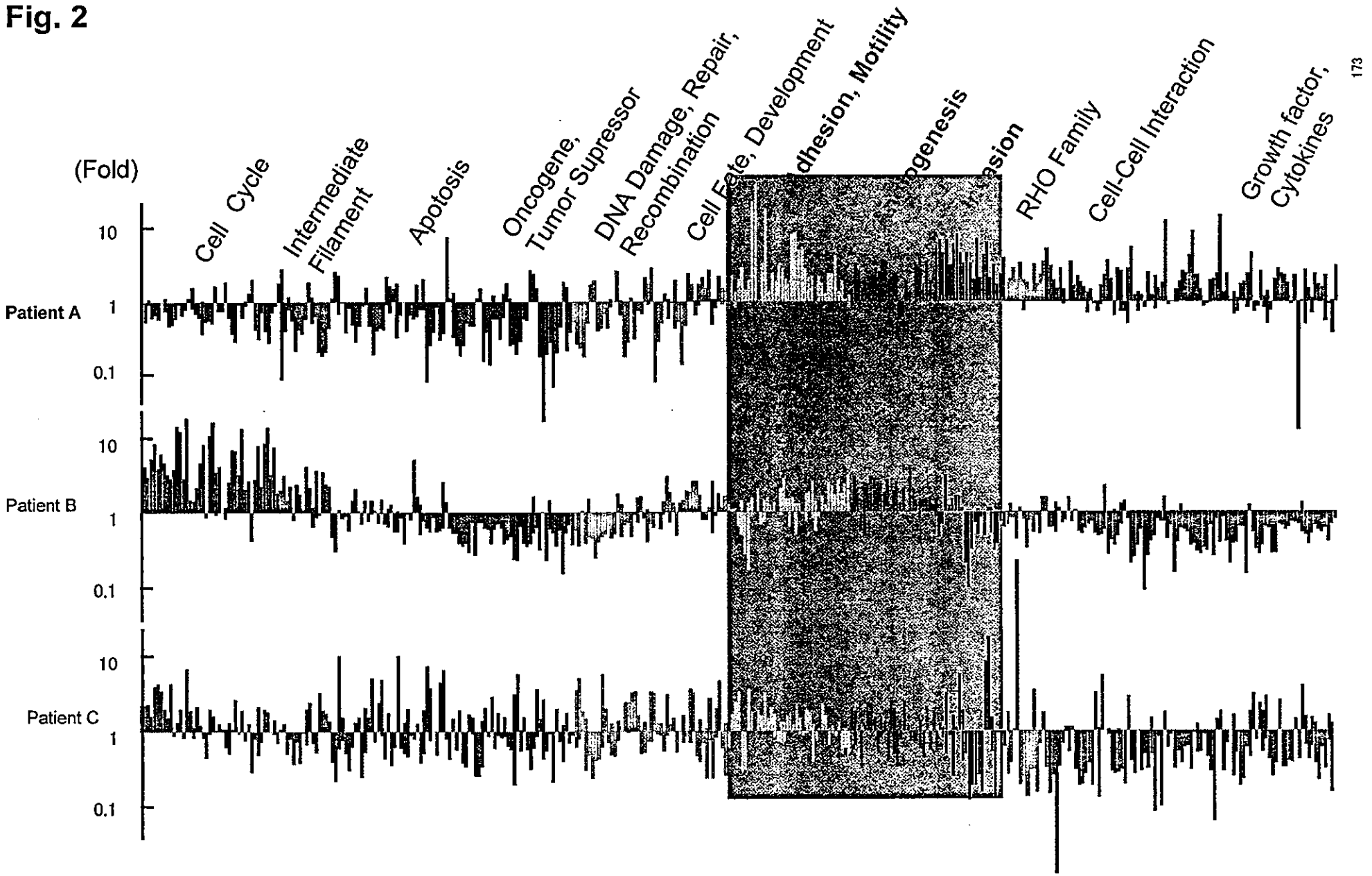
Fig. 8. Experimental design: sampling of PBL and tissue samples in correlative study in clinical phase I study of a falnesyltransferase inhibitor (FTI). . Peripheral Blood Lymphocytes and tumor samples were collected predose, post-dose day 2 and post dose day 8. Gene alteration after administration of FTI was analyzed for proof the pharmacodynamic effect of FTI.

Fig.9a The cDNA filter-array with a set of 775 genes chosen for predicting chemosensitivity analysis Fig. 9b. Gene expression change of tumor tissue and PBL in the melanoma patient after administration of FTI . Specific gene groups were modulated by FTI. Changes in gene expression influenced by FTI were not only observed in the tumor samples but also in the peripheral blood lymphocytes. This findings suggest that drug modulated changes of gene expression in peripheral blood lymphocytes could be useful as surrogate markers in pharmacogenomic studies.

Fig. 1

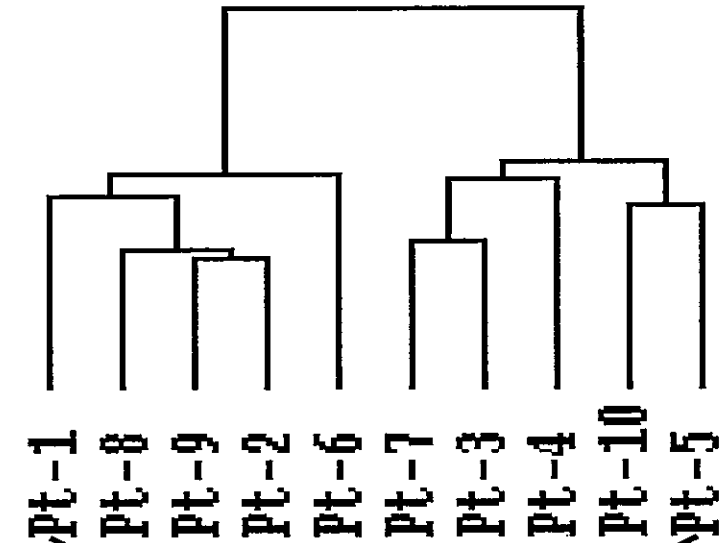
Pt. C (Normal Tissues)
Pt. C (Cancer Tissues)
Pt. B (Normal Tissues)
Pt. B (Cancer Tissues)
Pt. A (Normal Tissues)
Pt. A (Cancer Tissues)

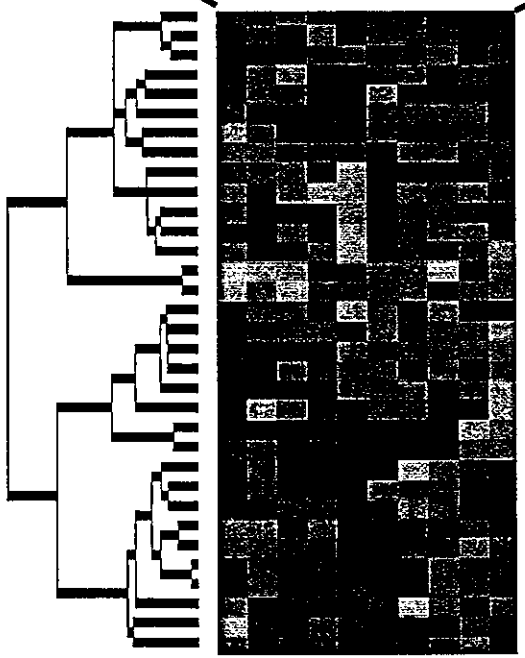
Fig. 2



Expression profile of cancer tissues as compared with normal tissues.

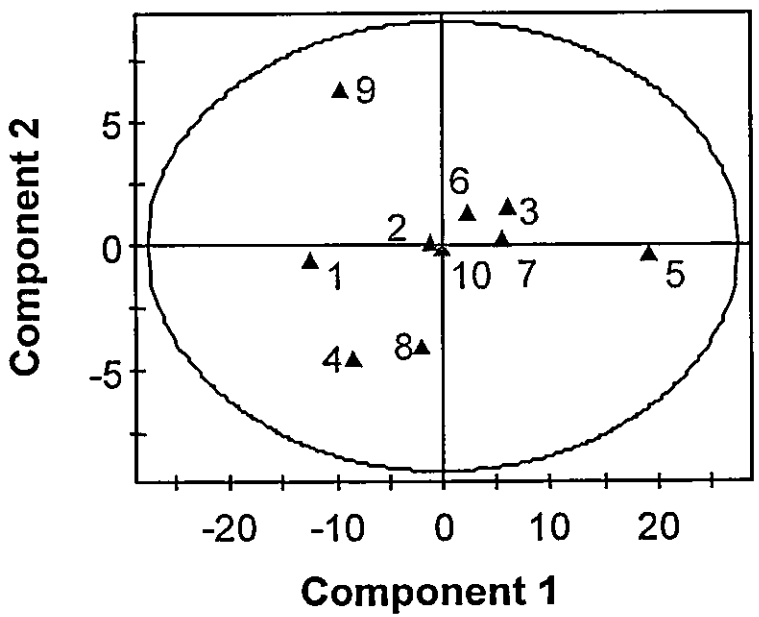
Fig 3A





stathmin; phosphoprotein p19 (PP19); oncoprotein 18 (OP18); leukemia-associated phosphoprotein p18 (LAP18); prosolin; metablastin
DNA cytosine-5-methyltransferase (DNA metase; MCMT)
G1/S-specific cyclin E (CCNE)
protein kinase C gamma type (PKC-gamma)
cyclin-dependent kinase 4 inhibitor 2B (CDKN2B); p14-INK4B; multiple tumor suppressor 2 (MTS2)
mitogen-activated protein kinase kinase 7 (MAP kinase kinase 7; MAPKK7; MAP2K7; PRKMK7); MAPK/ERK kinase 7 (MEK7); c-jun N-terminal kinas
cytochrome P450 IIC8 (CYP2C8); mephenytoin 4-hydroxylase
DNA mismatch repair protein MSH2
serum- & glucocorticoid-regulated serine/threonine protein kinase (SGK)
B-raf proto-oncogene (RAFB1)
metastasis-associated protein 1 (MTA1)
mitogen-activated protein kinase kinase 2 (MAP kinase kinase 2; MAPKK2; MAP2K2; PRKMK2); MAPK/ERK kinase 2 (MEK2)
ubiquitin-conjugating enzyme E2D 3 (UBE2D3); UBCH5C
early growth response protein 1 (EGR1); transcription factor ETR103; KROX24; zinc finger protein 225 (ZNF225); AT225
TRAF-interacting protein (I-TRAF) + TRAF family member-associated NF-kappaB activator TANK
colorectal mutant cancer protein (MCC)
sulfonylurea receptor (SUR); ATP-binding cassette subfamily C (CFTR/MRP) member 8 (ABCC8)
microtubule-associated protein 1B
ATP-binding cassette subfamily C member 3 (ABCC3); multidrug resistance-associated protein 5 (MRP5)
anthracycline resistance-associated protein (ARA)
microsomal glutathione S-transferase 1 (MGST1); GST12
fibroblast growth factor 3 (FGF3); HBGF3; int2 proto-oncogene precursor
ataxia telangiectasia (ATM)
mitogen-activated protein kinase kinase 4 (MAP kinase kinase 4; MKK4; PRKMK4); c-jun N-terminal kinase kinase 1 (JNKK1); JNK-activating
protein kinase C epsilon type (NPKC-epsilon)
ribosomal protein kinase B (RSKB)
coagulation factor VIIIc procoagulant component (F8C)
cyclin-dependent kinase 5 activator isoform p39I precursor (CDK5 activator; NCK5AI)
cadherin 11 precursor (CDH11); osteoblast-cadherin (OB-cadherin); OSF4
mitogen-activated protein kinase kinase 6 (MAP kinase kinase 6; MAPKK6; MAP2K6; PRKMK6); MAPK/ERK kinase 6 (MEK6); SAPKK3
fatty acid synthase
retinoblastoma-related protein p107
CDC6-related protein

Fig 3B


Component 2
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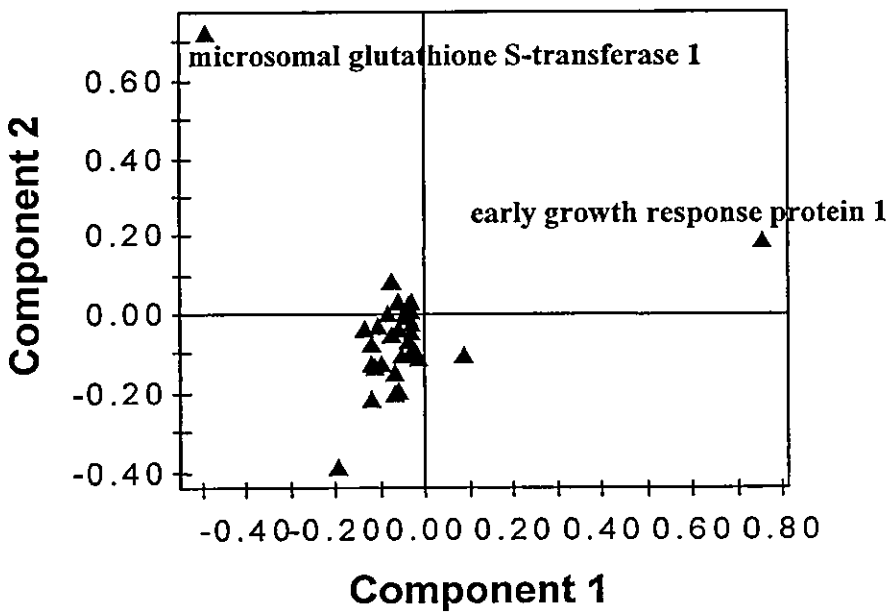
Component 1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early growth response protein 1
Component 2
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-0.40
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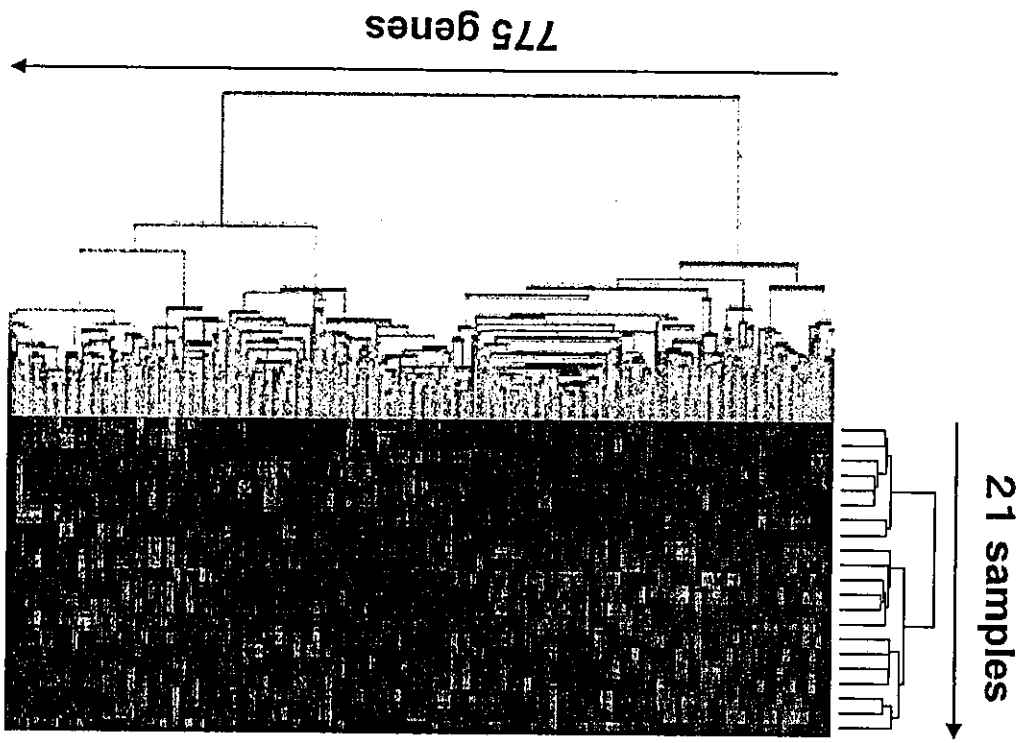
Component 1

Fig 4



PCNSL 3
PCNSL 2
PCNSL 1
PCNSL 5
PCNSL 6
PCNSL 4
Glioblastoma 2
Oligodendroglioma 3
Glioblastoma 5
Glioblastoma 6
Oligodendroglioma 4
Glioblastoma 7
Glioblastoma 1
Oligodendroglioma 2
Normal tissue 2
Normal tissue 1
Oligodendroglioma 5
Normal tissue 3
Glioblastoma 4
Glioblastoma 3
Oligodendroglioma 1