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The role of HOXB2 and HOXB3 in acute myeloid leukemia

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Abstract

Acute myeloid leukemia (AML) is a heterogeneous aggressive disease and the most common form of adult leukemia. Mutations in the type III receptor tyrosine kinase FLT3 are found in more than 30% of patients. Drugs against FLT3 have been developed for the treatment of AML, but they lack specificity, show poor response and lead to the development of a resistant phenotype upon treatment. Therefore, a deeper understanding of FLT3 signaling will facilitate identification of additional pharmacological targets in FLT3-driven AML. In this report, we identify HOXB2 and HOXB3 as novel regulators of oncogenic FLT3-ITD-driven AML. We show that HOXB2 and HOXB3 expression is upregulated in a group of AML patients carrying FLT3-ITD. Overexpression of HOXB2 or HOXB3 in mouse pro-B cells resulted in decreased FLT3-ITD-dependent cell proliferation as well as decreased colony formation and increased apoptosis. Expression of HOXB2 or HOXB3 resulted in a significant decrease in FLT3-ITD-induced AKT, ERK, p38 and STAT5 phosphorylation. Our data suggest that HOXB2 and HOXB3 act as tumor suppressors in FLT3-ITD driven AML.

Keywords Acute myeloid leukemia; HOXB2; HOXB3; FLT3; STAT5; colony formation

Introduction

Acute myeloid leukemia (AML) is a heterogeneous aggressive hematopoietic disorder which is the most common adult acute leukemia accounting for around 80% of cases. Genetic alterations lead to abrogated differentiation of hematopoietic cells. Therefore, the self-renewal ability of those cells is increased, and regulation of normal cell proliferation is disturbed. The major genetic changes include mutation in genes affecting cell proliferation (FLT3, KIT, NRAS/KRAS, JAK/STAT and PTPN11), myeloid differentiation (RUNX1/AML1 and CEBPA), cell cycle regulation or apoptosis (TP53, NPM1), and up-regulation of genes involved in stem-cell maintenance (HOXA, HOXB) [1-4].

The homeobox (HOX) genes are a family of homeodomain-containing transcription factors mainly involved in development. The human HOX genes are clustered in four different chromosomes, 7p15 (HOXA), 17q21 (HOXB), 12q13 (HOXC) and 2q31 (HOXD). HOXA family genes have been thoroughly studied in respect to AML [5-7]. While some studies suggest that HOXB family genes are up-regulated in certain types of AML, their roles in AML have not yet been defined [8-13]. A recent report suggests that HOXB4 expression is elevated in a group of AML patients and higher HOXB4 expression correlated with better prognosis [8]. Another report suggests that over-expression of HOXB6 in NB4 cells or in HL60 cells caused inhibition of the granulocytic and monocytic maturation, respectively [12].

The type III receptor tyrosine kinase FLT3 is expressed in almost all AML, and about 35% of AML patients carry an oncogenic *FLT3* mutation [14]. Among the several mutations that have been found, the internal tandem duplication (ITD) of the sequence that encodes the juxtamembrane domain is the most common mutation in FLT3. The presence of FLT3-ITD mutation portends a poor prognosis in AML. FLT3 mutations also occur in other types of leukemia to a lesser extent, including acute lymphoblastic leukemia [15]. While wild-type FLT3 requires its ligand FL for activation, oncogenic mutants are constitutively active. Activated FLT3 recruits SH2 domain-containing protein through phosphotyrosine residues resulting in activation of AKT, ERK, p38, and STAT5 [16-20].

In this report, we show that expression of HOXB2 and HOXB3 genes is upregulated in AML patients carrying the FLT3-ITD mutations. Loss of HOXB2 or HOXB3 expression in patients carrying FLT3-ITD mutations results in enrichment of oncogenic pathways. Overexpression of HOXB2 or HOXB3 significantly inhibits FLT3-ITD-induced cell proliferation and colony formation and further increases apoptosis.

Materials and methods

Plasmids and Antibodies: Plasmids expressing human HOXB2 and HOXB3 were generated by ligating the open reading frame (ORF) of the corresponding gene into the retroviral vector pMSCVneo. FLT3-ITD plasmid was described previously [21]. Anti-FLT3 antibody were described previously [22]. Anti-phosphotyrosine antibody 4G10 was purchased from Millipore (Life Technologies, Carlsbad, CA) and Anti-phospho p38 and anti-p38 antibodies were from BD Biosciences (Franklin Lakes, New Jersey). Anti-phospho-ERK1/2, anti-ERK2, anti-STAT5 and anti-AKT antibodies were from Santa-Cruz Biotechnology (Dallas, Texas) and anti-phospho AKT was from Epitomics (Abcam, Cambridge, UK). Anti- β -actin antibody was from Sigma-Aldrich (St. Louis, MO).

Cell culture and transfection: The murine pro-B cell line Ba/F3 was cultured in RPMI-1640 medium (Hyclone, Thermo Scientific, Waltham, MA) supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies, Carlsbad, CA), 10 ng/ml murine interleukin 3 (IL3) and 1% penicillin and streptomycin. Generation of Ba/F3-FLT3-ITD cells was described previously [23]. FLT3-ITD-transfected Ba/F3 cells were then further transfected with the pMSCV-neo-HOXB2 or pMSCV-neo-HOXB3 construct or empty pMSCV-neo vector. Cells were selected with 0.8 mg/ml G-418 for 2 weeks. Transfected cells were maintained in the same medium like Ba/F3 medium as recommended before [24]. Cells were grown at 37°C in a humidified atmosphere containing 5% CO₂.

Immunoprecipitation and western blotting: Cells were starved of serum and cytokines for 4 hours and were washed once with cold PBS before lysis with Triton X-100 based lysis buffer. Cell lysates were

mixed with SDS and DTT containing loading buffer in a 1:1 ratio and boiled before separation by SDS-PAGE. For immunoprecipitation 1 µg anti-STAT5 antibody was added in cell lysates and was kept for 1 hour on ice followed by purification on protein G Dynabeads and SDS-PAGE analysis.

Apoptosis, cell proliferation, and colony formation assay: Cells were washed three times to remove cytokine before all experiments. Annexin V and 7-aminoactinomycin D (7-AAD) apoptosis kit (BD Biosciences) was used to measure apoptosis in cytokine depleted cells. Cells positive for annexin V and both annexin V/7-AAD were counted as apoptotic cells. To measure cell proliferation, 10,000 cells were seeded in each well of a 96-well plate and incubated for 48 hours. AlamarBlue (Molecular Probe) was used to measure cell viability. Semisolid methylcellulose medium (Stem Cell Technologies) was used for colony formation assay. Around 500 cells were seeded and cultured for seven days before counting colonies.

Microarray data analysis: The data set GSE14468 was used which was generated from a cohort of 598 newly diagnosed AML patients [25]. Gene expression was compared using significance analysis of microarrays (SAM) tools [26] and gene set enrichment analysis (GSEA) [27]. One-way ANOVA was used for statistical analysis. In statistical significance tests, "ns" represents not significant, "*" represents $p < 0.05$, "***" represents $p < 0.01$, and "****" represents $p < 0.001$.

Results

HOXB family proteins are upregulated in FLT3-ITD positive AML:

To understand the molecular difference between oncogenic FLT3-ITD positive and negative AML, we analyzed gene expression data of AML patients. We used expression data from bone marrow aspirates or peripheral blood samples of 598 cases of de novo AML. Using SAM tool we checked the differential gene expression. We observed that several HOXB family genes were upregulated in FLT3-ITD positive patients (Table S1). HOXB2 displayed 2.3-fold upregulation, HOXB3 4.4-fold, HOXB5 1.4-

fold and HOXB6 2.2 fold upregulation (Fig. S1A). Therefore, our data suggest that expression of several HOXB genes is deregulated in FLT3-ITD-driven AML.

HOXB2 and HOXB3 are independent prognostic markers in AML:

The HOXB family includes 10 genes, HOXB1-9 and 13. Since several HOXB-family genes were deregulated in FLT3-ITD driven AML, we checked whether expression of HOXB-family genes has any prognostic significance in AML. We transformed relative expression values to the Z-score and divided patients into two groups depending on higher and lower HOXB genes expression. We observed that higher expression of either HOXB2 (Fig. 1A) or HOXB3 (Fig. 1B) but not HOXB5 (Fig. S1B) correlated with poor prognosis compared to lower HOXB2 (P=0.0053) and HOXB3 (P=0.0147) expression. This also holds true for higher expression of HOXB6 (Fig. S1C), HOXB7 (Fig. S1D), HOXB8 (Fig. S1E) and HOXB9 (Fig. S1F). Lower HOXB2 (Fig. 1C) and HOXB3 (Fig. 1D) further correlated with better event-free survival (P=0.0234 and P=0.0432 respectively). Although HOXB2 and HOXB3 displayed prognostic significance in the total patient group independent of FLT3 mutations, we were unable to show any prognostic significance in only FLT3-ITD-dependent AML (data not shown) probably due to limited number of patient samples in each group. Since both HOXB2 and HOXB3 gene expression were upregulated in FLT3-ITD-dependent AML and since both genes expression profiles displayed independent prognostic significance, we checked whether HOXB2 and HOXB3 expression correlate with each other. We observed a strong correlation in between expression of the two genes ($r^2=0.8633$) suggesting that patients having higher HOXB2 expression will also have a higher HOXB3 expression and *vice versa* (Fig. 1E). In addition to FLT3-ITD positive AML patients (Fig. 1F), HOXB2 and HOXB3 expression was upregulated in patients carrying the NPM1 mutation (Fig. 1G). Although HOXB2 and HOXB3 expression correlated with FLT3-ITD and NPM1 mutations, expression neither correlated with patients age (Fig. S1G and S1H) nor with the patients sex (Fig. S1I). However, expression of both HOXB2 (Fig. S1J) and HOXB3 (Fig. S1K) was significantly downregulated in the FAB M3 group patients.

Loss of HOXB2 or HOXB3 expression correlates with enrichment of oncogenic pathways:

Since HOXB2 and HOXB3 expression were upregulated in FLT3-ITD positive patients, we wanted to analyze whether the loss of HOXB2 or HOXB3 expression results in the enrichment of any oncogenic pathways. Therefore, we analyzed enrichment of oncogenic pathways using gene set enrichment analysis (GSEA). We observed enrichment of several oncogenic pathways including loss of RB and p107 function, loss of SNF function and E2F3 pathways in FLT3-ITD positive AML patients with lower HOXB2 (Fig. 2A) or HOXB3 (Fig. 2B) expression. These results indicate a possible link between the loss of HOXB2 or HOXB3 expression and enhancement of oncogenic signaling in FLT3-ITD-driven AML.

HOXB2 or HOXB3 over-expression inhibits colony formation and cell proliferation and induces apoptosis:

Results from GSEA suggest that HOXB2 or HOXB3 plays a role in FLT3-ITD-dependent AML patients. To determine the role of HOXB2 and HOXB3 in FLT3-ITD signaling we generated two cell lines by stably transfecting the pro-B cell line Ba/F3 with FLT3-ITD together with HOXB2 or HOXB3, or empty control vector. Expression of FLT3-ITD and HOXB2 or HOXB3 was verified by western blotting (data not shown). Expression of HOXB2 or HOXB3 significantly reduced the size (Fig. 3A) and number (Fig. 3B) of colonies in semi-solid medium. Furthermore, cells expressing HOXB2 or HOXB3 displayed reduced FLT3-ITD-dependent cell proliferation (Fig. 3C) and significantly enhanced apoptosis (Fig. 4D) compared to the empty vector. These findings suggest that expression of HOXB2 and HOXB3 is essential for controlling FLT3-ITD-induced biological events.

HOXB2 or HOXB3 over-expression inhibits FLT3-ITD-induced phosphorylation of AKT, ERK1/2, p38, and STAT5:

Since we observed that HOXB2 and HOXB3 altered FLT3-ITD-dependent cell proliferation, apoptosis and colony formation, we analyzed FLT3 downstream signaling using phospho-specific antibodies. We observed that HOXB2 and HOXB3 overexpression blocked FLT3-ITD-dependent AKT (Fig. 4A),

ERK1/2 (Fig. 4B), p38 (Fig. 4C) and STAT5 (Fig. 4D) phosphorylation in Ba/F3 cells. Thus, we suggest that overexpression of HOXB2 or HOXB3 initiates a transcriptional program that inhibits FLT3-ITD downstream signaling.

Discussion

In this study, we aimed to address the role of HOXB2 and HOXB3 in FLT3-ITD-dependent AML. Expression of HOXB2 and HOXB3 was upregulated in FLT3-ITD-dependent patients, and both HOXB2 and HOXB3 can be used as an independent prognostic marker in AML. Patients carrying lower HOXB2 or HOXB3 have enriched oncogenic pathways enrichment and overexpression of HOXB2 or HOXB3 resulted in inhibition FLT3-ITD signaling as well as biological effects.

Although aberrant expression of homeobox genes is quite common in many cancers, the role of HOXB2 and HOXB3 in human cancer has not been thoroughly studied [28]. HOXB2 has been shown to be overexpressed in breast cancer, and overexpression of HOXB2 was correlated with better prognostic outcome [29]. HOXB2 acts as a tumor suppressor in breast cancer cells. Knockdown of HOXB2 using shRNA resulted in increased tumor growth [29]. We observed that overexpression of HOXB2 in Ba/F3 cells expressing FLT3-ITD resulted in negative regulation of FLT3-ITD-induced signaling as well as the corresponding biological outcomes such as cell proliferation and colony formation and enhancement of apoptosis. Although our observation in Ba/F3 cells in line with the observation in breast cancer cell lines [29], other studies in cervical cancer [30], lung adenocarcinomas [31] and pancreatic cancer [32] suggest overexpression of HOXB2 was associated with cancer progression. Therefore, we suggest that role of HOXB2 in cancer is context dependent.

HOXB3 expression correlated with HOXB2 expression in AML patients suggesting that HOXB3 display similar function to HOXB2 in AML. Although expression of HOXB3 increased cell proliferation in several tumors [33-35], we observed that overexpression of HOXB3 resulted in reduced cell proliferation and colony formation as well as increased apoptosis suggesting similar function to HOXB2 in AML.

Taken together, our study suggests that HOXB2 and HOXB3 have a tumor suppressor role in FLT3-driven AML. Since HOXB2 and HOXB3 are capable of limiting cell proliferation and colony formation as well as inducing apoptosis, overexpression of HOXB2 and HOXB3 keeps the oncogenic FLT3-ITD signaling in balance [36, 37]. It is well-known from a number of cell types that too strong oncogenic signaling can lead to induction of apoptosis[38, 39], so the tumors needs to keep the oncogenic signals at a moderate level in order to be transforming. Future studies are aiming at elucidating the downstream effectors of HOXB2 and HOXB3 and their role in oncogenic transformation.

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Figure legends

Figure 1: Overall survival of AML patients with higher and lower HOXB2 or HOXB3 expression: Z-score was used to divide higher and lower HOXB2 or HOXB3 expressing AML patients. (A) Overall survival of AML patients with higher or lower HOXB2 expression. . (B) Overall survival of AML patients with higher or lower HOXB3 expression. (C) The event-free survival of AML patients with higher or lower HOXB2 expression. (D) The event-free survival of AML patients with higher or lower HOXB3 expression. (E) Correlation in between HOXB2 and HOXB3 expression. (F) Expression of HOXB2 and HOXB3 in FLT3-ITD positive and negative AML patients. (G) Expression of HOXB2 and HOXB3 in NPM1 mutation-positive and negative AML patients.

Figure 2: GSEA shows enrichment of oncogenic pathways in lower HOXB2 or HOXB3 expressing AML patients: Z-score was used to divide higher and lower HOXB2 or HOXB3 expressing FLT3-ITD positive AML patients. (A) Loss of HOXB2 expression correlated with enrichment of several oncogenic pathways.

(B) AML patients with lower HOXB3 expression showed enrichment of several oncogenic pathways compared to patients with higher HOXB3 expression.

Figure 3: Expression of HOXB2 or HOXB3 inhibits cell proliferation, colony formation, and enhanced apoptosis: (A) About 500 stably transfected Ba/F3 cells were used to determine colony formation potential in the semi-solid medium. (B) The number of colonies in stably transfected Ba/F3 cells in each well of 24 well plate. (C) FLT3-ITD dependent cell viability in presence and absence of HOXB2 or HOXB3 expression was measured after 48 hours using AlamarBlue. (D) Apoptosis induced by overexpression of HOXB2 or HOXB3 was measured using Annexin-V and 7-AAD kit in cytokine depleted cells.

Figure 4: Expression of HOXB2 or HOXB3 decreases FLT3-ITD-induced downstream signaling: Total cell lysates were used for SDS-PAGE and Western blotting analysis with AKT (A), ERK (B) and p38 (C) antibodies. (D) Cell lysates were immunoprecipitated with an anti-STAT5 antibody followed by western blotting analysis.

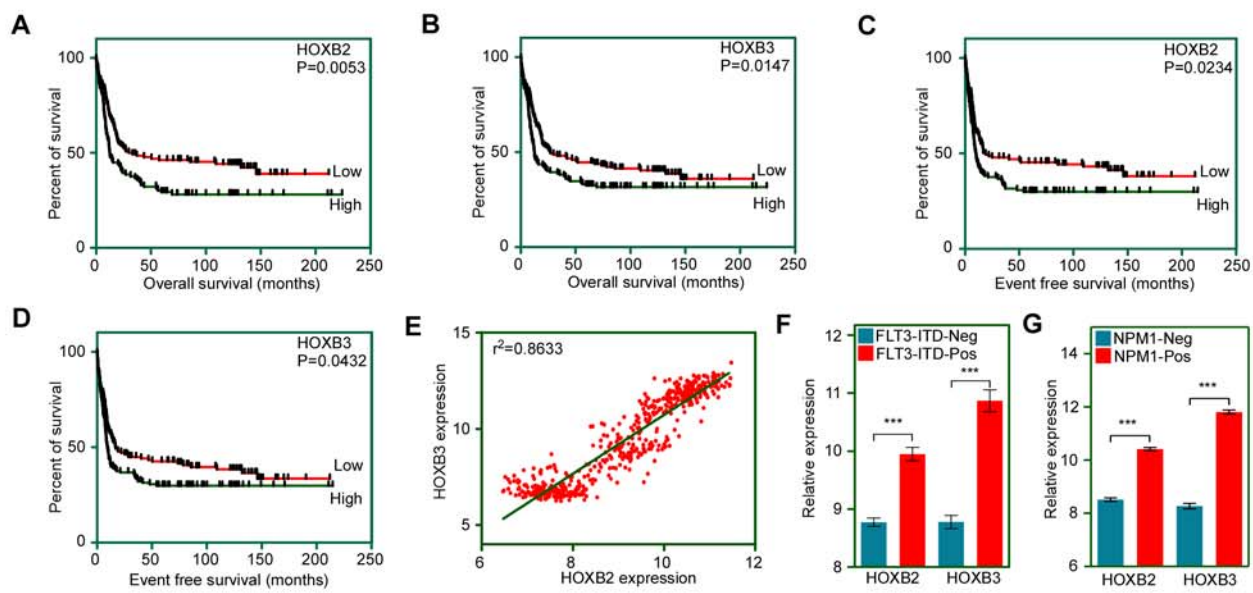


Figure 1

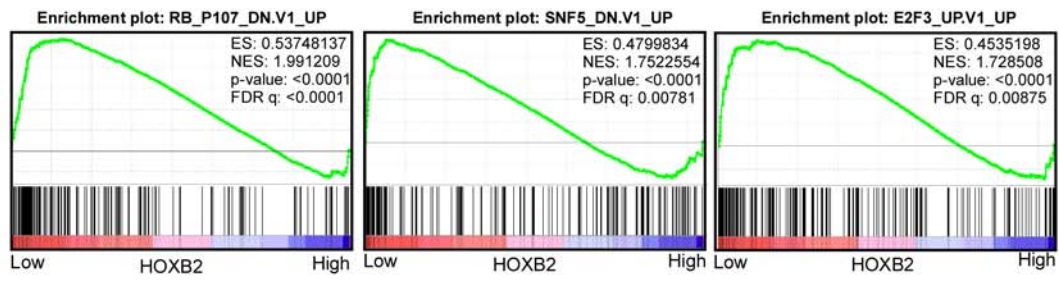
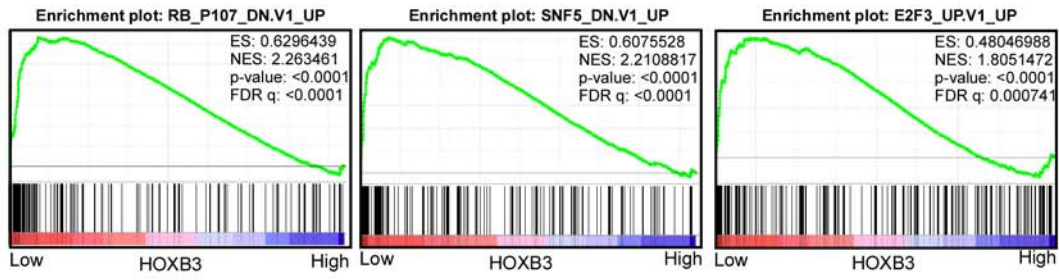
A**B**

Figure 2

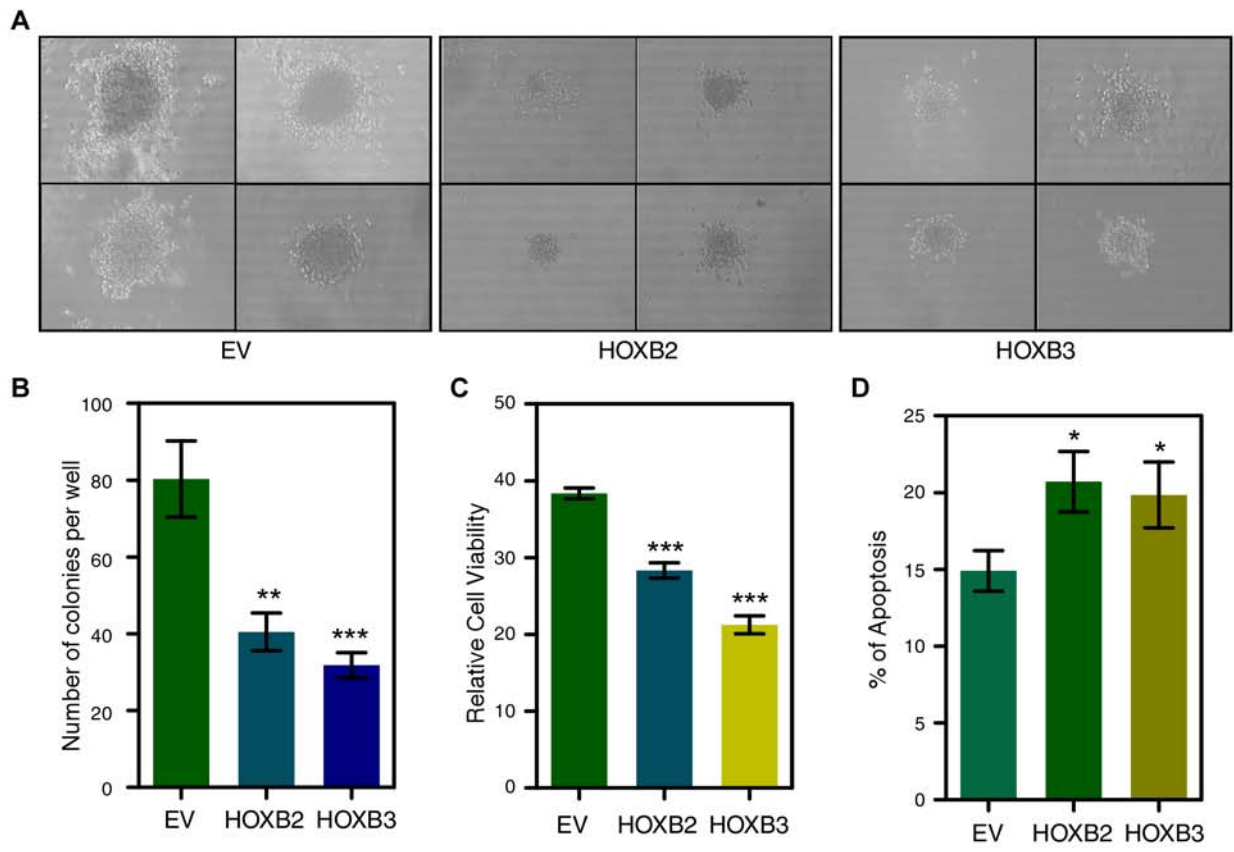


Figure 3

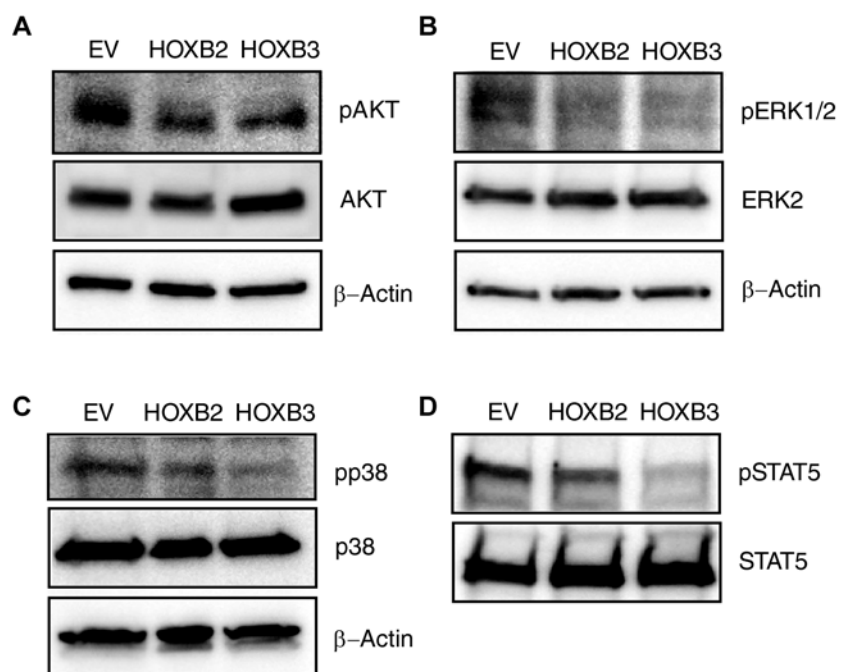


Figure 4

Figure S1: Prognostic significance of HOXB-family proteins.

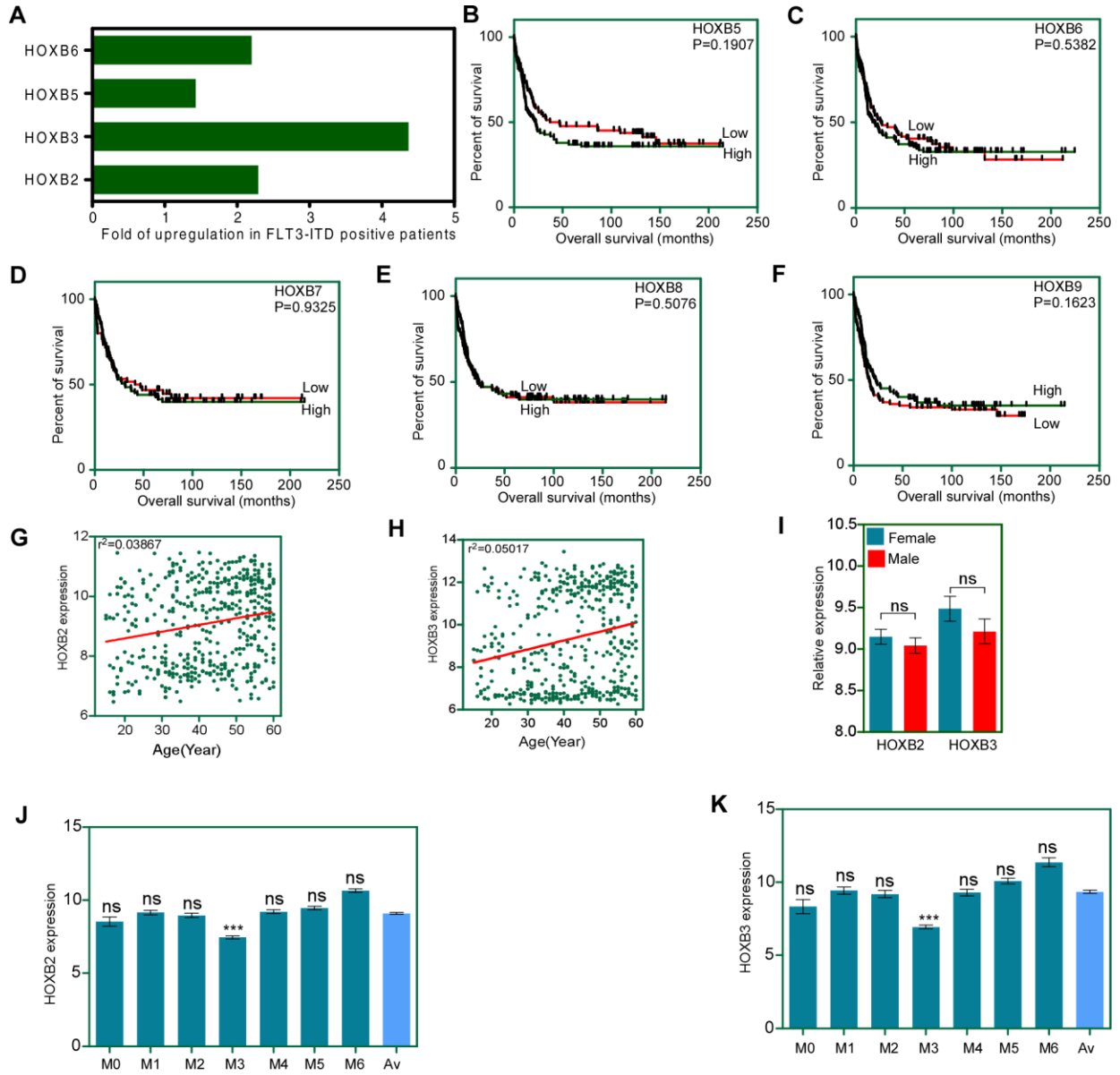


Table S1: Up-regulated genes in FLT3-ITD positive samples

Gene ID	Gene Name	Score (d)	Numerator (r)	Denominator (s+s0)	Fold Change	q-value (%)	Local fdr (%)
209392_at	ENPP2	9.03	1.54	0.17	2.91	0.00	0.00
228372_at	C10orf128	8.21	1.23	0.15	2.35	0.00	0.00
235391_at	FAM92A1	8.02	1.03	0.13	2.04	0.00	0.00
228904_at	HOXB3	8.02	2.13	0.27	4.37	0.00	0.00
227461_at	STON2	7.76	1.33	0.17	2.51	0.00	0.00
236892_s_at	HOXB-AS3	7.75	1.41	0.18	2.65	0.00	0.00
235852_at	STON2	7.70	1.23	0.16	2.34	0.00	0.00
228011_at	FAM92A1	7.68	0.95	0.12	1.93	0.00	0.00
200923_at	LGALS3BP	7.62	1.52	0.20	2.87	0.00	0.00
229437_at	MIR155 /// MIR155HG	7.52	1.44	0.19	2.72	0.00	0.00
204341_at	TRIM16	7.46	0.82	0.11	1.77	0.00	0.00
213110_s_at	COL4A5	7.20	1.76	0.24	3.39	0.00	0.01
211144_x_at	TARP /// TRGC2	7.17	1.34	0.19	2.53	0.00	0.01
215806_x_at	TARP /// TRGC2	7.17	1.36	0.19	2.56	0.00	0.01
216920_s_at	TARP /// TRGC2	7.14	1.34	0.19	2.52	0.00	0.01
209813_x_at	TARP	7.13	1.34	0.19	2.53	0.00	0.01
1553808_a_at	NKX2-3	7.12	1.58	0.22	3.00	0.00	0.01
201663_s_at	SMC4	7.01	0.92	0.13	1.89	0.00	0.02
201664_at	SMC4	6.92	0.92	0.13	1.89	0.00	0.02
215388_s_at	CFH /// CFHR1	6.91	0.91	0.13	1.88	0.00	0.02
219615_s_at	KCNK5	6.89	0.81	0.12	1.75	0.00	0.02
213800_at	CFH	6.87	0.71	0.10	1.64	0.00	0.03
210839_s_at	ENPP2	6.76	0.82	0.12	1.77	0.00	0.03
203372_s_at	SOCS2	6.73	1.57	0.23	2.97	0.00	0.03
203151_at	MAP1A	6.65	0.88	0.13	1.84	0.00	0.04
203373_at	SOCS2	6.65	1.43	0.21	2.69	0.00	0.04
1555600_s_at	APOL4	6.58	0.71	0.11	1.63	0.00	0.04
205453_at	HOXB2	6.55	1.20	0.18	2.29	0.00	0.05
232424_at	PRDM16	6.43	1.02	0.16	2.03	0.00	0.06
203897_at	LYRM1	6.39	0.74	0.12	1.67	0.00	0.06
206341_at	IL2RA	6.39	0.71	0.11	1.64	0.00	0.06
205366_s_at	HOXB6	6.30	1.14	0.18	2.20	0.00	0.07
210425_x_at	GOLGA8A /// GOLGA8B /// LOC100508892	6.29	1.06	0.17	2.08	0.00	0.07
236738_at	C3orf80	6.25	1.28	0.20	2.42	0.00	0.07
204438_at	MRC1	6.15	1.23	0.20	2.34	0.00	0.08
212070_at	GPR56	6.11	0.96	0.16	1.95	0.00	0.08

219304_s_at	PDGFD	6.10	0.92	0.15	1.89	0.00	0.08
204082_at	PBX3	6.08	1.30	0.21	2.46	0.00	0.08
210424_s_at	GOLGA8A /// GOLGA8B /// LOC100508892	6.00	0.83	0.14	1.78	0.00	0.09
204030_s_at	IQCJ-SCHIP1 /// SCHIP1	5.95	1.32	0.22	2.49	0.00	0.09
211269_s_at	IL2RA	5.92	0.70	0.12	1.62	0.00	0.10
208029_s_at	LAPTM4B	5.91	1.21	0.20	2.31	0.00	0.10
209014_at	MAGED1	5.90	0.72	0.12	1.65	0.00	0.10
214039_s_at	LAPTM4B	5.86	1.50	0.26	2.84	0.00	0.10
232979_at	MIR10A	5.82	0.65	0.11	1.57	0.00	0.10
242269_at	FLJ42875	5.80	0.69	0.12	1.61	0.00	0.10
213844_at	HOXA5	5.78	1.45	0.25	2.74	0.00	0.10
201427_s_at	SEPP1	5.74	1.72	0.30	3.29	0.00	0.10
241464_s_at	---	5.70	0.83	0.14	1.77	0.00	0.10
205190_at	PLS1	5.69	0.79	0.14	1.73	0.00	0.10
239237_at	LOC100506776	5.61	0.85	0.15	1.80	0.00	0.11
1554679_a_at	LAPTM4B	5.56	1.15	0.21	2.21	0.00	0.10
205227_at	IL1RAP	5.48	0.77	0.14	1.70	0.00	0.10
204485_s_at	TOM1L1	5.46	0.71	0.13	1.64	0.00	0.10
204224_s_at	GCH1	5.45	1.05	0.19	2.07	0.00	0.10
208798_x_at	GOLGA8A	5.44	1.04	0.19	2.05	0.00	0.10
1559597_at	---	5.42	0.94	0.17	1.93	0.00	0.10
243010_at	MSI2	5.40	0.79	0.15	1.72	0.00	0.09
236553_at	LOC100507520	5.38	0.68	0.13	1.60	0.00	0.09
208767_s_at	LAPTM4B	5.38	0.92	0.17	1.89	0.00	0.09
201328_at	ETS2	5.37	0.77	0.14	1.70	0.00	0.09
225237_s_at	MSI2	5.36	1.03	0.19	2.05	0.00	0.09
211031_s_at	CLIP2	5.36	0.80	0.15	1.75	0.00	0.09
239232_at	MSI2	5.36	0.76	0.14	1.69	0.00	0.09
208791_at	CLU	5.32	1.07	0.20	2.10	0.00	0.09
235521_at	HOXA3	5.30	1.22	0.23	2.33	0.00	0.08
226134_s_at	---	5.28	1.03	0.19	2.04	0.00	0.08
230670_at	IGSF10	5.28	0.71	0.13	1.63	0.00	0.08
226098_at	IFT80	5.24	0.64	0.12	1.55	0.00	0.07
235900_at	SPNS3	5.22	0.70	0.13	1.62	0.00	0.07
208792_s_at	CLU	5.22	0.99	0.19	1.98	0.00	0.07
1558956_s_at	IFT80	5.20	0.64	0.12	1.55	0.00	0.06
210365_at	LOC100506403 /// RUNX1	5.20	0.74	0.14	1.67	0.00	0.06
232088_x_at	LOC100271722	5.18	0.84	0.16	1.79	0.00	0.06
225240_s_at	MSI2	5.16	0.96	0.19	1.95	0.00	0.06

207223_s_at	PTBP3	5.16	0.57	0.11	1.48	0.00	0.05
214697_s_at	PTBP3	5.14	0.61	0.12	1.52	0.00	0.05
226670_s_at	PABPC1L	5.12	0.64	0.12	1.56	0.00	0.04
1559987_at	---	5.10	0.72	0.14	1.65	0.00	0.04
206310_at	SPINK2	5.03	1.23	0.24	2.35	0.00	0.02
209905_at	HOXA10-HOXA9 /// HOXA9	5.03	1.34	0.27	2.53	0.00	0.01
219218_at	BAHCC1	4.99	0.76	0.15	1.69	0.00	0.00
221942_s_at	GUCY1A3	4.99	0.87	0.18	1.83	0.00	0.00
202660_at	ITPR2	4.99	0.70	0.14	1.62	0.00	0.00
228365_at	CPNE8	4.98	1.06	0.21	2.09	0.00	0.00
206945_at	LCT	4.97	0.51	0.10	1.42	0.00	0.00
212820_at	DMXL2	4.94	0.97	0.20	1.96	0.00	0.00
227400_at	NFIX	4.93	0.58	0.12	1.49	0.00	0.00
1566764_at	MACC1	4.92	0.78	0.16	1.72	0.00	0.00
209409_at	GRB10	4.86	0.80	0.17	1.75	0.00	0.00
202862_at	FAH	4.85	0.60	0.12	1.51	0.00	0.00
227235_at	GUCY1A3	4.84	1.01	0.21	2.02	0.00	0.00
230743_at	HOXB-AS3	4.83	0.51	0.11	1.43	0.00	0.00
224901_at	SCD5	4.83	0.72	0.15	1.65	0.00	0.00
243579_at	MSI2	4.82	0.51	0.11	1.42	0.00	0.00
237246_at	---	4.80	0.62	0.13	1.53	0.00	0.00
214651_s_at	HOXA10-HOXA9 /// HOXA9 /// MIR196B	4.79	1.57	0.33	2.97	0.00	0.00
1553311_at	C20orf197	4.78	0.72	0.15	1.65	0.00	0.00
206298_at	ARHGAP22	4.76	0.67	0.14	1.59	0.00	0.00
238058_at	LOC150381	4.76	0.65	0.14	1.57	0.00	0.00
237594_at	---	4.75	0.68	0.14	1.60	0.00	0.00
212646_at	RFTN1	4.75	0.58	0.12	1.50	0.00	0.00
201069_at	MMP2	4.75	0.88	0.19	1.84	0.00	0.00
1557527_at	---	4.74	0.68	0.14	1.60	0.00	0.00
208797_s_at	GOLGA8A	4.72	0.56	0.12	1.48	0.00	0.00
200670_at	XBP1	4.72	0.51	0.11	1.43	0.00	0.00
202662_s_at	ITPR2	4.71	0.50	0.11	1.42	0.00	0.00
201329_s_at	ETS2	4.68	0.63	0.13	1.55	0.00	0.00
241342_at	TMEM65	4.68	0.71	0.15	1.63	0.00	0.00
206582_s_at	GPR56	4.67	0.56	0.12	1.47	0.00	0.00
207034_s_at	GLI2	4.66	0.48	0.10	1.40	0.00	0.00
206478_at	KIAA0125	4.65	1.01	0.22	2.01	0.00	0.00
201242_s_at	ATP1B1	4.64	0.97	0.21	1.96	0.00	0.00
206574_s_at	PTP4A3	4.63	0.61	0.13	1.52	0.00	0.00
206950_at	SCN9A	4.61	0.58	0.13	1.50	0.00	0.00

1552364_s_at	MSI2	4.57	0.62	0.13	1.53	0.00	0.00
209386_at	TM4SF1	4.56	0.75	0.16	1.68	0.00	0.00
210145_at	PLA2G4A	4.56	0.76	0.17	1.69	0.00	0.00
229530_at	GUCY1A3	4.56	0.82	0.18	1.77	0.00	0.00
1555037_a_at	IDH1	4.56	0.68	0.15	1.60	0.00	0.00
212364_at	MYO1B	4.55	0.54	0.12	1.46	0.00	0.00
213217_at	ADCY2	4.55	0.49	0.11	1.40	0.00	0.00
213395_at	MLC1	4.54	0.74	0.16	1.67	0.00	0.00
213413_at	STON1	4.54	0.54	0.12	1.45	0.00	0.00
212276_at	LPIN1	4.51	0.54	0.12	1.46	0.00	0.00
201487_at	CTSC	4.50	0.51	0.11	1.42	0.00	0.00
239580_at	GUCY1A3	4.50	0.63	0.14	1.55	0.00	0.00
205653_at	CTSG	4.50	1.08	0.24	2.11	0.00	0.00
205600_x_at	HOXB5	4.50	0.51	0.11	1.43	0.00	0.00
225647_s_at	CTSC	4.49	0.58	0.13	1.50	0.00	0.00
219412_at	RAB38	4.47	0.53	0.12	1.44	0.00	0.00
201193_at	IDH1	4.46	0.60	0.14	1.52	0.00	0.00
231838_at	PABPC1L	4.46	0.49	0.11	1.41	0.00	0.00
209360_s_at	LOC100506403 /// RUNX1	4.45	0.52	0.12	1.44	0.00	0.00
206067_s_at	WT1	4.44	0.74	0.17	1.67	0.00	0.00
217620_s_at	PIK3CB	4.39	0.48	0.11	1.40	0.00	0.00
226065_at	PRICKLE1	4.39	0.59	0.13	1.50	0.00	0.00
205899_at	CCNA1	4.38	1.05	0.24	2.07	0.00	0.00
213150_at	HOXA10	4.38	1.06	0.24	2.09	0.00	0.00
235753_at	HOXA7	4.37	0.76	0.17	1.69	0.00	0.00
202932_at	YES1	4.37	0.67	0.15	1.59	0.00	0.00
209193_at	PIM1	4.35	0.54	0.12	1.46	0.00	0.00
202948_at	IL1R1	4.35	0.61	0.14	1.53	0.00	0.00
225233_at	MSI2	4.34	0.60	0.14	1.51	0.00	0.00
212386_at	TCF4	4.34	0.85	0.20	1.80	0.00	0.00
205624_at	CPA3	4.34	1.13	0.26	2.19	0.00	0.00
217975_at	WBP5	4.34	0.91	0.21	1.88	0.00	0.00
223939_at	SUCNR1	4.33	0.90	0.21	1.87	0.00	0.00
229199_at	SCN9A	4.32	0.51	0.12	1.43	0.00	0.00
229971_at	GPR114	4.31	0.53	0.12	1.45	0.00	0.00
209537_at	EXTL2	4.30	0.49	0.11	1.40	0.00	0.00
202933_s_at	YES1	4.28	0.65	0.15	1.57	0.00	0.00
205518_s_at	CMAHP	4.28	0.66	0.15	1.58	0.00	0.00
244881_at	LMLN	4.28	0.46	0.11	1.38	0.00	0.00
1559214_at	---	4.26	0.74	0.17	1.68	0.00	0.00
244457_at	---	4.25	0.61	0.14	1.53	0.00	0.00

239963_at	---	4.25	0.75	0.18	1.69	0.00	0.00
202718_at	IGFBP2	4.22	0.86	0.20	1.82	0.00	0.00
1558770_a_at	PIK3R6	4.21	0.46	0.11	1.37	0.00	0.00
219602_s_at	PIEZO2	4.20	0.59	0.14	1.51	0.00	0.00
204069_at	MEIS1	4.20	0.95	0.23	1.93	0.00	0.00
205582_s_at	GGT5	4.19	0.42	0.10	1.34	0.00	0.00
1559067_a_at	LOC158402	4.19	0.54	0.13	1.45	0.00	0.00
222303_at	---	4.18	0.66	0.16	1.58	0.00	0.00
206847_s_at	HOXA7	4.18	0.49	0.12	1.40	0.00	0.00
238658_at	---	4.17	0.43	0.10	1.35	0.00	0.00
201243_s_at	ATP1B1	4.17	0.61	0.15	1.53	0.00	0.00
223075_s_at	AIF1L	4.16	0.67	0.16	1.59	0.00	0.00
221781_s_at	DNAJC10	4.15	0.43	0.10	1.35	0.00	0.00
212274_at	LPIN1	4.14	0.45	0.11	1.37	0.00	0.00
230064_at	---	4.11	0.56	0.14	1.47	0.00	0.00
219036_at	CEP70	4.11	0.62	0.15	1.54	0.00	0.00
214228_x_at	TNFRSF4	4.11	0.46	0.11	1.38	0.00	0.00
1560500_at	---	4.09	0.49	0.12	1.40	0.00	0.00
226784_at	TWISTNB	4.09	0.54	0.13	1.45	0.00	0.00
201952_at	ALCAM	4.09	0.63	0.15	1.55	0.00	0.00
232151_at	MACC1	4.08	0.51	0.13	1.42	0.00	0.00
202661_at	ITPR2	4.08	0.46	0.11	1.37	0.00	0.00
242172_at	---	4.06	0.64	0.16	1.55	0.00	0.00
242476_at	---	4.04	0.67	0.17	1.59	0.00	0.00
209695_at	PTP4A3	4.04	0.46	0.11	1.38	0.00	0.00
226069_at	PRICKLE1	4.03	0.57	0.14	1.48	0.00	0.00
1552623_at	HSH2D	4.03	0.65	0.16	1.57	0.00	0.00
222146_s_at	TCF4	4.03	0.64	0.16	1.55	0.00	0.00
236488_s_at	---	4.03	0.48	0.12	1.40	0.00	0.00
212387_at	TCF4	4.02	0.64	0.16	1.56	0.00	0.00
213891_s_at	TCF4	4.01	0.67	0.17	1.59	0.00	0.00