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14. ABSTRACT The main goal of this work was to test and further develop a hypothesis that structural modifications and/or interference between two heterochromatin proteins: MNEI and HP1 lead to abnormal gene regulation and impaired myeloid differentiation, CML acceleration, blast crises and/or secondary acute leukemia. In specific aim 1, we separated monomeric and high molecular forms of MNEI by chromatography and gel electrophoresis and probed their primary structure by mass spectroscopy and Western blotting indicating the presence of MNEI-elastase complex also containing an unmodified peptide specific for the high molecular form. We raised antibodies against 3 distinct high molecular forms of MNEI. We found that these antibodies recognize elevated levels of high molecular MNEI co-expressed with HP1 in a subset of CML cases including blastic form of CML. In specific Aim 2 we constructed cell lines derived from a blastic CML cell model K562 expressing MNEI and HP1. We showed that expression of MNEI did not interfere with constitutive heterochromatin but significantly inhibited cell proliferation. Using high throughput gene expression analysis and chromatin immunoprecipitation, we found that HP1 and MNEI similarly target multiple chromosomal loci. Our results suggest that MNEI interacts with chromosomal loci vacated by HP1 during normal myeloid differentiation and that association with the same set of gene targets with HP1 may impair gene regulation in cases where both MNEI isoforms and HP1 are expressed.

15. SUBJECT TERMS
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INTRODUCTION

Chronic myelogeneous leukemia (CML) initially develops through the chronic phase when the affected myeloid progenitors excessively proliferate but then undergo complete differentiation and become mature blood cells. When the myeloid progenitors respond poorly to the proliferation suppressing therapy, they eventually loose the ability to fully differentiate, and the chronic diseases transform into accelerated forms, blast crises, or acute secondary leukemia. The general goal of this work is to understand the molecular basis of the incomplete myeloid differentiation resulting from CML in order to improve the prognostics of the disease transition from chronic to accelerated forms and to design therapies capable of preventing or reversing the abnormal differentiation leading to the disease acceleration and death. To achieve this goal, we focused our study on two epigenetic heterochromatin factors associated with normal myeloid differentiation and CML: heterochromatin protein HP1 and MNEI. In terminally differentiated cells, HP1 is dramatically reduced but another heterochromatin-associated protein, MNEI, accumulates in chromatin of mature normal granulocytes. Our previous studies suggested that MNEI replaces HP1 to promote chromatin condensation and heterochromatin spreading in terminally differentiated myeloid cells. Based on two key observations that an altered high molecular isoform of MNEI accumulates in CML-derived cells and that MNEI and HP1 are co-expressed in accelerated phase and blastic CML, we hypothesized that the structural modification of MNEI and its interference with HP1 leads to abnormal regulation of genes critical for myeloid differentiation and leading to partial cell de-differentiation, CML acceleration, blast crises and/or secondary acute leukemia.

BODY

During the reported period (17 months), the work involved the principal investigator (Dr. Sergei Grigoryev, 20% effort), the co-investigator (Dr. David Claxton, 2% effort), and the postdoctoral scholar (Dr. Evgenya Popova, 100% effort). The initial period of performance (1 March 2006 – 28 July 2007) was extended at no cost to 28 July 2008 with understanding that the final report is due on 28 August 2008. The project was divided into two tasks (specific aims):

Task 1: *To determine the primary structure of MNEI isoforms and their association with chromatin and HP1 in normal and CML-derived myeloid blood cells.* In this set of experiments, we worked on isolating and identifying the primary structure of the high molecular forms of MNEI: MNEI^{72} and MNEI^{65}. Dr. Claxton provided cryopreserved blood samples where we

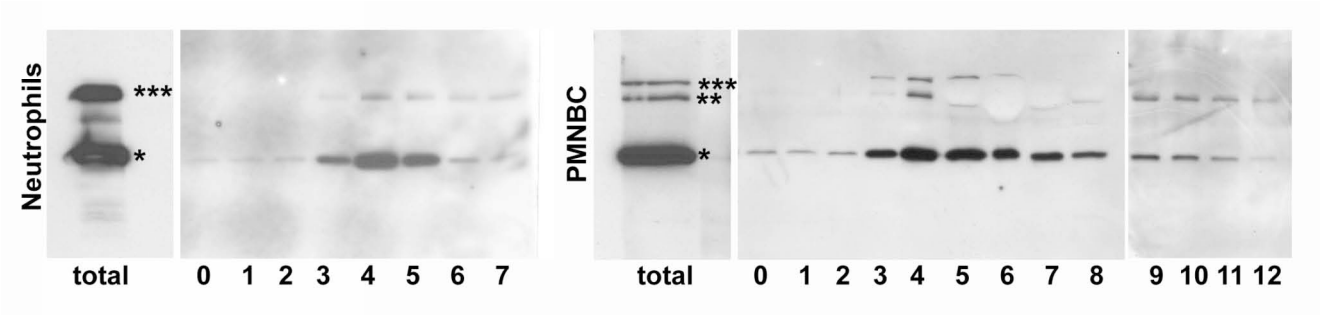

Figure 1. SDS-PAGE and Western blotting with anti-MNEI antibodies of total nuclear protein from Neutrophils (left) and PMNC (right) and protein fractions (fraction numbers indicated at the bottom) separated by ion exchange chromatography on HiTrap Q HP column (Pharmacia)

detected high level of MNEI^{65} using Western blotting. We also collected discard white blood cells from normal blood donors that contained high levels of MNEI^{72}. During the first stage of this work we had our IRB Protocol for work with discard normal blood and CML patients to be examined and found to comply with USAMRMC human subject protection regulations on 12 June 2006. We then isolated nuclei from human neutrophils and peripheral blood mononuclear cells (PMNC), produced nuclear extracts by treatment with 0.35 M NaCl and separated MNEI^{72} and MNEI^{65} using ion-exchange chromatography on FPLC system (Pharmacia). Initially, the MNEI yield was insufficient for analysis but we overcame this obstacle by modifying the elution buffer and including appropriate dialysis before loading on the chromatography column. The column eluates were analyzed by Western blotting with anti-MNEI antibodies (Fig. 1) showing a very abundant band of monomeric MNEI^{42} (*) and higher molecular weight bands for MNEI^{72} (***) and MNEI^{65} (**). The fractionated proteins were separated on SDS-PAGE, bands of interest were excised, alkylated, and trypsin digested. Mass spectrometry was conducted in-house at the Penn State College of Medicine Macromolecular Core Facility equipped with Applied Biosystems 4800 Proteomics Analyzer (MALDI TOF-TOF) with collaboration of Dr. Bruce Stanley, the facility director. We compared the molecular weights of peptides from the isolated protein with those theoretical trypsin-derived peptides of MNEI in databases using PeptIdent software and identified and mapped the peptides originating from the monomeric MNEI and its high molecular weight MNEI^{72}. These peptides appear to cover the beginning and the end of predicted MNEI sequence (Fig. 2) confirming that the complete MNEI molecules are present in the low- and the high molecular weight MNEI forms.

The yield, and especially the representation of MNEI^{65} peptides in mass-spectrometry was significantly lower than for MNEI^{72} and so far we were not able to unambiguously identify its binding partner from the masspec data. The molecular weight of one MNEI isoform (72 kDa) indicated that this band represents the complex of MNEI with its natural target in myeloid cells, neutrophil elastase. Additional information that became available from the literature confirmed

that MNEI forms an SDS-stable complex with neutrophil elastase (*1*) and that the MNEI-neutrophil elastase complex is translocated to the nucleus (*2*). We confirmed this hypothesis by forming MNEI-elastase complex that by its electrophoretic mobility is similar to MNEI^{72}. We concluded that MNEI-neutrophil elastase complex is formed in myeloid cells, and is likely to be found in the mature granulocytes of CML patients in the form MNEI^{72}. Further experiments showed that both MNEI^{72} and MNEI^{65} bands reacted with antibodies to MNEI-elastase complex.

meqlssantr faldlflals ennpagnifi spfsissama mvflgtrgnt aaqlsktfhf
ntveevhsrf qslnadinkr gasyilklan rlygektynf lpeflvstqk tygadlasvd
fqhasedark tinqwvkgqt egkipellas gmvdnmtklv lvnaiyfkgn wkdkfmkeat
tnapfrlnkk drktvkmmyq kkkfaygyie dlkcrvlelp yqgeelsmvi llpddiedes
tglkkieeql tleklhewtk penldfievn vslprfklee sytlnsdlar lgvqdlfnss
kadlsgmsga rdifiskivh ksfvevneeg teaaaatagi atfcmlmpee nftadhpflf
firhnssgsi lflgrfssp

Figure 2. MNEI protein sequence showing the peptides identified by mass spectrometry of MNEI^{42} only (single underline), MNEI^{72} and MNEI^{42} (double underline) and MNEI^{72} only (thick red line) as well as the nuclear localization sequence peptide (dashed blue line) exposed in MNEI- elastase complex (*2*).

After initial analysis of the high molecular forms of MNEI and positive identification of MNEI it was still unclear if the other high molecular weight form(s) could be attributed to RNA sequence alterations (alternative splicing, mutations) rather than posttranslational modifications or protein homo- and heterodimers. This was also an important to examine since a recent publication (*3*) has revealed MNEI mutations associated with other (non-leukemia) types of cancer. To address this possible sequence variations, we used RT-PCR using pairs of primers: Lei20F/Lei25R and Lei24F/Lei13R (see appendix, Table II) to amplify and sequence entire MNEI transcripts including potential mRNA splice variants of MNEI. Screening of normal blood and a number of leukemia samples including all samples expressing the MNEI^{65} MNEI^{72} isoforms (*4*) showed no variations from the canonic human MNEI sequence. We thus can practically rule out the possibility that the high molecular weight forms of MNEI represent splice variants or mutant proteins. This is an important confirmation of our initial hypothesis that posttranslational protein modifications are responsible for MNEI alterations seen in CML blood.

After coming to the conclusion that DNA and RNA sequence variations are unlikely to cause the observed protein modifications, we used the masspectrometry data to build the maps of MNEI peptides. Importantly, the MNEI^{72} map contains a peptide (FQSLNADINKR) that is absent from the monomeric form. Because the monomeric MNEI peptides are much more abundant than MNEI^{72} peptides, we concluded that this peptide is modified in the monomeric form. To test the expression of the MNEI isoforms we raised the following rat polyclonal antibodies: *MNEI28* against the whole MNEI-elastase complex; *MNEI29* against synthetic MNEI peptide RFQSLNADINKR; *MNEI30* against synthetic MNEI peptide

KKDRKTVKMMYQKKK exposed in MNEI-elastase complex and targeting MNEI into the nucleus (*2*). The antibodies were raised commercially at Cocalico Biologicals, Inc (Reamstown, PA). We used Western blotting to test these antibodies against isolated MNEI and blood samples derived from various myeloid cells and CML patients (Fig 3). Our results show that all three antibodies recognized $MNEI^{65}$ and $MNEI^{72}$ isoforms expressed in a subset of CML cases including both the blastic form of CML and also several chronic CML cases (Fig. 3). Since the levels of the MNEI forms recognized by α-*MNEI28*, *29*, and *30* do not strongly correlate with the levels of the $MNEI^{65}$ and $MNEI^{72}$ isoforms recognized by total α-MNEI, it is apparent that the new antibodies each recognize a subset of the total population of MNEI high molecular isoforms.

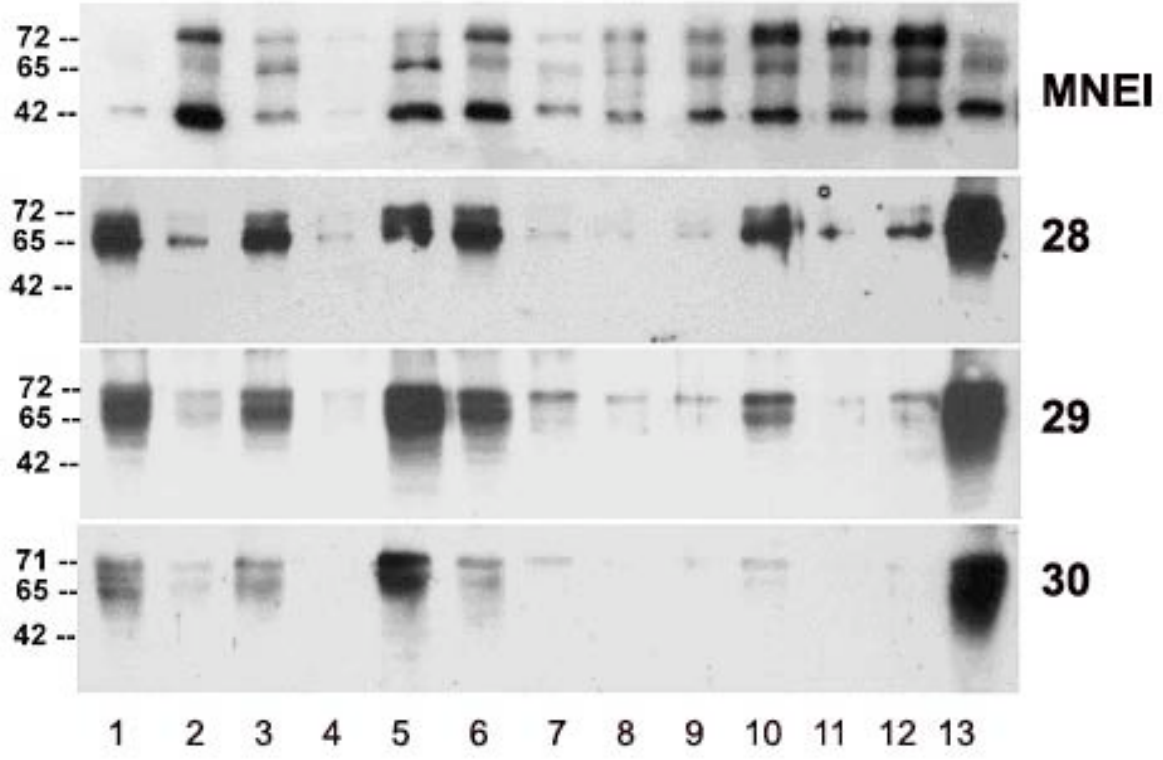

Figure 3. Western blotting of protein from the nuclei of peripheral blood mononuclear cells isolated from patients diagnosed with: CML, blastic form (1, 3); CML, accelerated phase (2); CML, chronic phase (5, 6, 8-10, 12); proliferative form of chronic myelomonocytic leukemia (4), unclassified myeloproliferative disorder (7); acute myeloid leukemia (11, 13). Nuclear samples were normalized to equal loading of histones before electrophoresis. The blots were probed with rabbit polyclonal antibodies against total MNEI (top panel) and rat polyclonal antibodies *MNEI28* against MNEI-neutrophil elastase complex, rat polyclonal antibodies *MNEI29* against MNEI peptide RFQSLNADINKR, and rat polyclonal antibodies *MNEI30* against MNEI peptide KKDRKTVKMMYQKKK as indicated.

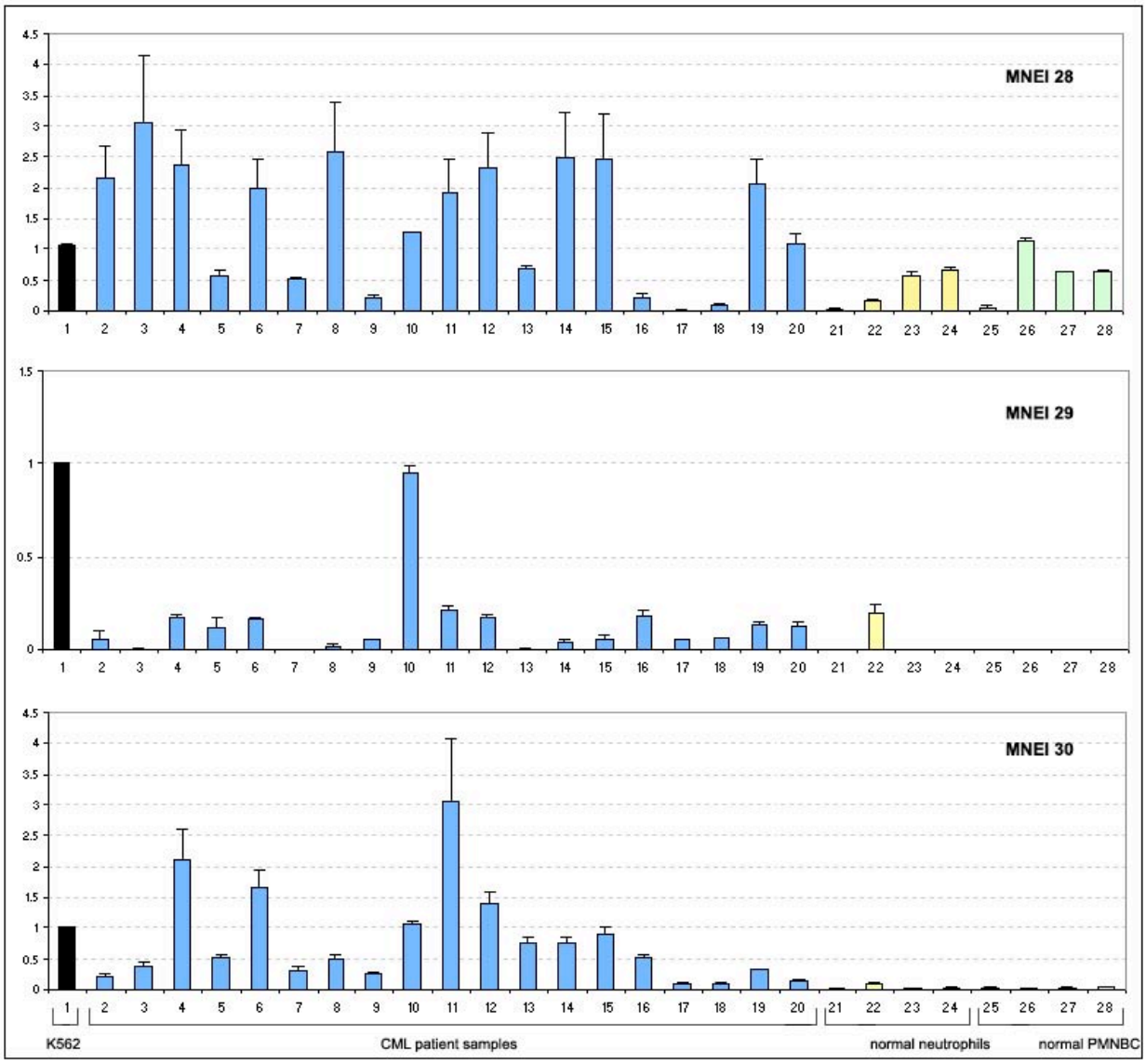

Figure 4. Histograms showing relative expression levels (normalized to 1.0 in K562 cells) of MNEI isoforms recognized by *MNEI28*, *MNEI29*, and *MNEI30* antibodies. Nuclear protein samples were isolated from K562 cells (1); peripheral blood mononuclear cells isolated from patients diagnosed with CML chronic phase (2,3, 7-12, 14, 15-21); CML blastic form (4); CML, accelerated phase (5, 6); proliferative form of chronic myelomonocytic leukemia (9, 13), and unclassified myeloproliferative disorder (15) and subjected to gel electrophoresis and Western blotting. Levels of expression were determined from scanning and digitizing multiple Western blotting auotoradiographs after enhanced chemiluminescent detection with OptiQuant software as we described previously (3). Nuclear samples were equalized for equal loading of histones before electrophoresis. The blots were probed with

To monitor the levels of MNEI isoforms across a large number of nuclear samples from normal myeloid cells and leukemia blood samples and determine the extent of its correlation with CML and other MPD, we conducted semi-quantitative Western blotting as we did before with our anti-total MNEI and HP1 antibodies (3). In this analysis we used CML patient samples that we already used in our previous work (3), new CML samples collected in the CI laboratory, as well granulocytes and PMNC obtained from blood of four different normal donors. It was important to include both granulocytes as well PMNC in the study because blood samples are taken from different patients may contain variable compositions of polymorphonuclear

granulocytes and mononuclear blood cells. Histograms representing the relative expression levels of the three MNEI antigens across a panel of CML and normal samples vs. the expression level in K562 cells (designated as 1.0) show that while *MNEI28* reacts with the nuclei from both CML patients and normal blood cell samples, *MNEI29* and *MNEI30* react strongly with MNEI[65] derived from multiple CML patients and show very little reaction with protein from granulocytes or PMNC from normal donor blood (Fig. 4).

Table I. Relative expression levels of total MNEI, HP1β and three MNEI isoforms in the nuclei of K562 (1); peripheral blood mononuclear cells isolated from patients diagnosed with CML chronic phase (3, 7-12, 14, 16-20); CML blast crisis (2, 4, 6); CML accelerated phase (5); proliferative form of chronic myelomonocytic leukemia (9, 13), and unclassified myeloproliferative disorder (15). Levels of expression were determined by scanning Western blots probed with previously described anti-MNEI and anti-HP1β (3) as well as with new antibodies *MNEI28*, *MNEI29*, and *MNEI30* antibodies as indicated. Total MNEI levels were normalized to 1.0 in normal granulocytes, all other antigen levels were normalized to 1.0 in K562 cells as we described before (3). Color code indicate high expression level (red), intermediate expression (yellow) , and low expression (blue).

#	Sample type	MNEI	HP1beta	MNEI28	MNEI29	MNEI30
1	K562 cell culture	0.06	1	1.06	1	1
2	CML-BC	0.81	0.03	2.17	0.06	0.21
3	CML	1.57	0.02	3.06	0	0.39
4	CML-BC	0.23	0.47	2.37	0.17	2.1
5	CML(AC)	1.83	0	0.58	0.12	0.5
6	CML-BC	1.01	0.28	1.98	0.16	1.66
7	CML	1.63	0.04	0.52	0	0.31
8	CML	1.46	0.06	2.58	0.01	0.49
9	CMML	0.23	0.06	0.22	0.05	0.24
10	CML	1.58	0.06	1.28	0.95	1.06
11	CML	1.45	0.01	1.91	0.2	3.07
12	CML	1.45	0	2.31	0.17	1.38
13	CMML	1.59	0.07	0.68	0	0.75
14	CML	1.03	0.05	2.48	0.03	0.75
15	MPD	1.37	0.04	2.44	0.05	0.89
16	CML	0.37	0.44	0.22	0.18	0.52
17	CML	0.96	0.05	0.01	0.05	0.08
18	CML	1.43	0.06	0.09	0.06	0.09
19	CML(Ph-ve)	2.6	0.04	2.06	0.13	0.34
20	CML	3.6	0.02	1.08	0.13	0.14
21	Gran 020608 normal	1.12	0.05	0.03	0	0.03
22	Gran 113005 normal	1	0.03	0.15	0.2	0.08
23	Gran 072605 normal	1.05	0.01	0.58	0	0.02
24	Gran 072605normal	0.79	0.02	0.65	0	0.03
25	PMNC 020608normal	0.07	0.08	0.05	0	0.04
26	PMNC 030707normal	0.22	0.17	1.15	0	0.02
27	PMNC 072605normal	0.22	0.07	0.62	0	0.03
28	PMNC 072605normal	0.15	0.02	0.62	0	0.05
	Median :	1.05	0.05	0.68	0.05	0.31
	Group I =	0 - 0.69	0 - 0.03	0 - 0.45	0 - 0.03	0 - 0.20
	Group II =	0.70 - 1.39	0.04 - 0.07	0.46 - 0.90	0.04 - 0.07	0.21 - 0.41
	Group III =	1.40 - max	0.08 - max	0.91 - max	0.08 - max	0.42- max
Disease correlation (r):		0.471	0.14798	0.473965	0.270974	0.449464
	p value:	<0.00953	<0.4053	<0.01804	<0.1779	<0.01667

When compared to the expression levels of the total MNEI and HP1β including those suggested previously to correlate with CML acceleration, it becomes clear that the levels of total MNEI and two new antibodies, *MNEI28* and *MNEI30* show significant correlation with disease (total MNEI: r=0.48, p<0.01; *MNEI28*: r=0.44, p<0.02; *MNEI30*: r=0.45, p<0.02) while *MNEI29* and HP1β levels show weaker and less significant correlations (Table I). No special alteration in the MNEI isoforms expression has been found to be associated with accelerated CML forms as well as with the proliferative CMML and MPD samples included in the study. Note, however, that the blastic and the accelerated forms of CML (samples 2, 4-6) fall into the high and intermediate expression groups detected by *MNEI29* and *MNEI30* suggesting that these antibodies may be used for identifying structural and differentiation abnormalities in myeloid cells potentially leading to CML acceleration

To examine MNEI isoforms distribution in the nuclei of the CML-derived blood cells, we conducted immunofluorescence experiments with the blood cell samples (including those cells that we already used in our previous work (3) as well as new CML samples collected in the CI laboratory) by staining cells with new antibodies against the three MNEI isoforms in combination with Hoechst 33258 staining of total chromatin DNA. An example of such staining is shown on figure 5. In all cells tested, antigens recognized by antibodies *MNEI28* and *MNEI30* were distributed across both nuclei and cytoplasm (Fig. 5, panels 1,2, and 5, 6). In contrast, *MNEI29* showed a predominately nuclear staining consistent with the presence of the unmodified MNEI peptide RFQSLNADINKR specifically in the nuclear MNEI (panels 3,4).

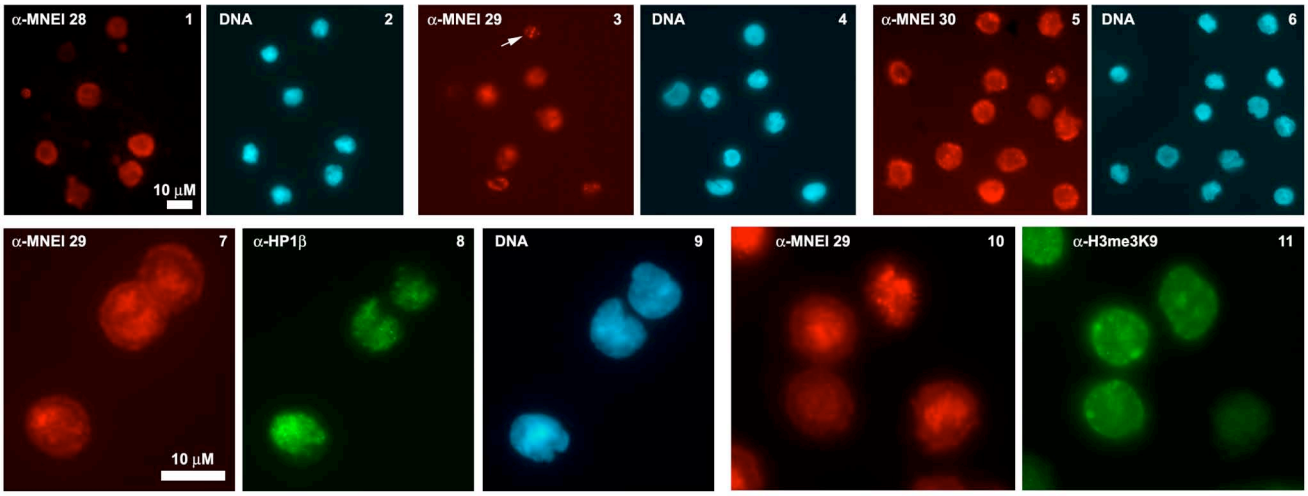

Figure 5. Intracellular localization of MNEI isoforms in blood cells. Immunofluorescence microscopy of blast crisis CML-derived blood cells (Sample #4 on Table I) stained with antibodies against *MNEI28* (1), *MNEI29* (3, 7, 10), *MNEI30* (5), HP1β (8), histone $H3me_3K9$ (11) and Hoechst 33258 for DNA (2, 4, 6, 9). Panels 1-6 mag. 400x; panels 7-11 mag. 1000x; scale bars, 10 μm.

For the blood cell samples showing coexpression of HP1β and *MNEI29* isoform (Table I), we did double immunofluorescence staining with anti-*MNEI29* and HP1β, and with anti-*MNEI29*

and histone H3me3K9 methylation, as well as total chromatin DNA. An example of such staining is shown on Fig. 5, panels 7-11. In most of the cells, *MNEI29* (red, panels 7, 10) was co-localized with HP1β signal (green, panel 8) but not with H3me3K9 (green, panel 11). However, in some morphologically distinct cells, *MNEI29* formed characteristic foci (arrows on panel 3). In all such cells HP1β was dramatically reduced and H3me3K9 evenly redistributed in the nucleus suggesting global rearrangement of heterochromatin in these cells. This is consistent with our hypothesis that the increased accumulation of nuclear MNEI may interfere with HP1β and lead to a global heterochromatin rearrangement. The nature of cells expressing highly focal MNEI remain to be determined.

To examine nuclear MNEI distribution across a multiple morphologically-sorted or HP1-co-expression sorted nuclei, *MNEI29* and HP1β immunofluorescence signals from every nucleus in several fields were digitized using image capturing with CCD camera and analysis of intensities in a multiple cell population was conducted using Image-Pro Plus software (Media Cybernetics). Two examples of scattergrams showing the distribution of *MNEI29* isoforms and HP1β in the cell population from two blast crisis CML (samples 4 and 6 on Table I) highly expressing HP1 show a relatively even distribution of *MNEI29* and HP1β across the cellular population (Figure 6). Because *MNEI29* expression was not significantly correlated with CML (Table I) and the other two antigens, *MNEI28* and *MNEI30*, were not nuclear, it was not possible to relate MNEI isoform immunofluorescence levels in the nuclei with CML progression.

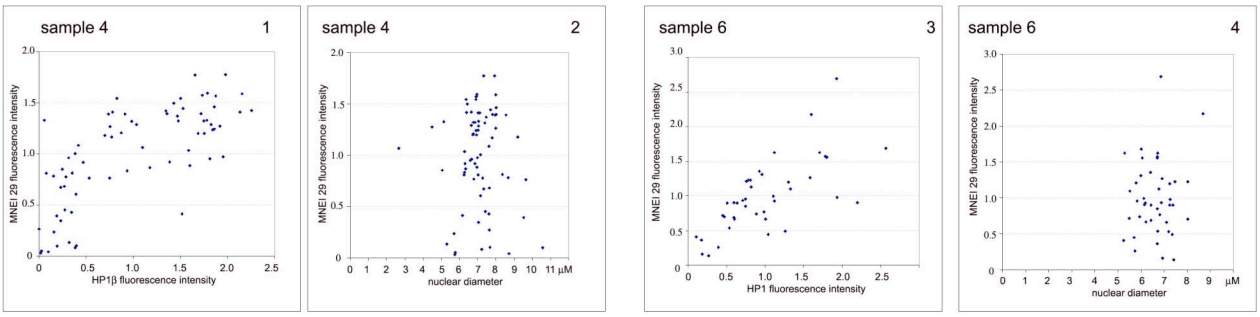

Figure 6. Scattergrams showing distribution of *MNEI29* and HP1b relative fluorescence intensities (average intensity designated as 1.0) and cell diameters measures for populations of cells stained by antibodies from samples 4 and 6 (CML, blast crisis). Fluorescence intensities were measured after immunostaining with antibodies against *MNEI29* and HP1β. Nuclear diameters were measure on nuclear DNA stained by Hoechst 33258.

In summary, our work on Task 1 resulted in revealing two structural isoforms of MNEI, *MNEI28* and *MNEI30* (representing a complex of MNEI with elastase and antigenic exposure of the MNEI nuclear localization signal peptide respectively) that can serve as markers of abnormal myeloid proliferation associated with CML. This study also confirms our hypothesis that certain structural alterations of MNEI distinguish normal chromatin from chromatin in CML blood cells.

We also identified a peptide modified in cytoplasmic MNEI and raised a new antibody against the unmodified peptide that turned to be specific for the nuclear form of MNEI. Nuclear MNEI was co-expressed and co-localized with HP1β in a number of CML-derived cells but not with constitutive heterochromatin consistent with our original hypothesis suggesting that nuclear MNEI and HP1 co-expression under pathological conditions may be associated with their interaction leading to heterochromatin spreading to normally euchromatin chromosomal loci.

Task 2. *To determine the chromosomal loci and genes directly affected by MNEI/HP1 interference.*

We constructed MNEI-expressing and HP1-expressing vectors by subcloning the sequences human MNEI cDNA (Genbank Acc# M93056) and the $MNEI_{P14T->R}$ mutant as well as HP1α (Genbank acc# AF21690_1) plasmids that we already have in the pEGFP-C3 expression vector and test the constructs by sequencing. We used this vector because it delivered a high level of protein expression without induction and the GFP protein conjugation highly improved the selection process for raising the cell lines and also served as an excellent control for the expression level with and without the protein fusion.

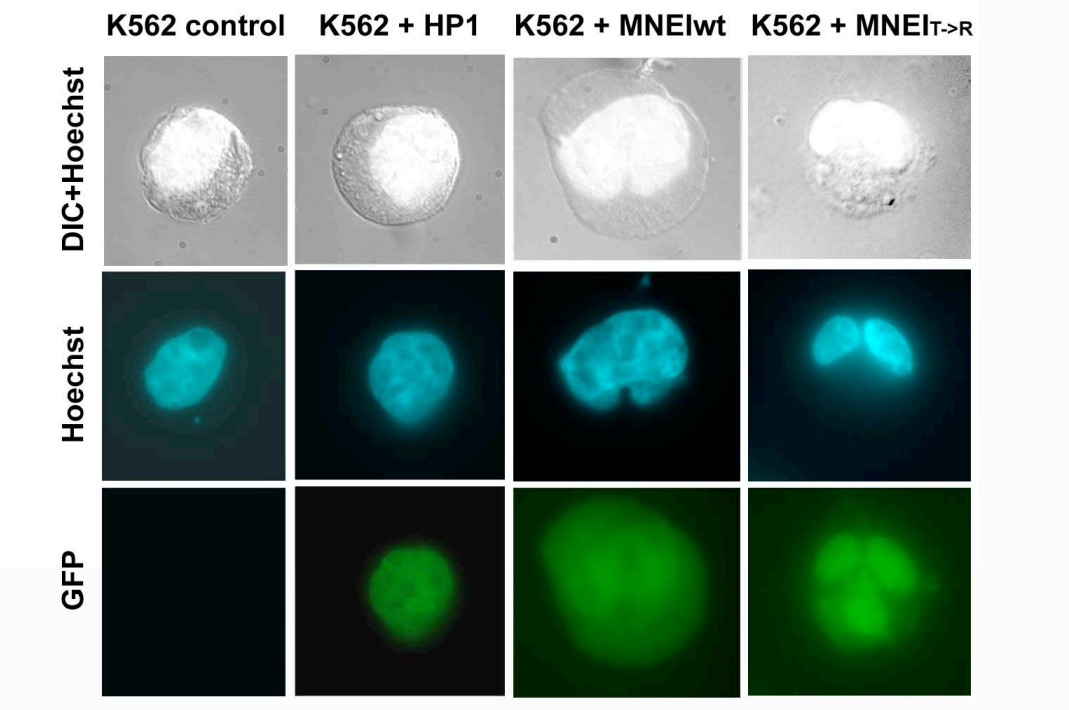

Figure 7. K562 cells – control and transfected with HP1α-GFP, $MNEI_{wt}$-GFP, and $MNEI_{T->R}$-GFP visualized using differential interference (DIC) microscopy, and direct fluorescence with Hoechst 33258 (blue) showing DNA and GFP fluorescence (green) showing the localization of HP1α and MNEI as indicated. The control cell does not have green fluorescence.

We transfected the wild-type MNEI and $MNEI_{T->R}$ as well as the HP1 constructs into K562 and U937 cells and selected the transfected cells using neomycine resistance marker and

then selected the clones expressing MNEI and HP1α as well as control vectors expressing GFP from the same promoter using both GFP fluorescence and imunofluorescence with specific antibodies. Both cell types produced a high level of expression with both types of genes seen as GFP fluorescence with nuclear localization for HP1α and nucleocytoplasmic for MNEI (Fig. 7). For K562 cells, we observed that HP1α and $MNEI_{T->R}$ expression were not deleterious for the cells over a long (more than several weeks) incubation period and we successfully established the HP1α and the MNEI-expressing clones using selection with neomycine. In contrast, with U937 in spite of testing several alternative transfection protocols (Lipofectamine, GenePorter, FuGENE) and achieving a very high level of initial transfection using electroporation with Cell Line Nucleofector kit V (Amaxa Inc.) method, we could not obtain cells that would survive while transfected with expression vector. The survival rate did not depend on the nature of the protein and we concluded that this type of cells was not amenable for stable transfection under our conditions. Because even in the absence of MNEI and HP1α expression, the control vector alone was sufficient to eliminate the transfected U937 cells from population especially under neomycin selection we concluded that even without protein induction our transfection procedures were deleterious for these cells. We therefore focused our research on the work with K562 cell lines expressing MNEI and HP1α.

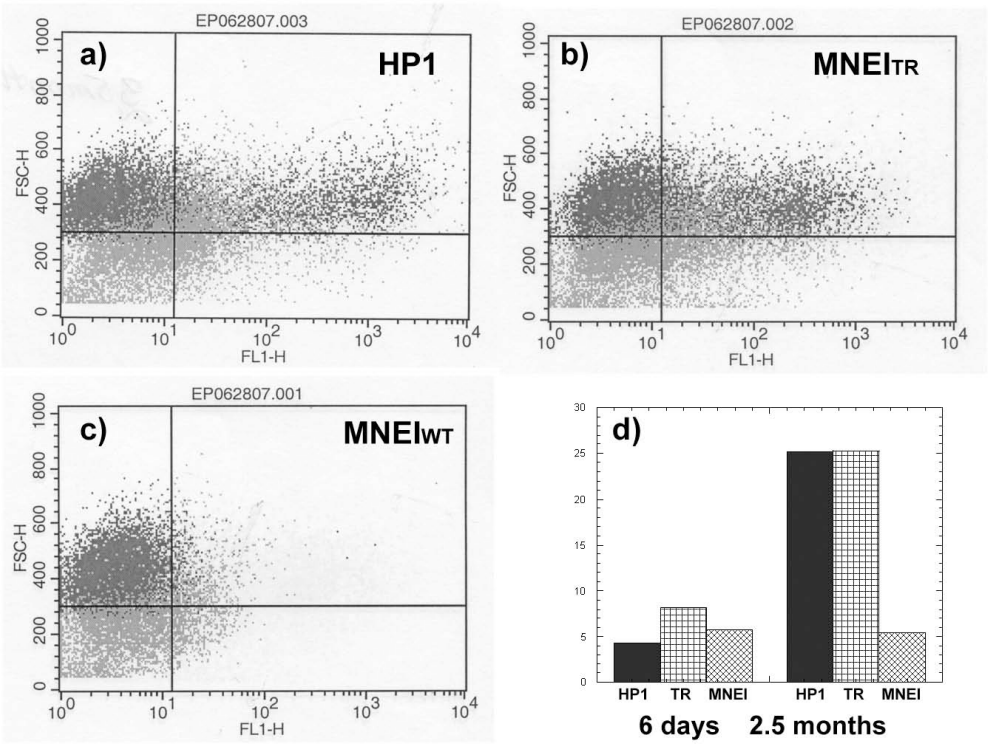

Figure 8. Top panels: results of FACS analysis of K562 cells expressing a) HP1-GFP, b) $MNEI_{T->R}$-GFP, and c) $MNEI_{wt}$-GFP after a period of 2.5 months selection with G-418. Upper right quadrants represent alive GFP-fluorescent cells. Panel d: percent of live cells population expressing HP1-GFP, $MNEI_{T->R}$-GFP, and $MNEI_{wt}$-GFP after a period of 6 days or 2.5 months selection with G-418.

We examined chromatin condensation, as localization of epigenetic chromatin factors in the MNEI- and HP1α-expressing cells using immunofluorescence detection of endogenous methylated histone H3 (H3me3K9) and another HP1 isoform, HP1β. We found that in these cells, MNEI or HP1α expression did not interfere with nuclear localization of HP1β or with constitutive heterochromatin marker H3me3(K9). We then characterized the growth rates, and nuclear localization of MNEI and HP1α by direct fluorescence of GFP using FACS sorting Expression of either HP1α or $MNEI_{T->R}$ was not deleterious for K562 so that we could propagate cell cultures expressing these proteins for more than 2.5 months without problem (Fig. 8 a,b). Expression of wild type MNEI while initially as efficient as with $MNEI_{T->R}$, upon prolonged incubation lead to a gradually decreasing number of living cells expressing $MNEI_{wt}$ with a significant number of cells still expressing MNEI after 2.5 months in culture without selection (Fig. 8 c,d). In all subsequent experiments we used cultures stably expressing GFP-HP1α, GFP-MNEI and GFP control vector and sorted by FACS to select for only GFP-expressing cells.

To determine specific chromosomal loci and genes affected by MNEI/HP1α co-expression, we conducted expression microarray hybridization experiments with RNA isolated from MNEI-, HP1α- and control GFP-expressing K562 clones. For hybridization, we used HumanWG-6 v3.0 whole-genome gene expression arrays (Illumina) that allows one to assay simultaneously more than 48,000 transcripts contained in the National Center for Biotechnology Information Reference Sequence database. This analysis was conducted at the Hershey Medical Center Functional Genomics core facility. Among 29,388 transcripts detected in our K562-derived clones we found 111 activated and 1094 repressed by MNEI and 104 activated and 1029 repressed by HP1α (>1.4-fold difference). Remarkably, for the both factors the majority of the expression level changes involved repression consistent with their presumed role as chromatin repressors and the majority of the changes caused by HP1 and MNEI appeared to be similar: Venn diagram of the genes changed more than 1.4-fold revealed a strong overlap between MNEI- and HP1-expressing cells (Fig. 9). This similarity is pronounced even stronger for highly changed genes (3-fold and more). A list of the 323 transcripts corresponding to the blue overlap zone in the Venn diagram showing coordinated changes caused by MNEI and HP1 overexpression is shown in table III included in the appendix. Among the transcripts strongly repressed by MNEI and HP1α there are a major hemapoietic transcriptional regulator ETS1 activating granulocyte macrophage colony-stimulating factor transcription (*5*) and a number of other genes, STC2 (*6*), G0S2 (*7*), ITGB2 (*8*), ANG1 (*9*) whose repression is associated with leukemia cell proliferation. Among much fewer activated transcripts, increased expression of one

(AXL) is associated with drug resistance and adverse prognosis in AML (*10*). We also noticed that several coordinately regulated genes occupying adjacent positions on human chromosomes (such as variably charged X-linked mRNA) confirming our hypothesis that HP1 and MNEI can interact with the same chromatin loci and interactively affect gene expression. Functional significance of many genes regulated by HP1α and MNEI requires a further study.

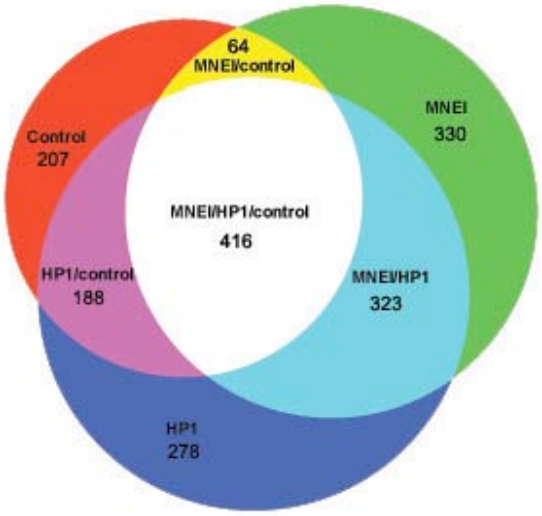

Figure 9. Venn diagram showing coordinated expression level changes (more than 1.4-fold) for RNA isolated from: control GFP-expressing K562 cells (red circle for non-overlapping transcripts); HP1β-expressing K562 cells (green circle for non-overlapped genes) and MNEI-expressing K562 cells (blue circle for non-overlapped genes).

We used ChIP (chromatin immunoprecipitation) in order to identify candidate genes directly binding MNEI as a result of HP1α or MNEI expression and to determine whether the levels of the principle heterochromatin marker, histone H3me3K9 methylation, or MNEI itself on selected chromosomal loci involved in CML could be affected by MNEI or HP1α expression. We have successfully developed conditions for chromatin immunoprecipitation (ChIP) for MNEI in combination with H3me3K9 based on the protocol that we formerly used for another nuclear serpin, MENT (*11*). Immunoprecipitated DNA concentrations were determined using real-time PCR detection (SYBR green detection system) with constitutive heterochromatic DNA probe (α-satellite), a silent control gene probe (β-globin), and probes for the promoter and coding regions of two key genes associated with CML: junB whose inactivation leads to a CML-like myeloproliferative disorder and blast crisis (*12*) and c-myc whose deregulated expression inhibits terminal differentiation in myeloid cells (*13*). Immunoprecipitation without antibodies and with pre-immune sera was used as control for background ChIP levels in each set of experiments. The results of the ChIP analysis (Fig. 10) show that histone H3me3K9 was not significantly changed on constitutive heterochromatin (α-satellite) and slightly changed only on c-myc promoter in both HP1α- and MNEI-expressing cells. There was a significant increase in MNEI levels associated with junB promoter and the coding region in both HP1 and MNEI-expressing cells and also increase in MNEI on c-myc coding regions. Thus, our ChIP analysis show that HP1α and MNEI overexpression does not affect constitutive heterochromatin but rater promotes MNEI

association with genes functionally associated with CML. This result is in an excellent agreement with colocalization of HP1β and MNEI in euchromatin in the cells derived from CML patients (see Fig. 5, panels 7-11) and with their coordinated effect on gene expression (Fig. 9 and Table III, see appendix) confirming our hypothesis that the HP1 isoforms and MNEI may regulate a similar set of genes in CML-derived cells.

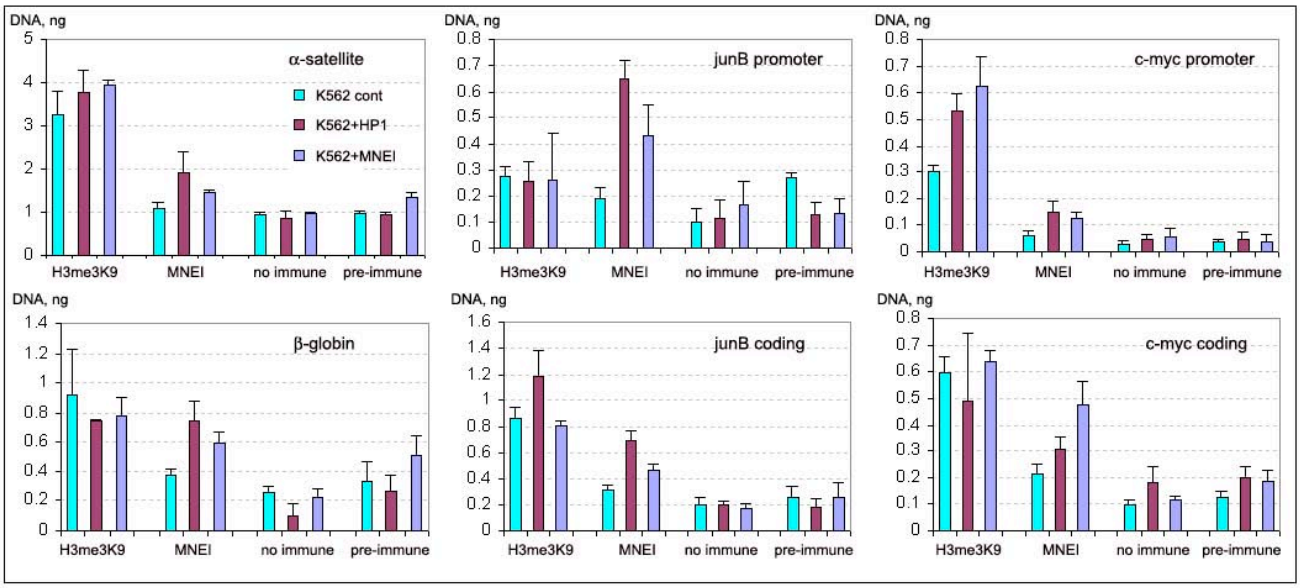

Figure 10. Chromatin immunoprecipitation (ChIP). Chromatin proteins were crosslinked with formaldehyde *in situ* and isolated from K562 cells expressing control GFP vector (K562 cont), HP1α (K562+HP1), and MNEI (K562+MNEI) as indicated by the color key. ChIP reactions were conducted as described (5) with ChIP grade anti-H3me$_3$K9 antibodies from Abcam (ab8898) and anti-MNEI antibodies described before (4). Control experiments were conducted without added antibodies (no immune) and with pre-immune sera as indicated below the X-axes to establish the background ChIP levels. Concentrations of immunoprecipitated DNA (nanograms) shown at the Y-axes were determined by real-time PCR (SYBR green detection system) with standard DNA primers for six sets of primers specific for coding region of β-globin gene (Quiagen, cat#QT00244489), coding region of junB (Quiagen, cat#QT00201341), and custom sunthesized primers for α-satellite repeats, promoter of junB, and promoter and coding regions of c-myc (Table II in the appendix).

KEY RESEARCH ACCOMPLISHMENTS

- we isolated and conducted mass-spectrometric mapping of high molecular weight isoforms of MNEI associated with CML.

- The high molecular form of MNEI was found to represent independently expressed complex of MNEI with several proteins including MNEI-elastase and to contain an unmodified peptide RFQSLNADINKR not found in monomeric MNEI.

- we established conditions for highly efficient and quantitative Reverse Transcription-PCR of MNEI to show that MNEI mRNA sequences were not altered in CML patients and

conclude that the CML-linked changes are associated with posttranslational protein modifications.

- We raised antibodies against MNEI/elastase complex and specific peptides exposed in the modified MNEI. We found that these antibodies recognize MNEI65 isoforms elevated in a subset of CML-derived cells including the blastic form of CML.

- MNEI expression was established in K562 cells and used to demonstrate that MNEI accumulation in the nucleus does not interfere HP1 localization in a short term but that expression of wild-type MNEI significantly inhibits cell proliferation over prolonged incubation and this inhibition required intact protease inhibitory activity of MNEI.

- We conducted a high-throughput analysis of gene expression levels affected by HP1α and MNEI to reveal a set of genes coordinately regulated by HP1α and MNEI. No genes oppositely regulated by HP1α and MNEI were found suggesting that these two proteins fulfill a similar gene regulatory function in undifferentiated and differentiated myeloid cells respectively.

- ChIP analysis shows that HP1α and MNEI similarly regulate MNEI level at CML-associated junB and c-myc genes and that MNEI level variations are not associated with changes in constitutive heterochromatin marker, histone H3me3K9.

REPORTABLE OUTCOMES

- a preliminary account of this work was reported at the “Road to a cure: the chronic myelogeneous leukemia research program meeting”, December 06, Orlando, Florida.

- Sequences of PCR primers and Reverse Transcription-PCR and real time PCR conditions optimized for quantitative MNEI expression analysis and ChIP analysis with has been designed and tested (see Table II in the appendix).

- 11 vectors expressing high levels of HP1 and MNEI without deleterious cell effects have been constructed and tested.

- 4 different lines of K562 cells stably expressing $MNEI_{wt}$ (4 clones), $MNEI_{T->R}$ (4 clones), and HP1 (1 clone) and control GFP vector (2 clones) have been constructed, selected, and stably propagated in culture.

- 3 different rat polyclonal antibody preparations were raised and tested to specifically recognize high molecular weight MNEI isoforms.

- 1205 genes regulated by MNEI and 1133 transcripts regulated by HP1α overexpression in CML-derived K562 cells were identified. A list of 323 transcripts showing coordinated changes caused by MNEI and HP1 overexpression is included in the appendix (table III in the appendix).

CONCLUSION

In the reported period, we prepared and validated new tools such as vectors, expressing cell lines, and antibodies against MNEI^{65} that allowed us test the hypothesis linking co-expression of MNEI and HP1 with impaired myeloid differentiation and CML.

We confirmed principle elements of our initial hypothesis, in particular we concluded that structural modifications rather than mutation or splice variants alter MNEI in myeloid cells and that variations in the level of two MNEI^{65} isoforms are significantly correlated with CML progression, and that HP1α and MNEI are co-localized in euchromatin and similarly regulate expression levels of multiple genes consistent with functional replacement of HP1 by MNEI in differentiated myeloid cells.

High levels of HP1 and MNEI isoforms recognized by *MNEI29* and *MNEI30* antibodies in CML-derived blood cells may be used for identifying structural and differentiation abnormalities associated with CML and related myeloproliferative disorders.

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APPENDICES

Table II. Custom-made oligonucleotide primers used in PCR reactions:

Primers for reverse transcription -PCR of MNEI

Lei21R: CTTCTAAGGGGAAGAAAATCTCCCC

Lei20F: GCCTCCTTCCTGACCTCGCAC

Lei13R: ATGGGTGTAAACAGCCAACAG

Lei24F: AAGAAGCCACGACGAATGC

Primers for real-time quantitative PCR of α-satellite DNA:

Alpha.F79: GCATTCTCAGAAACTTCTTTGTGAT

Alpha.R171: CTACAAAAAGAGTGTTTCAAAACTGC

Primers for real-time quantitative PCR of junB promoter:

jun.F5537: CGTGGCCGCTGTTTACAAG

jun.R5783: TCCTGGCGTCGTTTCCC

Primers for real-time quantitative PCR of c-myc promoter:

Myc.F2775: CTGCCCATTTGGGGACAC

Myc.R2868: GGTGCTTACCTGGTTTTCCACTA

Primers for real-time quantitative PCR of c-myc coding region:

Myc.F4528: TCAACGTTAGCTTCACCAACAG

Myc.R4655: TTTCTTCCAGATATCCTCGCTG

Table III. A list of transcripts coordinately regulated by HP1α and MNEI overexpression in K562 cells. The first three columns show the changes in expression levels in K562 cells expressing GFP control vector, HP1α and MNEI as indicated. The next three columns show gene identifiers.

GFP	HP1	MNE1			
x-fold difference			Genbank	Gene Symbol	Definition
0.86	0.04	0.05	NM_032858.1	MAEL	Homo sapiens maelstrom homolog (Drosophila) (MAEL), mRNA.
0.89	0.05	0.42	NM_020871.2	LRCH2	Homo sapiens leucine-rich repeats and calponin homology (CH) domain containing 2 (LRCH2), mRNA.
1.18	0.08	0.13	NM_182758.1	WDR72	Homo sapiens WD repeat domain 72 (WDR72), mRNA.
0.91	0.08	0.25	NM_006055.1	LANCL1	Homo sapiens LanC lantibiotic synthetase component C-like 1 (bacterial) (LANCL1), mRNA.
0.68	0.07	0.06	XM_943431.1	VCX3A	PREDICTED: Homo sapiens variable charge, X-linked 3A, transcript variant 3 (VCX3A), mRNA.
0.75	0.08	0.06	NM_016378.2	VCX2	Homo sapiens variable charge, X-linked 2 (VCX2), mRNA.
0.72	0.07	0.06	NM_001001888.1	VCX-C	Homo sapiens variably charged X-C (VCX-C), mRNA.
1.02	0.11	0.09	NM_006997.2	TACC2	Homo sapiens transforming, acidic coiled-coil containing protein 2 (TACC2), transcript variant 4, mRNA.
0.84	0.09	0.08	NM_013452.2	VCX	Homo sapiens variable charge, X-linked (VCX), mRNA.
1.00	0.11	0.20	NM_031442.2	TMEM47	Homo sapiens transmembrane protein 47 (TMEM47), mRNA.
0.73	0.09	0.08	NM_004679.2	VCY	Homo sapiens variable charge, Y-linked (VCY), mRNA.
0.84	0.13	0.13	XM_946075.1	VCX2	PREDICTED: Homo sapiens variable charge, X-linked 2 (VCX2), mRNA.
0.99	0.15	0.21	NM_198179.1	GPR103	Homo sapiens G protein-coupled receptor 103 (GPR103), mRNA.
0.95	0.16	0.22	NM_001431.1	EPB41L2	Homo sapiens erythrocyte membrane protein band 4.1-like 2 (EPB41L2), mRNA.
1.04	0.17	0.20	NM_006059.2	LAMC3	Homo sapiens laminin, gamma 3 (LAMC3), mRNA.
1.04	0.18	0.29	NM_005238.2	ETS1	Homo sapiens v-ets erythroblastosis virus E26 oncogene homolog 1 (avian) (ETS1), mRNA.
1.07	0.19	0.09	NM_174900.2	ZFP42	Homo sapiens zinc finger protein 42 (ZFP42), mRNA.
0.98	0.17	0.13	NM_031453.2	FAM107B	Homo sapiens family with sequence similarity 107, member B (FAM107B), mRNA.
0.91	0.16	0.40	XM_941615.1	LOC652224	PREDICTED: Homo sapiens similar to zinc finger, CCHC domain containing 9 (LOC652224), mRNA.
1.04	0.19	0.19	AA886944		nz51h02.s1 NCI_CGAP_Pr12 Homo sapiens cDNA clone IMAGE:1291347, mRNA sequence
0.99	0.22	0.26	NM_152380.2	TBX15	Homo sapiens T-box 15 (TBX15), mRNA.
0.91	0.20	0.43	XM_929311.1	LOC646388	PREDICTED: Homo sapiens similar to zinc finger, CCHC domain containing 9 (LOC646388), mRNA.
1.09	0.25	0.30	NM_003714.2	STC2	Homo sapiens stanniocalcin 2 (STC2), mRNA.
0.85	0.20	0.17	NM_015714.2	G0S2	Homo sapiens G0/G1switch 2 (G0S2), mRNA.
0.94	0.22	0.23	NM_148923.2	CYB5A	Homo sapiens cytochrome b5 type A (microsomal) (CYB5A), transcript variant 1, mRNA.
0.87	0.21	0.30	XM_926689.1	LOC653285	PREDICTED: Homo sapiens similar to hypothetical protein LOC255313 (LOC653285), mRNA.
1.03	0.25	0.24	XM_927536.1	ALDH1L2	PREDICTED: Homo sapiens aldehyde dehydrogenase 1 family, member L2 (ALDH1L2), mRNA.
0.92	0.23	0.23	NM_145006.2	SUSD3	Homo sapiens sushi domain containing 3 (SUSD3), mRNA.
0.93	0.24	0.27	NM_019043.3	APBB1IP	Homo sapiens amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein (APBB1IP), m
0.82	0.21	0.25	NM_016383.2	LUZP4	Homo sapiens leucine zipper protein 4 (LUZP4), mRNA.
1.08	0.28	0.39	NM_173844.1	MALT1	Homo sapiens mucosa associated lymphoid tissue lymphoma translocation gene 1 (MALT1), transcript variant 2
1.00	0.27	0.43	NM_019038.2	TDRD4	Homo sapiens tudor domain containing 4 (TDRD4), mRNA.
1.01	0.28	0.22	NM_152680.1	FLJ32028	Homo sapiens hypothetical protein FLJ32028 (FLJ32028), mRNA.
1.06	0.30	0.34	NM_016231.2	NLK	Homo sapiens nemo-like kinase (NLK), mRNA.
0.98	0.28	0.38	NM_206857.1	RTN1	Homo sapiens reticulon 1 (RTN1), transcript variant 2, mRNA.
0.75	0.22	0.18	NM_012443.2	SPAG6	Homo sapiens sperm associated antigen 6 (SPAG6), transcript variant 1, mRNA.
1.06	0.31	0.27	NM_130446.1	KLHL6	Homo sapiens kelch-like 6 (Drosophila) (KLHL6), mRNA.
1.15	0.35	0.58	NM_005773.2	ZNF256	Homo sapiens zinc finger protein 256 (ZNF256), mRNA.
0.98	0.30	0.30	NM_006675.3	TSPAN9	Homo sapiens tetraspanin 9 (TSPAN9), mRNA.
0.96	0.30	0.17	NM_025218.2	ULBP1	Homo sapiens UL16 binding protein 1 (ULBP1), mRNA.
1.05	0.33	0.31	NM_138995.1	MYO3B	Homo sapiens myosin IIIB (MYO3B), mRNA.
0.92	0.29	0.24	XM_927974.1	LOC145814	PREDICTED: Homo sapiens hypothetical protein LOC145814, transcript variant 2 (LOC145814), mRNA.
1.02	0.32	0.33	NM_012105.3	BACE2	Homo sapiens beta-site APP-cleaving enzyme 2 (BACE2), transcript variant a, mRNA.
0.93	0.29	0.29	NM_024711.3	GIMAP6	Homo sapiens GTPase, IMAP family member 6 (GIMAP6), transcript variant 1, mRNA.
1.09	0.34	0.57	AF038185		Homo sapiens clone 23700 mRNA sequence
1.03	0.33	0.45	NM_001024847.1	TGFBR2	Homo sapiens transforming growth factor, beta receptor II (70/80kDa) (TGFBR2), transcript variant 1, mRNA.
1.07	0.34	0.42	NM_152407.2	GRPEL2	Homo sapiens GrpE-like 2, mitochondrial (E. coli) (GRPEL2), mRNA.
1.14	0.37	0.37	NM_002293.2	LAMC1	Homo sapiens laminin, gamma 1 (formerly LAMB2) (LAMC1), mRNA.
1.28	0.43	0.31	NM_000211.1	ITGB2	Homo sapiens integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage anti
1.00	0.34	0.32	NM_053056.1	CCND1	Homo sapiens cyclin D1 (PRAD1: parathyroid adenomatosis 1) (CCND1), mRNA.
1.12	0.38	0.30	NM_006934.2	SLC6A9	Homo sapiens solute carrier family 6 (neurotransmitter transporter, glycine), member 9 (SLC6A9), transcript vari
1.30	0.44	0.39	NM_001995.2	ACSL1	Homo sapiens acyl-CoA synthetase long-chain family member 1 (ACSL1), mRNA.
0.94	0.32	0.38	NM_016377.2	AKAP7	Homo sapiens A kinase (PRKA) anchor protein 7 (AKAP7), transcript variant gamma, mRNA.

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1.21	**0.41**	**0.44**	NM_022763.2	**FNDC3B**	Homo sapiens fibronectin type III domain containing 3B (FNDC3B), mRNA.
1.09	**0.38**	**0.26**	NM_133443.1	**GPT2**	Homo sapiens glutamic pyruvate transaminase (alanine aminotransferase) 2 (GPT2), mRNA.
1.00	**0.35**	**0.41**	XM_940375.1	**KBTBD9**	PREDICTED: Homo sapiens kelch repeat and BTB (POZ) domain containing 9 (KBTBD9), mRNA.
1.10	**0.39**	**0.42**	AK123847		Homo sapiens cDNA FLJ41853 fis, clone NT2RI3004161
0.96	**0.35**	**0.31**	NM_182662.1	**AADAT**	Homo sapiens aminoadipate aminotransferase (AADAT), transcript variant 2, mRNA.
1.05	**0.38**	**0.39**	NM_181782.2	**NCOA7**	Homo sapiens nuclear receptor coactivator 7 (NCOA7), mRNA.
1.04	**0.38**	**0.50**	AV760463		AV760463 MDS Homo sapiens cDNA clone MDSEEH11 5, mRNA sequence
0.83	**0.30**	**0.38**	AK123661		Homo sapiens cDNA FLJ41667 fis, clone FEBRA2028366
1.11	**0.40**	**0.59**	NM_001146.3	**ANGPT1**	Homo sapiens angiopoietin 1 (ANGPT1), mRNA.
1.06	**0.39**	**0.38**	NM_052813.2	**CARD9**	Homo sapiens caspase recruitment domain family, member 9 (CARD9), mRNA.
1.00	**0.36**	**0.23**	NM_172234.1	**IL17RB**	Homo sapiens interleukin 17 receptor B (IL17RB), transcript variant 2, mRNA.
0.99	**0.36**	**0.31**	NM_006216.2	**SERPINE2**	Homo sapiens serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2 (SEF
0.93	**0.34**	**0.37**	NM_002849.2	**PTPRR**	Homo sapiens protein tyrosine phosphatase, receptor type, R (PTPRR), transcript variant 1, mRNA.
0.96	**0.36**	**0.41**	NM_182535.1	**LOC200261**	Homo sapiens hypothetical protein LOC200261 (LOC200261), mRNA.
0.83	**0.31**	**0.39**	NM_005246.1	**FER**	Homo sapiens fer (fps/fes related) tyrosine kinase (phosphoprotein NCP94) (FER), mRNA.
1.04	**0.39**	**0.51**	XM_371798.4	**C6orf52**	PREDICTED: Homo sapiens chromosome 6 open reading frame 52 (C6orf52), mRNA.
1.05	**0.40**	**0.37**	NM_002583.2	**PAWR**	Homo sapiens PRKC, apoptosis, WT1, regulator (PAWR), mRNA.
1.01	**0.39**	**0.36**	NM_014429.2	**MORC1**	Homo sapiens MORC family CW-type zinc finger 1 (MORC1), mRNA.
1.04	**0.40**	**0.45**	XM_945440.1	**LOC650114**	PREDICTED: Homo sapiens hypothetical protein LOC650113, transcript variant 1 (LOC650114), mRNA.
1.16	**0.45**	**0.56**	NM_002147.2	**HOXB5**	Homo sapiens homeo box B5 (HOXB5), mRNA.
1.12	**0.44**	**0.47**	NM_003980.3	**MAP7**	Homo sapiens microtubule-associated protein 7 (MAP7), mRNA.
1.04	**0.41**	**0.42**	NM_001806.2	**CEBPG**	Homo sapiens CCAAT/enhancer binding protein (C/EBP), gamma (CEBPG), mRNA.
0.91	**0.36**	**0.43**	NM_018711.2	**SVOP**	Homo sapiens SV2 related protein homolog (rat) (SVOP), mRNA.
1.01	**0.40**	**0.55**	NM_005391.1	**PDK3**	Homo sapiens pyruvate dehydrogenase kinase, isozyme 3 (PDK3), mRNA.
0.85	**0.34**	**0.32**	XM_936687.1	**MGC39900**	PREDICTED: Homo sapiens hypothetical protein MGC39900 (MGC39900), mRNA.
0.96	**0.39**	**0.36**	NM_022052.1	**NXF3**	Homo sapiens nuclear RNA export factor 3 (NXF3), mRNA.
1.06	**0.43**	**0.45**	NM_000633.2	**BCL2**	Homo sapiens B-cell CLL/lymphoma 2 (BCL2), nuclear gene encoding mitochondrial protein, transcript variant a
1.10	**0.45**	**0.46**	NM_002356.4	**MARCKS**	Homo sapiens myristoylated alanine-rich protein kinase C substrate (MARCKS), mRNA.
1.00	**0.41**	**0.40**	NM_012446.2	**SSBP2**	Homo sapiens single-stranded DNA binding protein 2 (SSBP2), mRNA.
1.11	**0.46**	**0.40**	NM_001030287.1	**ATF3**	Homo sapiens activating transcription factor 3 (ATF3), transcript variant 3, mRNA.
1.00	**0.42**	**0.46**	XM_931355.1	**LOC653135**	PREDICTED: Homo sapiens similar to Nucleosome binding protein 1 (Nucleosome binding protein 45) (NBP-45
1.13	**0.47**	**0.55**	NM_014725.2	**STARD8**	Homo sapiens START domain containing 8 (STARD8), mRNA.
1.02	**0.43**	**0.43**	NM_003243.2	**TGFBR3**	Homo sapiens transforming growth factor, beta receptor III (betaglycan, 300kDa) (TGFBR3), mRNA.
1.11	**0.47**	**0.40**	NM_001025.4	**RPS23**	Homo sapiens ribosomal protein S23 (RPS23), mRNA.
1.08	**0.46**	**0.61**	BX116110		BX116110 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGp998J083861, mRNA sequence
1.02	**0.43**	**0.40**	NM_018664.1	**SNFT**	Homo sapiens Jun dimerization protein p21SNFT (SNFT), mRNA.
0.99	**0.43**	**0.45**	NM_001003722.1	**GLE1L**	Homo sapiens GLE1 RNA export mediator-like (yeast) (GLE1L), transcript variant 1, mRNA.
0.95	**0.41**	**0.40**	NM_006255.3	**PRKCH**	Homo sapiens protein kinase C, eta (PRKCH), mRNA.
1.10	**0.48**	**0.45**	BG484767		602505656F1 NIH_MGC_77 Homo sapiens cDNA clone IMAGE:4619364 5, mRNA sequence
1.23	**0.54**	**0.26**	NM_133367.2	**PAQR8**	Homo sapiens progestin and adipoQ receptor family member VIII (PAQR8), mRNA.
0.89	**0.39**	**0.36**	NM_032717.3	**HMFN0839**	Homo sapiens hypothetical protein MGC11324 (HMFN0839), mRNA.
0.99	**0.44**	**0.39**	NM_005738.2	**ARL4**	Homo sapiens ADP-ribosylation factor-like 4 (ARL4), transcript variant 1, mRNA.
1.01	**0.45**	**0.43**	NM_181657.1	**LTB4R**	Homo sapiens leukotriene B4 receptor (LTB4R), mRNA.
1.01	**0.45**	**0.58**	BX105743		BX105743 NCI_CGAP_GC6 Homo sapiens cDNA clone IMAGp998H135716, mRNA sequence
0.96	**0.43**	**0.49**	NM_006135.1	**CAPZA1**	Homo sapiens capping protein (actin filament) muscle Z-line, alpha 1 (CAPZA1), mRNA.
0.98	**0.44**	**0.48**	BX110978		BX110978 Soares_testis_NHT Homo sapiens cDNA clone IMAGp998B021785, mRNA sequence
0.94	**0.42**	**0.51**	NM_032010.1	**MAP1B**	Homo sapiens microtubule-associated protein 1B (MAP1B), transcript variant 2, mRNA.
1.07	**0.48**	**0.47**	NM_015651.1	**PHF19**	Homo sapiens PHD finger protein 19 (PHF19), transcript variant 1, mRNA.
1.04	**0.47**	**0.51**	NM_138777.2	**MRRF**	Homo sapiens mitochondrial ribosome recycling factor (MRRF), nuclear gene encoding mitochondrial protein, tra
0.90	**0.41**	**0.29**	NM_033212.2	**MGC10992**	Homo sapiens hypothetical protein LOC92922 (MGC10992), mRNA.
0.96	**0.44**	**0.53**	BX393727		BX393727 Homo sapiens NEUROBLASTOMA COT 25-NORMALIZED Homo sapiens cDNA clone CS0DC001Y
1.06	**0.48**	**0.65**	NM_024055.2	**SLC30A5**	Homo sapiens solute carrier family 30 (zinc transporter), member 5 (SLC30A5), transcript variant 2, mRNA.
1.08	**0.49**	**0.45**	AB074162		Homo sapiens primary neuroblastoma cDNA, clone:Nbla10527, full insert sequence
0.96	**0.44**	**0.43**	NM_004810.2	**GRAP2**	Homo sapiens GRB2-related adaptor protein 2 (GRAP2), mRNA.
1.11	**0.51**	**0.50**	NM_006636.2	**MTHFD2**	Homo sapiens methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclo

0.91	**0.42**	**0.44**	NM_000136.2	**FANCC**	Homo sapiens Fanconi anemia, complementation group C (FANCC), mRNA.
1.07	**0.50**	**0.31**	NM_080546.2	**SLC44A1**	Homo sapiens solute carrier family 44, member 1 (SLC44A1), transcript variant 2, mRNA.
0.87	**0.41**	**0.44**	NM_004099.4	**STOM**	Homo sapiens stomatin (STOM), transcript variant 1, mRNA.
0.95	**0.45**	**0.38**	NM_005831.3	**CALCOCO2**	Homo sapiens calcium binding and coiled-coil domain 2 (CALCOCO2), mRNA.
0.97	**0.46**	**0.39**	NM_005808.2	**CTDSPL**	Homo sapiens CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like (CTDS
1.10	**0.52**	**0.53**	NM_014667.1	**VGLL4**	Homo sapiens vestigial like 4 (Drosophila) (VGLL4), mRNA.
1.05	**0.50**	**0.71**	NM_033644.2	**FBXW11**	Homo sapiens F-box and WD-40 domain protein 11 (FBXW11), transcript variant 2, mRNA.
0.94	**0.44**	**0.65**	BM983264		UI-CF-DU1-aav-c-16-0-UI.s1 UI-CF-DU1 Homo sapiens cDNA clone UI-CF-DU1-aav-c-16-0-UI 3, mRNA sequer
1.13	**0.54**	**0.50**	NM_032119.1	**MASS1**	Homo sapiens monogenic, audiogenic seizure susceptibility 1 homolog (mouse) (MASS1), mRNA.
0.97	**0.46**	**0.54**	NM_052901.2	**SLC25A25**	Homo sapiens solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25 (SLC25A25), nucle
1.03	**0.49**	**0.50**	NM_182943.2	**PLOD2**	Homo sapiens procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2), transcript variant 1, mRNA.
1.07	**0.51**	**0.58**	NM_001015882.1	**bA16L21.2.1**	Homo sapiens DnaJ-like protein (bA16L21.2.1), mRNA.
1.12	**0.54**	**0.44**	NM_003506.2	**FZD6**	Homo sapiens frizzled homolog 6 (Drosophila) (FZD6), mRNA.
1.07	**0.52**	**0.54**	NM_015447.1	**CAMSAP1**	Homo sapiens calmodulin regulated spectrin-associated protein 1 (CAMSAP1), mRNA.
0.92	**0.45**	**0.56**	NM_002721.3	**PPP6C**	Homo sapiens protein phosphatase 6, catalytic subunit (PPP6C), mRNA.
1.08	**0.52**	**0.35**	NM_032118.2	**WDR54**	Homo sapiens WD repeat domain 54 (WDR54), mRNA.
1.03	**0.50**	**0.63**	BU193649		AGENCOURT_7951823 NIH_MGC_82 Homo sapiens cDNA clone IMAGE:6102907 5, mRNA sequence
1.04	**0.51**	**0.49**	NM_052965.1	**C1orf19**	Homo sapiens chromosome 1 open reading frame 19 (C1orf19), mRNA.
1.05	**0.51**	**0.45**	NM_015198.2	**COBL**	Homo sapiens cordon-bleu homolog (mouse) (COBL), mRNA.
0.96	**0.47**	**0.44**	NM_033339.3	**CASP7**	Homo sapiens caspase 7, apoptosis-related cysteine peptidase (CASP7), transcript variant gamma, mRNA.
0.88	**0.43**	**0.46**	NM_006313.1	**USP15**	Homo sapiens ubiquitin specific peptidase 15 (USP15), mRNA.
0.96	**0.47**	**0.56**	NM_016353.2	**ZDHHC2**	Homo sapiens zinc finger, DHHC-type containing 2 (ZDHHC2), mRNA.
1.07	**0.53**	**0.42**	NM_033086.1	**FGD3**	Homo sapiens FYVE, RhoGEF and PH domain containing 3 (FGD3), mRNA.
1.02	**0.51**	**0.52**	NM_007229.1	**PACSIN2**	Homo sapiens protein kinase C and casein kinase substrate in neurons 2 (PACSIN2), mRNA.
0.95	**0.48**	**0.62**	NM_019591.2	**ZNF26**	Homo sapiens zinc finger protein 26 (KOX 20) (ZNF26), mRNA.
0.97	**0.49**	**0.60**	CR740473		CR740473 NCI_CGAP_Pan1 Homo sapiens cDNA clone IMAGp971H06120 ; IMAGE:2223527 5, mRNA seque
1.02	**0.51**	**0.60**	NM_033281.5	**MRPS36**	Homo sapiens mitochondrial ribosomal protein S36 (MRPS36), nuclear gene encoding mitochondrial protein, mR
1.02	**0.52**	**0.58**	XM_942687.1	**LOC654189**	PREDICTED: Homo sapiens similar to heterogeneous nuclear ribonucleoprotein A3, transcript variant 1 (LOC65
1.02	**0.52**	**0.56**	NM_003486.5	**SLC7A5**	Homo sapiens solute carrier family 7 (cationic amino acid transporter, y+ system), member 5 (SLC7A5), mRNA.
0.99	**0.50**	**0.14**	NM_004321.4	**KIF1A**	Homo sapiens kinesin family member 1A (KIF1A), mRNA.
1.04	**0.53**	**0.51**	XM_379668.3	**LOC286208**	PREDICTED: Homo sapiens hypothetical protein LOC286208, transcript variant 1 (LOC286208), mRNA.
0.95	**0.49**	**0.57**	NM_001010862.1	**SPIN3**	Homo sapiens spindlin family, member 3 (SPIN3), mRNA.
0.98	**0.50**	**0.47**	NM_012233.1	**RAB3GAP1**	Homo sapiens RAB3 GTPase activating protein subunit 1 (catalytic) (RAB3GAP1), mRNA.
0.98	**0.51**	**0.51**	NM_014811.3	**KIAA0649**	Homo sapiens KIAA0649 (KIAA0649), mRNA.
1.06	**0.55**	**0.56**	NM_001007246.1	**BRWD1**	Homo sapiens bromodomain and WD repeat domain containing 1 (BRWD1), transcript variant 3, mRNA.
1.02	**0.53**	**0.56**	NM_032199.1	**ARID5B**	Homo sapiens AT rich interactive domain 5B (MRF1-like) (ARID5B), mRNA.
1.06	**0.55**	**0.60**	BX115738		BX115738 Soares melanocyte 2NbHM Homo sapiens cDNA clone IMAGp998D07589, mRNA sequence
1.07	**0.55**	**0.55**	NM_002509.2	**NKX2-2**	Homo sapiens NK2 transcription factor related, locus 2 (Drosophila) (NKX2-2), mRNA.
0.91	**0.47**	**0.48**	NM_015635.2	**GAPVD1**	Homo sapiens GTPase activating protein and VPS9 domains 1 (GAPVD1), mRNA.
1.03	**0.54**	**0.56**	NM_018414.2	**ST6GALNAC1**	Homo sapiens ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1, 3)-N-acetylgalactosaminide alpha-2,6-sialy
1.10	**0.58**	**0.48**	NM_005311.3	**GRB10**	Homo sapiens growth factor receptor-bound protein 10 (GRB10), transcript variant 1, mRNA.
0.97	**0.51**	**0.52**	NM_012261.2	**C20orf103**	Homo sapiens chromosome 20 open reading frame 103 (C20orf103), mRNA.
1.02	**0.54**	**0.33**	NM_194272.1	**RBPMS2**	Homo sapiens RNA binding protein with multiple splicing 2 (RBPMS2), mRNA.
0.95	**0.51**	**0.52**	NM_001419.2	**ELAVL1**	Homo sapiens ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R) (ELAVL1), mRNA.
1.07	**0.57**	**0.51**	NM_170696.1	**ALDH1A2**	Homo sapiens aldehyde dehydrogenase 1 family, member A2 (ALDH1A2), transcript variant 2, mRNA.
0.99	**0.53**	**0.41**	NM_001008397.1	**LOC493869**	Homo sapiens similar to RIKEN cDNA 2310016C16 (LOC493869), mRNA.
0.90	**0.48**	**0.32**	NM_032147.1	**USP44**	Homo sapiens ubiquitin specific peptidase 44 (USP44), mRNA.
0.93	**0.50**	**0.52**	BM684327		UI-E-EJ1-aji-b-16-0-UI.s1 UI-E-EJ1 Homo sapiens cDNA clone UI-E-EJ1-aji-b-16-0-UI 3, mRNA sequence
0.99	**0.54**	**0.68**	NM_031412.2	**GABARAPL1**	Homo sapiens GABA(A) receptor-associated protein like 1 (GABARAPL1), mRNA.
1.01	**0.55**	**0.63**	NM_004560.2	**ROR2**	Homo sapiens receptor tyrosine kinase-like orphan receptor 2 (ROR2), mRNA.
1.00	**0.54**	**0.49**	NM_207170.1	**SYF2**	Homo sapiens SYF2 homolog, RNA splicing factor (S. cerevisiae) (SYF2), transcript variant 2, mRNA.
1.11	**0.61**	**0.66**	NM_022461.2	**AZI2**	Homo sapiens 5-azacytidine induced 2 (AZI2), mRNA.
1.02	**0.56**	**0.71**	NM_006000.1	**TUBA1**	Homo sapiens tubulin, alpha 1 (testis specific) (TUBA1), mRNA.
1.12	**0.61**	**0.62**	BE552200		hy04c12.x1 NCI_CGAP_GC6 Homo sapiens cDNA clone IMAGE:3196342 3, mRNA sequence
1.03	**0.56**	**0.44**	NM_015549.1	**PLEKHG3**	Homo sapiens pleckstrin homology domain containing, family G (with RhoGef domain) member 3 (PLEKHG3), n
1.10	**0.60**	**0.58**	NM_031482.3	**ATG10**	Homo sapiens ATG10 autophagy related 10 homolog (S. cerevisiae) (ATG10), mRNA.
0.98	**0.54**	**0.61**	NM_005915.4	**MCM6**	Homo sapiens MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae) (MC

0.95	**0.52**	**0.50**	NM_019020.2	**TBC1D16**	Homo sapiens TBC1 domain family, member 16 (TBC1D16), mRNA.
0.93	**0.51**	**0.59**	NM_003337.2	**UBE2B**	Homo sapiens ubiquitin-conjugating enzyme E2B (RAD6 homolog) (UBE2B), mRNA.
1.03	**0.57**	**0.65**	NM_152793.1	**Ells1**	Homo sapiens hypothetical protein Ells1 (Ells1), mRNA.
0.98	**0.55**	**0.66**	AW956608		EST368678 MAGE resequences, MAGD Homo sapiens cDNA, mRNA sequence
0.89	**0.50**	**0.09**	NM_003602.2	**FKBP6**	Homo sapiens FK506 binding protein 6, 36kDa (FKBP6), mRNA.
0.95	**0.53**	**0.48**	NM_016830.2	**VAMP1**	Homo sapiens vesicle-associated membrane protein 1 (synaptobrevin 1) (VAMP1), transcript variant 3, mRNA.
1.06	**0.60**	**0.64**	NM_138712.2	**PPARG**	Homo sapiens peroxisome proliferative activated receptor, gamma (PPARG), transcript variant 1, mRNA.
1.06	**0.60**	**0.62**	NM_032300.2	**TCHP**	Homo sapiens trichoplein, keratin filament binding (TCHP), mRNA.
0.96	**0.54**	**0.62**	BU596411		AGENCOURT_8943469 NIH_MGC_142 Homo sapiens cDNA clone IMAGE:6452955 5, mRNA sequence
1.02	**0.58**	**0.69**	NM_033551.2	**LARP1**	Homo sapiens La ribonucleoprotein domain family, member 1 (LARP1), transcript variant 2, mRNA.
1.04	**0.59**	**0.61**	NM_138773.1	**LOC91137**	Homo sapiens hypothetical protein BC017169 (LOC91137), mRNA.
1.11	**0.63**	**0.73**	NM_021998.3	**ZNF6**	Homo sapiens zinc finger protein 6 (CMPX1) (ZNF6), mRNA.
0.94	**0.53**	**0.53**	XM_374004.2	**LOC389025**	PREDICTED: Homo sapiens hypothetical LOC389025 (LOC389025), mRNA.
1.00	**0.57**	**0.63**	AK023464		Homo sapiens cDNA FLJ13402 fis, clone PLACE1001456
1.03	**0.58**	**0.53**	NM_001839.2	**CNN3**	Homo sapiens calponin 3, acidic (CNN3), mRNA.
1.00	**0.57**	**0.51**	NM_005467.2	**NAALAD2**	Homo sapiens N-acetylated alpha-linked acidic dipeptidase 2 (NAALAD2), mRNA.
1.06	**0.61**	**0.66**	NM_024308.2	**MGC4172**	Homo sapiens short-chain dehydrogenase/reductase (MGC4172), mRNA.
0.97	**0.55**	**0.58**	XM_926036.1	**LOC653103**	PREDICTED: Homo sapiens similar to Ankyrin repeat domain protein 11 (Ankyrin repeat-containing cofactor 1) (
1.01	**0.58**	**0.61**	NM_052812.1	**TRIM15**	Homo sapiens tripartite motif-containing 15 (TRIM15), transcript variant 2, mRNA.
0.95	**0.55**	**0.63**	NM_001008215.1	**MGC52110**	Homo sapiens hypothetical protein MGC52110 (MGC52110), mRNA.
0.99	**0.57**	**0.62**	NM_001012756.1	**ZFP260**	Homo sapiens zinc finger protein 260 (ZFP260), mRNA.
0.99	**0.57**	**0.63**	NM_005869.2	**SDCCAG10**	Homo sapiens serologically defined colon cancer antigen 10 (SDCCAG10), mRNA.
1.06	**0.61**	**0.64**	BF509118		UI-H-BI4-aou-g-01-0-UI.s1 NCI_CGAP_Sub8 Homo sapiens cDNA clone IMAGE:3086376 3, mRNA sequence
0.98	**0.57**	**0.58**	NM_015570.1	**AUTS2**	Homo sapiens autism susceptibility candidate 2 (AUTS2), mRNA.
0.95	**0.55**	**0.67**	NM_030793.3	**FBXO38**	Homo sapiens F-box protein 38 (FBXO38), transcript variant 1, mRNA.
1.09	**0.63**	**0.69**	NM_020951.1	**ZNF529**	Homo sapiens zinc finger protein 529 (ZNF529), mRNA.
0.98	**0.57**	**0.59**	NM_001031744.1	**LOC158160**	Homo sapiens hypothetical protein LOC158160 (LOC158160), transcript variant 1, mRNA.
1.06	**0.62**	**0.56**	BC090942		Homo sapiens cDNA clone IMAGE:3079901
1.09	**0.64**	**0.65**	XM_925839.1	**LOC158301**	PREDICTED: Homo sapiens hypothetical protein LOC158301 (LOC158301), mRNA.
1.02	**0.60**	**0.60**	NM_018212.4	**ENAH**	Homo sapiens enabled homolog (Drosophila) (ENAH), transcript variant 2, mRNA.
0.98	**0.58**	**0.60**	XM_928577.1	**LOC645558**	PREDICTED: Homo sapiens hypothetical protein LOC645558 (LOC645558), mRNA.
1.13	**0.67**	**0.65**	NM_005886.1	**KATNB1**	Homo sapiens katanin p80 (WD repeat containing) subunit B 1 (KATNB1), mRNA.
1.07	**0.63**	**0.59**	NM_004059.3	**CCBL1**	Homo sapiens cysteine conjugate-beta lyase; cytoplasmic (glutamine transaminase K, kyneurenine aminotransfe
0.93	**0.55**	**0.59**	NM_002715.2	**PPP2CA**	Homo sapiens protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform (PPP2CA), mRNA.
1.05	**0.63**	**0.63**	XM_928675.1	**LOC653308**	PREDICTED: Homo sapiens similar to N-acylsphingosine amidohydrolase 2, transcript variant 1 (LOC653308), r
1.04	**0.62**	**0.64**	NM_144614.2	**MBD3L2**	Homo sapiens methyl-CpG binding domain protein 3-like 2 (MBD3L2), mRNA.
1.06	**0.63**	**0.64**	NM_001550.2	**IFRD1**	Homo sapiens interferon-related developmental regulator 1 (IFRD1), transcript variant 1, mRNA.
1.11	**0.66**	**0.56**	NM_014711.3	**CP110**	Homo sapiens CP110 protein (CP110), mRNA.
1.05	**0.63**	**0.65**	NM_181699.1	**PPP2R1B**	Homo sapiens protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform (PPP2R1B), tran
0.90	**0.54**	**0.43**	NM_006505.2	**PVR**	Homo sapiens poliovirus receptor (PVR), mRNA.
1.09	**0.66**	**0.72**	NM_002823.2	**PTMA**	Homo sapiens prothymosin, alpha (gene sequence 28) (PTMA), mRNA.
0.95	**0.57**	**0.49**	NM_007063.2	**TBC1D8**	Homo sapiens TBC1 domain family, member 8 (with GRAM domain) (TBC1D8), mRNA.
0.96	**0.59**	**0.53**	NM_018948.2	**ERRFI1**	Homo sapiens ERBB receptor feedback inhibitor 1 (ERRFI1), mRNA.
0.95	**0.58**	**0.60**	NM_130469.2	**JDP2**	Homo sapiens jun dimerization protein 2 (JDP2), mRNA.
0.96	**0.59**	**0.56**	NM_173570.2	**ZDHHC23**	Homo sapiens zinc finger, DHHC-type containing 23 (ZDHHC23), mRNA.
0.96	**0.58**	**0.55**	NM_006366.2	**CAP2**	Homo sapiens CAP, adenylate cyclase-associated protein, 2 (yeast) (CAP2), mRNA.
1.00	**0.61**	**0.64**	NM_019558.2	**HOXD8**	Homo sapiens homeobox D8 (HOXD8), mRNA.
1.02	**0.62**	**0.68**	NM_019118.2	**C1orf91**	Homo sapiens chromosome 1 open reading frame 91 (C1orf91), mRNA.
1.04	**0.64**	**0.73**	BI517762		603042006F1 NIH_MGC_116 Homo sapiens cDNA clone IMAGE:5182585 5, mRNA sequence
1.01	**0.62**	**0.63**	NM_005578.2	**LPP**	Homo sapiens LIM domain containing preferred translocation partner in lipoma (LPP), mRNA.
1.10	**0.68**	**0.68**	NM_153831.2	**PTK2**	Homo sapiens PTK2 protein tyrosine kinase 2 (PTK2), transcript variant 1, mRNA.
1.05	**0.65**	**0.72**	NM_015000.1	**STK38L**	Homo sapiens serine/threonine kinase 38 like (STK38L), mRNA.
0.96	**0.59**	**0.68**	XM_940433.1	**LOC651302**	PREDICTED: Homo sapiens similar to Zinc finger protein 192 (LD5-1) (LOC651302), mRNA.
1.06	**0.65**	**0.72**	NM_006643.2	**SDCCAG3**	Homo sapiens serologically defined colon cancer antigen 3 (SDCCAG3), mRNA.
0.99	**0.61**	**0.50**	NM_003622.2	**PPFIBP1**	Homo sapiens PTPRF interacting protein, binding protein 1 (liprin beta 1) (PPFIBP1), transcript variant 1, mRNA
0.91	**0.56**	**0.59**	NM_001032296.1	**STK24**	Homo sapiens serine/threonine kinase 24 (STE20 homolog, yeast) (STK24), transcript variant 2, mRNA.
0.94	**0.58**	**0.56**	NM_001605.1	**AARS**	Homo sapiens alanyl-tRNA synthetase (AARS), mRNA.

0.93	**0.58**	**0.48**	XR_001414.1	**LOC400713**	PREDICTED: Homo sapiens zinc finger-like, transcript variant 1 (LOC400713), misc RNA.
1.07	**0.66**	**0.72**	NM_002079.1	**GOT1**	Homo sapiens glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1) (GOT1), mRNA.
0.97	**0.60**	**0.61**	NM_173497.1	**HECTD2**	Homo sapiens HECT domain containing 2 (HECTD2), transcript variant 2, mRNA.
1.03	**0.64**	**0.65**	XM_937528.1	**C10orf73**	PREDICTED: Homo sapiens chromosome 10 open reading frame 73 (C10orf73), mRNA.
1.06	**0.66**	**0.66**	NM_020438.3	**DOLPP1**	Homo sapiens dolichyl pyrophosphate phosphatase 1 (DOLPP1), mRNA.
1.09	**0.68**	**0.71**	NM_139075.1	**TPCN2**	Homo sapiens two pore segment channel 2 (TPCN2), mRNA.
1.01	**0.63**	**0.44**	NM_020645.1	**NRIP3**	Homo sapiens nuclear receptor interacting protein 3 (NRIP3), mRNA.
1.04	**0.66**	**0.68**	NM_004707.2	**ATG12**	Homo sapiens ATG12 autophagy related 12 homolog (S. cerevisiae) (ATG12), mRNA.
1.07	**0.67**	**0.70**	NM_017953.2	**C1orf181**	Homo sapiens chromosome 1 open reading frame 181 (C1orf181), mRNA.
1.09	**0.69**	**0.71**	BG742924		602632014F1 NCI_CGAP_Skn3 Homo sapiens cDNA clone IMAGE:4777466 5, mRNA sequence
1.06	**0.67**	**0.51**	NM_024657.2	**MORC4**	Homo sapiens MORC family CW-type zinc finger 4 (MORC4), mRNA.
0.96	**0.61**	**0.62**	NM_005080.2	**XBP1**	Homo sapiens X-box binding protein 1 (XBP1), mRNA.
1.04	**0.66**	**0.72**	NM_181890.1	**UBE2D3**	Homo sapiens ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast) (UBE2D3), transcript variant 6, mR
0.90	**0.57**	**0.52**	NM_001066.2	**TNFRSF1B**	Homo sapiens tumor necrosis factor receptor superfamily, member 1B (TNFRSF1B), mRNA.
0.98	**0.62**	**0.59**	NM_001025366.1	**VEGF**	Homo sapiens vascular endothelial growth factor (VEGF), transcript variant 1, mRNA.
1.12	**0.71**	**0.64**	NM_025009.3	**CEP135**	Homo sapiens centrosomal protein 135kDa (CEP135), mRNA.
1.09	**0.69**	**0.71**	NM_007255.1	**B4GALT7**	Homo sapiens xylosylprotein beta 1,4-galactosyltransferase, polypeptide 7 (galactosyltransferase I) (B4GALT7),
1.05	**0.67**	**0.73**	XM_939884.1	**LOC648113**	PREDICTED: Homo sapiens similar to M-phase phosphoprotein, mpp8, transcript variant 1 (LOC648113), mRN
1.09	**0.70**	**0.77**	NM_033397.2	**KIAA1754**	Homo sapiens KIAA1754 (KIAA1754), mRNA.
0.98	**0.63**	**0.66**	NM_138781.2	**LOC113386**	Homo sapiens similar to envelope protein (LOC113386), mRNA.
0.95	**0.61**	**0.57**	NM_000610.3	**CD44**	Homo sapiens CD44 antigen (Indian blood group) (CD44), transcript variant 1, mRNA.
0.95	**0.61**	**0.57**	NM_001031684.1	**SFRS7**	Homo sapiens splicing factor, arginine/serine-rich 7, 35kDa (SFRS7), mRNA.
1.03	**0.66**	**0.64**	NM_001010925.1	**ANKRD19**	Homo sapiens ankyrin repeat domain 19 (ANKRD19), mRNA.
1.02	**0.66**	**0.72**	XM_934178.1	**LOC645037**	PREDICTED: Homo sapiens similar to GAGE-2 protein (G antigen 2), transcript variant 2 (LOC645037), mRNA.
1.05	**0.68**	**0.68**	NM_014285.4	**EXOSC2**	Homo sapiens exosome component 2 (EXOSC2), mRNA.
1.09	**0.71**	**0.68**	NM_014767.1	**SPOCK2**	Homo sapiens sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 2 (SPOCK2), mRNA.
1.06	**0.68**	**0.72**	NM_020117.8	**LARS**	Homo sapiens leucyl-tRNA synthetase (LARS), mRNA.
0.94	**0.61**	**0.66**	NM_016406.1	**UFC1**	Homo sapiens ubiquitin-fold modifier conjugating enzyme 1 (UFC1), mRNA.
1.00	**0.65**	**0.39**	NM_001004305.1	**LOC284757**	Homo sapiens hypothetical protein LOC284757 (LOC284757), mRNA.
0.95	**0.62**	**0.65**	NM_207036.1	**TCF12**	Homo sapiens transcription factor 12 (HTF4, helix-loop-helix transcription factors 4) (TCF12), transcript variant 1
1.07	**0.70**	**0.68**	CX869259		HESC4_33_E11.g1_A037 NIH_MGC_262 Homo sapiens cDNA clone IMAGE:7474271 5, mRNA sequence
0.98	**0.64**	**0.69**	BM932090		UI-E-EJ1-ajl-f-21-0-UI.r1 UI-E-EJ1 Homo sapiens cDNA clone UI-E-EJ1-ajl-f-21-0-UI 5, mRNA sequence
1.10	**0.72**	**0.70**	NM_022495.3	**C14orf135**	Homo sapiens chromosome 14 open reading frame 135 (C14orf135), mRNA.
0.95	**0.62**	**0.63**	NM_019000.2	**FLJ20152**	Homo sapiens hypothetical protein FLJ20152 (FLJ20152), mRNA.
1.10	**0.72**	**0.75**	NM_004128.1	**GTF2F2**	Homo sapiens general transcription factor IIF, polypeptide 2 (30kD subunit) (GTF2F2), mRNA.
0.99	**0.65**	**0.63**	NM_007011.4	**ABHD2**	Homo sapiens abhydrolase domain containing 2 (ABHD2), transcript variant 1, mRNA.
0.92	**0.61**	**0.54**	NM_001903.2	**CTNNA1**	Homo sapiens catenin (cadherin-associated protein), alpha 1, 102kDa (CTNNA1), mRNA.
0.93	**0.62**	**0.66**	NM_004477.2	**FRG1**	Homo sapiens FSHD region gene 1 (FRG1), mRNA.
1.01	**0.67**	**0.68**	NM_001017397.1	**TRIM36**	Homo sapiens tripartite motif-containing 36 (TRIM36), transcript variant 2, mRNA.
1.01	**0.67**	**0.61**	NM_025027.3	**ZNF606**	Homo sapiens zinc finger protein 606 (ZNF606), mRNA.
1.02	**0.68**	**0.60**	XM_936467.1	**BEXL1**	PREDICTED: Homo sapiens brain expressed X-linked-like 1 (BEXL1), mRNA.
1.08	**0.72**	**0.71**	BX109986		BX109986 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone IMAGp998K14124, mRNA sequence
0.99	**0.66**	**0.68**	NM_002623.3	**PFDN4**	Homo sapiens prefoldin subunit 4 (PFDN4), mRNA.
1.06	**0.71**	**0.70**	BX107771		BX107771 NCI_CGAP_Lu24 Homo sapiens cDNA clone IMAGp998K245814, mRNA sequence
1.04	**0.70**	**0.65**	NM_004652.3	**USP9X**	Homo sapiens ubiquitin specific peptidase 9, X-linked (fat facets-like, Drosophila) (USP9X), transcript variant 1,
0.97	**0.65**	**0.56**	NM_014181.1	**HSPC159**	Homo sapiens HSPC159 protein (HSPC159), mRNA.
1.00	**0.68**	**0.68**	NM_013396.3	**USP25**	Homo sapiens ubiquitin specific peptidase 25 (USP25), mRNA.
0.95	**0.64**	**0.62**	D87470	**KIAA0280**	Human mRNA for KIAA0280 gene, partial cds
0.97	**0.66**	**0.62**	NM_003441.1	**ZNF141**	Homo sapiens zinc finger protein 141 (clone pHZ-44) (ZNF141), mRNA.
1.03	**0.70**	**0.63**	XR_001250.1	**LOC285550**	PREDICTED: Homo sapiens hypothetical protein LOC285550, transcript variant 13 (LOC285550), misc RNA.
1.06	**0.72**	**0.64**	NM_024083.2	**ASPSCR1**	Homo sapiens alveolar soft part sarcoma chromosome region, candidate 1 (ASPSCR1), mRNA.
0.98	**0.67**	**0.65**	XM_931465.1	**LOC51152**	PREDICTED: Homo sapiens melanoma antigen (LOC51152), mRNA.
1.00	**0.69**	**0.63**	NM_003598.1	**TEAD2**	Homo sapiens TEA domain family member 2 (TEAD2), mRNA.
1.04	**0.71**	**0.72**	NM_032782.3	**HAVCR2**	Homo sapiens hepatitis A virus cellular receptor 2 (HAVCR2), mRNA.
0.92	**0.64**	**0.62**	AK095855		Homo sapiens cDNA FLJ38536 fis, clone HCHON2001200
0.99	**0.68**	**0.67**	XM_927336.1	**LOC644113**	PREDICTED: Homo sapiens hypothetical protein LOC644113 (LOC644113), mRNA.
0.98	**0.68**	**0.69**	NM_031922.2	**REPS1**	Homo sapiens RALBP1 associated Eps domain containing 1 (REPS1), mRNA.

1.02	**0.71**	**0.69**	NM_015316.1	**PPP1R13B**	Homo sapiens protein phosphatase 1, regulatory (inhibitor) subunit 13B (PPP1R13B), mRNA.
1.07	**0.75**	**0.74**	NM_198550.1	**SEC63D1**	Homo sapiens SEC63 domain containing 1 (SEC63D1), mRNA.
0.97	**0.68**	**0.66**	XM_936535.1	**LOC648245**	PREDICTED: Homo sapiens hypothetical protein LOC648245, transcript variant 1 (LOC648245), mRNA.
0.94	**0.66**	**0.63**	NM_001675.2	**ATF4**	Homo sapiens activating transcription factor 4 (tax-responsive enhancer element B67) (ATF4), transcript variant
0.99	**0.69**	**0.61**	NM_003275.1	**TMOD1**	Homo sapiens tropomodulin 1 (TMOD1), mRNA.
0.97	**0.68**	**0.61**	NM_020447.2	**C15orf17**	Homo sapiens chromosome 15 open reading frame 17 (C15orf17), mRNA.
1.00	**0.70**	**0.67**	XM_926814.1	**LOC653158**	PREDICTED: Homo sapiens similar to hypothetical protein MGC40405, transcript variant 1 (LOC653158), mRN
0.99	**0.70**	**0.71**	NM_016114.3	**ASB1**	Homo sapiens ankyrin repeat and SOCS box-containing 1 (ASB1), mRNA.
0.99	**0.70**	**0.68**	NM_194325.1	**ZNF30**	Homo sapiens zinc finger protein 30 (KOX 28) (ZNF30), mRNA.
0.94	**0.66**	**0.65**	NM_175901.3	**LOC283932**	Homo sapiens hypothetical protein LOC283932 (LOC283932), mRNA.
1.00	**1.43**	**1.57**	NM_001031724.1	**CAB39L**	Homo sapiens calcium binding protein 39-like (CAB39L), transcript variant 1, mRNA.
1.08	**1.60**	**1.82**	NM_032637.2	**SKP2**	Homo sapiens S-phase kinase-associated protein 2 (p45) (SKP2), transcript variant 2, mRNA.
0.98	**1.49**	**1.42**	NM_003532.2	**HIST1H3E**	Homo sapiens histone 1, H3e (HIST1H3E), mRNA.
1.00	**1.55**	**1.43**	BE675385		7f08b08.x1 NCI_CGAP_CLL1 Homo sapiens cDNA clone IMAGE:3294039 3 similar to contains Alu repetitive el
1.04	**1.64**	**1.66**	NM_001031724.1	**CAB39L**	Homo sapiens calcium binding protein 39-like (CAB39L), transcript variant 1, mRNA.
1.01	**1.71**	**1.51**	NM_171828.1	**KCNMB3**	Homo sapiens potassium large conductance calcium-activated channel, subfamily M beta member 3 (KCNMB3)
0.95	**1.63**	**1.50**	NM_021194.2	**SLC30A1**	Homo sapiens solute carrier family 30 (zinc transporter), member 1 (SLC30A1), mRNA.
1.08	**1.88**	**1.70**	XM_935208.1	**LOC645625**	PREDICTED: Homo sapiens similar to Importin alpha-2 subunit (Karyopherin alpha-2 subunit) (SRP1-alpha) (RA
0.93	**1.63**	**2.38**	NM_001099.2	**ACPP**	Homo sapiens acid phosphatase, prostate (ACPP), mRNA.
0.98	**1.72**	**1.41**	NM_032348.2	**MXRA8**	Homo sapiens matrix-remodelling associated 8 (MXRA8), mRNA.
0.89	**1.57**	**1.76**	NM_030763.1	**NSBP1**	Homo sapiens nucleosomal binding protein 1 (NSBP1), mRNA.
0.95	**1.69**	**1.46**	NM_016657.1	**KDELR3**	Homo sapiens KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3 (KDELR3), transcrip
1.06	**1.94**	**1.59**	NM_025193.2	**HSD3B7**	Homo sapiens hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7 (HSD3B7), mRNA
0.95	**1.79**	**1.35**	NM_006086.2	**TUBB3**	Homo sapiens tubulin, beta 3 (TUBB3), mRNA.
0.98	**1.87**	**1.64**	NM_001699.3	**AXL**	Homo sapiens AXL receptor tyrosine kinase (AXL), transcript variant 2, mRNA.
1.08	**2.10**	**2.79**	NM_173515.2	**CNKSR3**	Homo sapiens CNKSR family member 3 (CNKSR3), mRNA.
0.90	**1.88**	**1.97**	XM_496754.2	**LOC441081**	PREDICTED: Homo sapiens similar to nuclear pore membrane protein 121 (LOC441081), mRNA.
1.00	**2.23**	**1.93**	NM_004616.2	**TSPAN8**	Homo sapiens tetraspanin 8 (TSPAN8), mRNA.
0.99	**2.20**	**2.02**	XM_927237.1	**LOC653219**	PREDICTED: Homo sapiens similar to G antigen, family D, 2 isoform 1a, transcript variant 1 (LOC653219), mRN
1.06	**2.45**	**2.22**	NM_170662.3	**CBLB**	Homo sapiens Cas-Br-M (murine) ecotropic retroviral transforming sequence b (CBLB), mRNA.
1.01	**2.35**	**2.45**	NM_001015878.1	**AURKC**	Homo sapiens aurora kinase C (AURKC), transcript variant 1, mRNA.
1.20	**2.86**	**3.14**	NM_003126.1	**SPTA1**	Homo sapiens spectrin, alpha, erythrocytic 1 (elliptocytosis 2) (SPTA1), mRNA.
1.12	**2.74**	**2.16**	NM_002130.4	**HMGCS1**	Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble) (HMGCS1), mRNA.
1.14	**2.89**	**2.53**	NM_153281.1	**HYAL1**	Homo sapiens hyaluronoglucosaminidase 1 (HYAL1), transcript variant 8, mRNA.
0.94	**2.42**	**1.95**	NM_006237.2	**POU4F1**	Homo sapiens POU domain, class 4, transcription factor 1 (POU4F1), mRNA.
0.84	**2.21**	**1.94**	NM_020411.1	**XAGE1**	Homo sapiens X antigen family, member 1 (XAGE1), transcript variant 1, mRNA.
0.92	**2.68**	**2.19**	NM_012244.2	**SLC7A8**	Homo sapiens solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 (SLC7A8), transcri
0.93	**2.80**	**2.81**	NM_001015878.1	**AURKC**	Homo sapiens aurora kinase C (AURKC), transcript variant 1, mRNA.
1.03	**3.34**	**2.55**	BG697582		602660818F1 NCI_CGAP_Skn3 Homo sapiens cDNA clone IMAGE:4803797 5, mRNA sequence
0.98	**3.45**	**2.47**	NM_016521.2	**TFDP3**	Homo sapiens transcription factor Dp family, member 3 (TFDP3), mRNA.
1.07	**5.35**	**2.66**	NR_002189.1	**LOC391257**	Homo sapiens SUMO1 pseudogene 1 (LOC391257) on chromosome 20.
0.93	**5.80**	**1.59**	NM_033128.1	**SCIN**	Homo sapiens scinderin (SCIN), mRNA.
1.01	**6.37**	**6.01**	XM_945981.1	**LOC654126**	PREDICTED: Homo sapiens similar to leucine rich repeat containing 37B, transcript variant 2 (LOC654126), mR
1.01	**6.52**	**9.60**	NM_130902.1	**COX7B2**	Homo sapiens cytochrome c oxidase subunit VIIb2 (COX7B2), mRNA.