JNK2 and p38 MAPK over-expressions do not represent key events in chronic myeloid leukemia transformation

M. MERKEROVA, H. BRUCHOVA*, R. BRDICKA

Institute of Hematology and Blood Transfusion, Department of Molecular Genetics, Prague, U nemocnice 1, 128 20 Prague 2, Czech Republic, e-mail: michaela.merkerova@uhkt.cz

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Over-expression of two members of MAP kinase family (JNK2 and p38) has been already observed in chronic myeloid leukemia (CML). In the present study, significance of this deregulation was investigated. Impacts of JNK2/p38 suppression on gene expression profile of CML cell lines (K562/KU-812) were studied using an experimental approach that combines siRNA-mediated specific inhibition of the genes and array-based expression analyses. After JNK2 depletion, 27 out of 588 tested genes showed significant expression changes, with 13 down-regulated genes and 14 up-regulated genes. Among others, expression of MSH2 and MSH6, mdm2, and caspase-2 was reduced and, on the other hand, MKK1 and MKK6, RFC2, cytokeratins K18 and K19, BAD, and DR5 expression was up-regulated. In the case of p38 silencing, 20 genes were considered as significantly deregulated (7 genes reduced, 13 over-expressed). These genes included caspase-10, SOD1, and Notch4 (down-regulation) and caspase-2 and caspase-3, CDC2, CDK4, and c-kit (up-regulation). In conclusion, comparison of expression profiles after JNK2 or p38 gene silencing revealed distinct sets of affected genes. The results implied an unequal impact of the MAPK deregulation on the CML cells. Further, we demonstrated that neither JNK2 nor p38 siRNA-mediated inhibition led to significant change of CML cell proliferation. It suggests that there are other important, likely upstream regulators essential for CML malignant cell growth/transformation; therefore, separate inhibition of JNK2 or p38 MAPK gene is not sufficient for a proliferation arrest.

Key words: CML; expression arrays; JNK2; MAPK; p38; siRNA

Mitogen-activated protein kinases (MAPKs) are serin-threonin kinases that are parts of signal transduction pathway connecting extracellular stimuli with cellular responses. MAPKs affect cell viability, regulate cell cycle and gene expression [1]. Three major mammalian MAPK subgroups have been identified: extracellular signal-regulated kinase (ERK), c-Jun NH_2-terminal kinase (JNK), and p38 MAPK [1-3].

Three genes encode the JNK subgroup: JNK1 – JNK3. JNK1 and JNK2 are ubiquitously expressed but JNK3 is more restricted to the brain, testis, and heart [2]. JNKs are strongly activated by diverse stress stimuli like UV and ionising radiation, heat and osmotic shock, inflammatory cytokines, and metabolic inhibitors [2]. The JNK pathway is involved in many forms of stress-induced apoptosis [4]. On the other hand, recent studies have provided evidence that JNKs play also a protective role and support cell survival. Under some circumstances, JNKs participate in cell proliferation, transformation processes and tumor progression [2,5,6]. For example, depletion of JNKs using antisense oligonucleotides inhibited growth of human glioblastoma cells associated with S phase arrest [6]. This regulatory complexity of JNK emerges probably from its diverse function depending on cell types, stimulus, spectrum of its substrates, or duration of activation. Although JNK1 and JNK2 have been generally considered to share functions in signal transduction pathway, recent studies have demonstrated differential effects of the isoforms [7-12]. Hreniuk *et al.* [7] showed that the JNK1 isoform plays a preferential role in stress-induced apoptosis. JNK2-null mice are resistant to mitogen-induced tumor growth and malignant transformation [11], and constitutively active JNK2 isoform plays a significant role in the behaviour of glial tumors [12]. It suggests that JNK2 inhibition may be used for treatment of hyperproliferative disorders such as cancer.

p38 belongs to a different subgroup of MAP kinases. p38 MAPK is also involved in cellular response to diverse stimuli,

*Corresponding author: hana.bruchova@uhkt.cz

including environmental stress and inflammatory cytokines. p38 is phosphorylated by MKK3 and/or MKK6 upstream kinases and in turn activate a variety of downstream targets, including MAPKAP2, the C/EBP family of transcription factors, p53, and other transcription factors. In this way, p38 MAPK is believed to play an important role in variety of cellular events, including cell growth, cell differentiation, and cell death [13].

Chronic myeloid leukemia (CML) is probably the most extensively studied hematological malignancy at present. It is widely known that BCR/ABL fusion gene, which encodes an aberrant tyrosine kinase, has a causal role in CML pathogenesis. BCR/ABL activates multiply signalling pathways, including MAPK, Ras, PI3-K, JAK/STAT, and Myc [14,15]. These days, imatinib mesylate, a selective tyrosine kinase inhibitor, is successfully used for treatment in BCR/ABL-positive CML. Due to imatinib resistance, several second generation inhibitors, as AMN107 or dasatinib, were tested [16-18]. Regardless, a comprehensive research is focused on identification of another genes deregulated in CML transformation [19,20]. New targets molecules are required and advanced understanding of processes associated with cell transformation and progression is still relevant.

In our previous array experiments, we observed over-expression of JNK2 and p38 MAP kinases in peripheral blood cells of BCR/ABL-positive CML patients at the time of their initial diagnosis [21-23]. Observed deregulation of JNK2 corresponded with observation that BCR/ABL kinase primarily activates JNK signal transduction pathway and this cascade has been implicated in BCR/ABL-mediated transformation [15]. In contrast, it has been demonstrated that activation of p38 MAPK pathway is essential for the antileukemic effects of imatinib mesylate as well as of interferon-α in BCR/ABL-expressing cells [24,25]; however, activation of JNKs is not altered by imatinib [26].

Thus, to reveal the processes leading to deregulation of these molecules becomes an important question that needs to be solved. To answer this question, we investigated gene expression profiles in JNK2/p38 knock-down CML cell lines treated with JNK2/p38 specific siRNAs.

Material and methods

Cell cultures. CML-derived cell lines K562 and KU-812 (ATCC) were maintained in RPMI 1640 medium supplemented with 10% foetal bovine serum and antibiotics (100U streptomycin/100U penicillin) in a humidified atmosphere at 37°C with 5% CO_2.

siRNA transfection. For silencing experiments, siRNAs targeted JNK2 MAPK (Qiagen, 1022632), p38 MAPK (Santa Cruz, sc-29433), and Silencer Negative control #1 siRNA (Qiagen, 1022076) were used. siRNAs were delivered into the cells (5 x 10^6 cells/35 mm plate) using the nucleofector technology (Cell Line Nucleofector Kit V, Amaxa Biosystems) according to manufacturers´ instructions. Gene expression in the transfected cells was analysed after 48 hour cultivation. For array analyses, chronic treatment protocol (two repeated siRNA transfections during 4 days, retransfection after 48 hours) was performed.

RNA extraction and qRT-PCR. Harvested cells were washed with PBS and total RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform method [27]. RNA was reverse transcribed using random hexanucleotides and M-MLV reverse transcriptase (Promega). qRT-PCR was carried out using RotorGene RG-3000 apparatus (Corbet Research) with following primers and probes (or SYBR Green I): JNK2-s 5´-AGCGAGACAAAATAAAAACAAGT-3´, JNK2-as 5´-TCCATTCTTCAATTGCATGTTC -3´, and JNK2-probe 5´-FAM-CTTCCAACTGGGCATCATAAATTTGA-TMR -3´; p38-s 5´- AGCTGACATAATTCACAGGGACC -3´, p38-as 5´-TATGCATCCCACTGACCAAATATCAA -3´, and p38-probe 5´- FAM-CCTGTCATTTCATCATCTGTGTGCCGA-TMR -3´; casp2-s 5´-AAAAGTTGCCGAAGATGAGACTG-3´ and casp2-as 5´-GAACCTCGTTTGGTGTTCCG-3´; c-kit-s 5´-GTTCTGCTCCTACTGCTTCGC-3´ and c-kit-as 5´-TAACAGCCTAATCTCGTCGCC-3´; EPS15-s 5´-AGGAGAGTGTAGAGTCAGGGA-3´ and EPS15-as 5´-TCCGTTTACAAGAATGCTGTGT-3´; MKK6-s 5´-CTCTCGGTCAAGTGAAGATGTG-3´ and MKK6-as 5´-ACACTGTATCCCTTCTGGTTGAG-3´; mdm2-s 5´-TGATGAAAGCCTGGCTCTGT-3´ and mdm2-as 5´-CTCTTCAGCTTGTGTTGAGT-3´; MSH2-s 5´-ACCAAAGGAATGTGTTTTACCCG-3´ and MSH-as 5´-CCGGTTGAGGTCCTGATAAATGT-3´; Notch4-s 5´-CACAACTCCTCCCCTTTGC-3´ and Notch4-as 5´-CCACAGCAAACTGCTGACAT-3´; RFC2-s 5´-CGAAGCCTACAAGATTCTTGCTC-3´ and RFC2-as 5´-ATGTGAGTGTATCCAATTTCCTTGA-3´; SOD1-s 5´-AGGATGAAGAGAGGCATGTTG-3´ and SOD1-as 5´-GCTTTTTCATGGACCACCAG-3´. Normalisation was done to the β_2-microglobulin control gene: β_2-microglobulin-s 5´- GAGTATGCCTGCCGTGTG-3´, β_2-microglobulin-as 5´-AATCCAAATGCGGCATCT-3´, and β_2-microglobulin-probe 5´-*FAM*-CCTCCATGATGCTGCTTACATGTCTC-*TAMRA*-3´.

Immunoblotting. The cells were lysed in Laemmli sample buffer and total cell protein extracts were loaded onto 10%SDS-PAGE. After blotting to a nitrocellulose membrane (Amersham), blocked membranes were incubated with following antibodies: anti-JNK2 (dilution 1:250, Santa Cruz), anti-p38 (dilution 1:250, Santa Cruz), anti-β-actin (1:2500, Sigma), and rabbit anti-mouse antibody conjugated with horseradish peroxidase (1:1000, Sigma). The signal was detected using BM Blue POD Substrate (Roche).

Cell proliferation assay. The chronically treated cells were subjected to the proliferation assay as described previously [28]. Cell viability was tested by trypan blue exclusion assay. The proliferation rate was determined by counting cells with a hemacytometer 48 hours after siRNA transfection. Relative cell numbers were normalised against siRNA-untreated control.

Expression array analysis. Atlas Human Cancer cDNA Expression Arrays (Clontech) were used to determine expression changes after silencing of two different MAPK genes. In order to eliminate potential non-specific effects, cells transfected with negative control siRNA were used as control samples for comparative analyses. Total RNA was isolated from cells chronically treated with siRNA. The reverse transcription and labelling with ^{32}P (Amersham) of 1μg of RNA and hybridisation procedures followed the protocol recommended by the array manufacturer. Gene expression was evaluated by AtlasImage 2.7 software (Clontech). Array experiments were performed in triplicates and the averages were counted by AtlasImage software. 1.5-fold or higher signal changes were considered to be significant.

Results

CML-derived cell line K562 with high level of JNK2 expression and KU-812, which highly expresses p38 MAPK [unpublished data], were used for the expression analyses. In these cells, silencing of the MAPKs was performed using validated JNK2-siRNA (nucleofection into K562 cells) and p38-siRNA (KU-812 cells). The efficiency to reduce the gene expression was assessed by qRT-PCR. 48 hours after siRNA transfections, we observed significantly decreased levels of both MAPK mRNAs. The quantity of JNK2 mRNA reduced by 81.2% in comparison to untreated control K562 cells (Figure 1A). In the case of p38, the mRNA level dropped by 65.4% in KU-812 (Figure 1B). Amounts of JNK2 and p38 MAPK proteins were studied 48 hours after siRNA transfections. The analyses revealed depletions of both proteins (Figure 2), which confirmed fullness of the gene silencing experiments.

During siRNA experiments, we measured proliferation of cells treated with tested siRNAs and there was to be seen slight decrease in proliferation rate of both samples after 4 day cultivation. However, these changes were not significant ($P > 0.05$) using unpaired Student´s t-test (GraphPad Prism Software version 4).

cDNA expression arrays with 588 probes were used to search for expression changes after silencing of two different MAPK genes. We tested differential expressions of the genes involved in cell proliferation, growth, apoptosis, oncogenesis *etc*. Atlas Human Cancer cDNA Expression Array was chosen since we previously used the same type for determination of deregulation of JNK2 and p38 MAPKs in CML [21-23]. K562 and KU-812 cells were chronically treated with JNK2/p38 siRNA as described in Methods. Before hybridisation, the gene suppressions were confirmed in the samples by qRT-PCR and western blots. After JNK2 depletion in K562 cells, 27 out of 588 genes showed significant expression changes, including 13 down-regulated genes and 14 up-regulated genes. In the case of p38 silencing in KU812, 20 genes were selected as significantly deregulated (7 genes reduced, 13 elevated). The results were summarized in Tables 1 – 4. Among others, MSH2 and MSH6, mdm2, and caspase-2

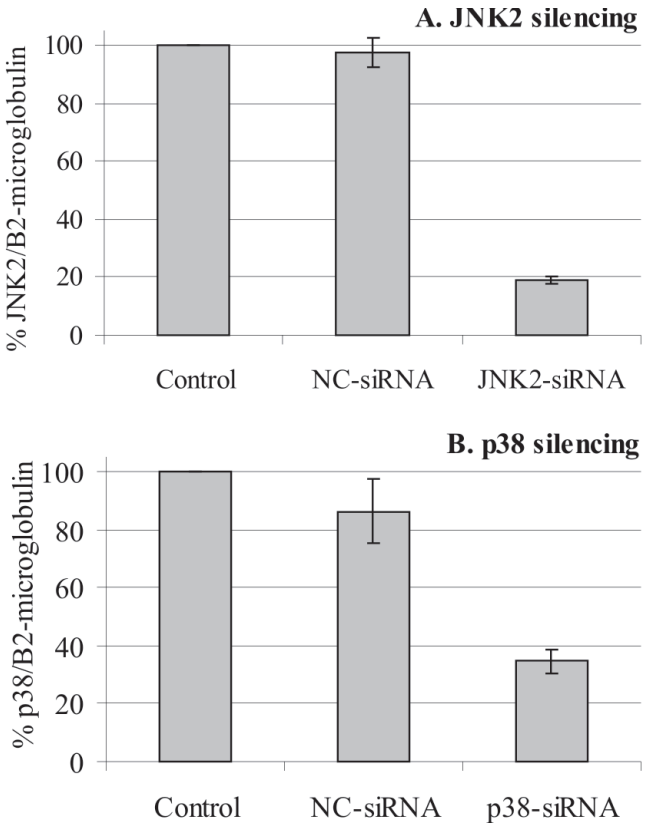

Figure 1. Determination of siRNA efficiencies. (A) Reduction of JNK2 mRNA expression in K562 treated with JNK2-siRNA. (B) Reduction of p38 mRNA in KU-812 treated with p38-siRNA. mRNA levels were measured using qRT-PCR 48 hours after nucleoporation. Normalised values of MAPK levels were compared to untreated control cells (Control). Values are means of five independent experiments ± SD.

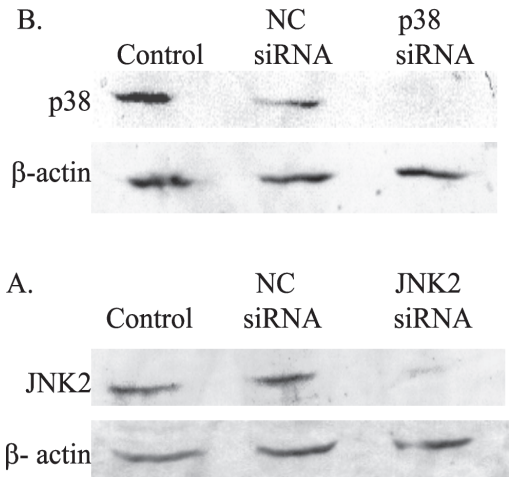

Figure 2. Western blot analyses of siRNA silencing. (A) K562 cells treated with JNK2-siRNA. (B) KU-812 cells treated with p38-siRNA. The upper panels show immunoblots with anti-MAPK-specific antibody (JNK2 or p38), and the lower panels show the same membranes reprobed with anti-β-actin antibody as a loading control.

Table 1. Up-regulated genes after JNK2 siRNA treatment in K562 cells.

GeneBank#	Ratio	Gene
L05624	1.570	dual-specificity mitogen-activated protein kinase kinase 1 (MKK 1)
U39657	1.965	dual-specificity mitogen-activated protein kinase kinase 6 (MKK 6)
U18422	1.792	transcription factor DP2; E2F dimerization partner 2
U69276	1.726	GRB-IR / GRB10
M26326	1.838	type I cytoskeletal 18 keratin; cytokeratin 18 (K18)
Y00503	1.541	type I cytoskeletal 19 keratin; cytokeratin 19 (K19; CK19)
U66879	2.403	BAD protein; bcl-2 binding component 6 (BBC6); bcl-2L8
AF016268	2.519	death receptor 5 (DR5); cytotoxic TRAIL receptor 2 (TRICK2A)
U37688	5.366	RATS1
U35835 + U47077	1.723	DNA-dependent protein kinase (DNA-PK) + DNA-PK catalytic subunit (DNA-PKCS)
M87338	1.885	replication factor C 40-kDa subunit (RFC40); RFC2
M96684	1.660	purine-rich single-stranded DNA-binding protein alpha (PURA)
M29366	3.166	ERBB-3 receptor protein-tyrosine kinase precursor; epidermal growth factor receptor
M34570	7.505	collagen 6 alpha 2 subunit (COL6A2)

Gene expression profiles of K562 cells treated with JNK2 siRNA and negative control siRNA were compared using AtlasImage 2.7 software. Array experiments were conducted in triplicates and the averages were counted by the AtlasImage software. Numbers represent the ratios of the average signal intensities of the samples treated with JNK2/NC siRNA.

Table 2. Down-regulated genes after JNK2 siRNA treatment in K562 cells.

GeneBank#	Ratio	Gene
L31951	0.290	c-jun N-terminal kinase 2 (JNK2)
X51688	0.364	G2/mitotic-specific cyclin A (CCNA; CCN1)
U01038	0.307	serine/threonine-protein kinase PLK1 (STPK13)
U00001	0.662	CDC27HS protein
U78798	0.560	TRAF6
U13021 + U13022	0.597	caspase-2 precursor (CASP2); ICH-1L protease + ICH-1S protease
U04045	0.389	DNA mismatch repair protein MSH2
U54777	0.444	DNA mismatch repair protein MSH6
Z12020	0.302	p53-associated mdm2 protein
M15990	0.434	C-yes proto-oncogene (YES1)
M60974	0.699	growth arrest & DNA-damage-inducible protein (GADD45)
U15979	0.692	delta-like protein precursor (DLK)
M29870	0.668	ras-related C3 botulinum toxin substrate 1; p21-rac1
M25639	0.280	macrophage migration inhibitory factor (MIF); glycosylation-inhibiting factor (GIF)

Gene expression profiles of K562 cells treated with JNK2 siRNA and negative control siRNA were compared using AtlasImage 2.7 software. Array experiments were conducted in triplicates and the averages were counted by the AtlasImage software. Numbers represent the ratios of the average signal intensities of the samples treated with JNK2/NC siRNA.

Table 3. Up-regulated genes after p38 siRNA treatment in KU-812 cells.

GeneBank#	Ratio	Gene
X05360	4.026	cyclin-dependent kinase 1 (CDK1, CDC2)
M14505	1.609	cell division protein kinase 4; cyclin-dependent kinase 4 (CDK4)
M81934	1.603	CDC25B; CDC25HU2; M-phase inducer phosphatase 2
U11791	1.696	cyclin H (CCNH); MO15-associated protein
U69276	3.037	GRB-IR / GRB10
AF015956	1.689	DAXX
U13021 + U13022	1.744	caspase-2 precursor (CASP2); ICH-1L protease + ICH-1S protease
U13737	1.728	caspase-3 (CASP3)
U18321 + X83544	3.062	ionizing radiation resistance-conferring protein + death-associated protein 3 (DAP3)
X06182	1.745	c-kit proto-oncogene
U04045	2.223	DNA mismatch repair protein MSH2
U77493	2.752	notch2
U07707	1.578	epidermal growth factor receptor substrate 15 (EPS15)

Gene expression profiles of KU-812 cells treated with p38 siRNA and negative control siRNA were compared using AtlasImage 2.7 software. Array experiments were conducted in triplicates and the averages were counted by the AtlasImage software. Numbers represent the ratios of the average signal intensities of the samples treated with p38/NC siRNA.

mRNAs were reduced and, on the other hand, MKK1 and MKK6, RFC2, cytokeratins K18 and K19, BAD, and DR5 showed higher expression after JNK2 inhibition. Concerning p38 silencing, the deregulated genes included caspase-10, SOD1, and Notch4 (down-regulation) and caspase-2 and caspase-3, CDC2, CDK4, and c-kit (up-regulation).

Validation of the array data was done by qRT-PCR. We determined mRNA expression levels for 5 genes deregulated after JNK2 silencing (MKK6, RFC2, caspase-2, MSH2, and mdm2) and for 5 genes deregulated after p38 inhibition (MSH2, c-kit, EPS15, SOD1, and notch4). qRT-PCR confirmed results obtained from cDNA arrays since relative expression levels determined by global analysis correlated to qRT-PCR results (Figure 3).

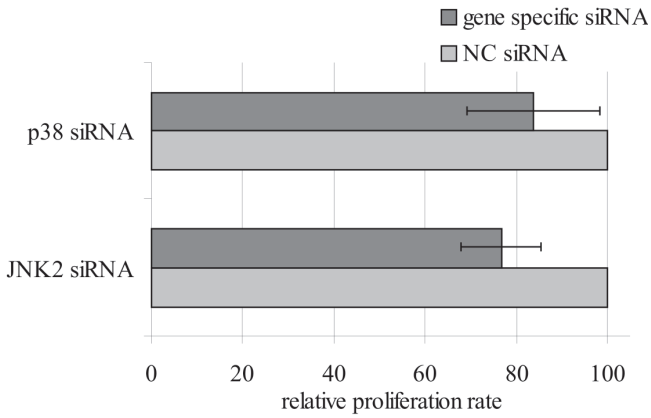

Figure 3. Cell proliferation after silencing of MAPKs. (A) K562 cells treated with JNK2-siRNA. (B) KU-812 cells with transfected p38-siRNA. All transfections were done by chronic treatment. Values are means of three independent experiments ± SD.

Discussion

Previously, it has been proved that activation of MAPK signalling pathway plays a crucial role in CML transformation. In several publications, we demonstrated over-expression of JNK2 and p38 MAPK in peripheral blood cells of BCR/ABL-positive CML [21-23]. In the present study, over-expression of these particular MAPKs by the siRNA-mediated silencing was investigated.

Firstly, we monitored cell growth after JNK2 and p38 inhibition in CML cell lines. Although protein levels were significantly reduced, neither JNK2 nor p38 siRNA effected cell viability and growth even after 4 days of chronic treatment. If neglecting the residual activity of MAPKs, our results suggest that these MAPKs do not represent key/essential kinases for CML transformation and another likely up-stream molecules play role in activation of signalling pathways involved in CML pathogenesis; therefore, our experiment on specific inhibition of a MAPK was not sufficient for substantial change in cell proliferation.

Deeper knowledge of intracellular events in CML cells may disclose the reasons of the inefficiency of MAPK silencing to inhibit the growth of the cells. To understand the background of deregulation of JNK2 and p38 in CML, changes in expression profiles caused by their siRNA-mediated silencing were monitored and we pointed out several genes with significant change at the expression level (Tables 1 – 4). The most important or interesting ones are discussed in the following paragraphs.

After p38 silencing, expressions of several cell cycle promoting factors were increased (CDC2 and CDK1 cyclin-dependent kinases, CDC25B, and cyclin H). For example, cyclin H is a part of CDK-activating kinase komplex, which is necessary for cellular proliferation. Therefore, the over-expression of cyclin H may be connected to up-regulation of CDC2 and CDK1. Concerning CDC25B phosphatase, it is a meiotic regulator that dephosphorylates CDC2. CDC25B has been shown to have oncogenic potential through its proliferative effect [29,30]. It was demonstrated that p38 binds and phosphorylates CDC25B and regulation of this phosphorylation by p38 is a critical event for initiating the G2/M checkpoint after ultraviolet radiation [31]. In summary, the observation that p38 inhibition leads to activation of several proliferation and cell cycle promoting factors is consistent with the fact that p38 kinase is involved in cell death.

Table 4. Down-regulated genes after p38 siRNA treatment in KU-812 cells.

GeneBank#	Ratio	Gene
L35253	0.520	mitogen-activated protein kinase p38 (MAPK p38)
U60519	0.277	caspase-10 precursor (CASP10)
K00065	0.604	cytosolic superoxide dismutase 1 (SOD1)
M96684	0.581	purine-rich single-stranded DNA-binding protein alpha (PURA)
U95299	0.238	notch4; hnotch4
M29366	0.438	ERBB-3 receptor protein-tyrosine kinase precursor; epidermal growth factor receptor
M62880	0.267	integrin beta 7 precursor (ITGB7)
X52541	0.600	early growth response protein 1 (hEGR1); transcription factor ETR103

Gene expression profiles of KU-812 cells treated with p38 siRNA and negative control siRNA were compared using AtlasImage 2.7 software. Array experiments were conducted in triplicates and the averages were counted by the AtlasImage software. Numbers represent the ratios of the average signal intensities of the samples treated with p38/NC siRNA.

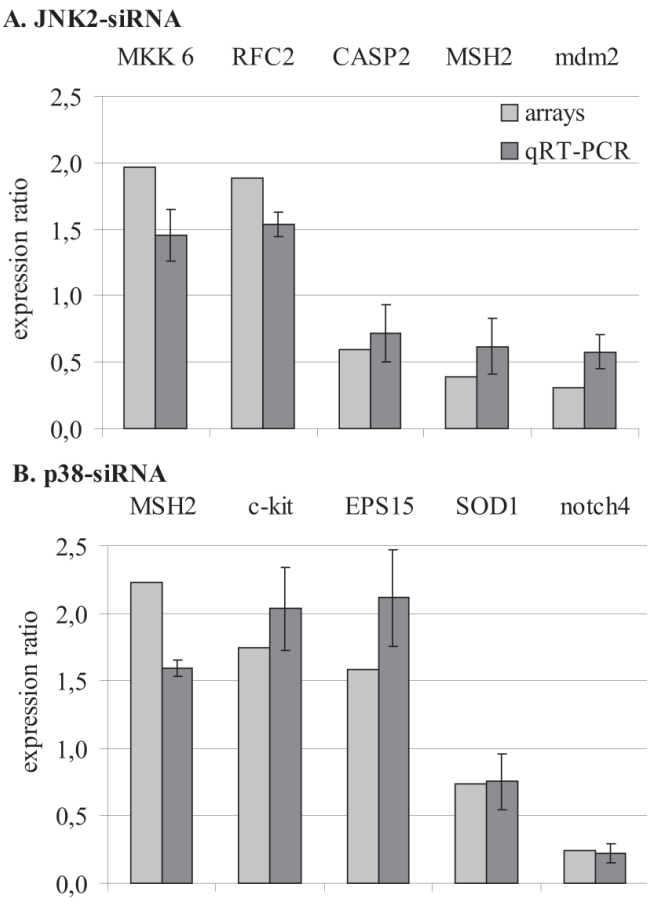

Figure 4. Validation of gene expression data. Comparison of the expression ratios obtained from array experiments and qRT-PCR. (A) K562 cells treated with JNK2-siRNA. (B) KU-812 cells treated with p38-siRNA. Values are means of three independent experiments ± SD. The values from qRT-PCR are normalised to β_2-microglobulin expression.

In contrast to p38 inhibition, JNK2-siRNA mediated gene silencing triggered only down-regulation of few cell cycle genes (cyclin A, PLK1, and CDC27Hs). Interestingly, cyclin A as well as PLK1 has been proposed as diagnostic markers for tumor-cell proliferation in oncology [32,33]. In this context, JNK2 inhibition plays rather an opposite role to p38 silencing in the cell cycle.

Further, two dual-specificity protein kinases – MKK1 and MKK6 were increased after JNK2 silencing. MKK1 and MKK6 are upstream kinases of MAPK; MKK1 is known to activate Erk and MKK6 activates p38. It may indicate that JNK2 inhibition leads to activation of other kinases that belong to different MAPK subgroups.

After JNK2 inhibition, two pro-apoptotic genes (BAD, DR5) were activated. Caspase-2 and Mdm2 gene expressions were reduced. Concerning caspase-2, understanding impact of its deregulation is complicated through production of two isoforms as results of alternative splicing: Ich-1L, which causes apoptosis, and Ich-1S, which prevents apoptosis [34].

In p38-siRNA treated cells, three pro-apoptotic factors were significantly over-expressed (DAXX, caspase-2, and caspase-3) and the expression of caspase-10 and ERG1 was reduced. Thus, JNK2 and p38 would appear to share both pro-apoptotic and anti-apoptotic roles in BCR/ABL-positive CML cells.

Another large group of deregulated genes after siRNA treatment consists of many factors associated with DNA repair and stress response (environmental stress, superoxide radicals, DNA damage, *etc.*). After JNK2 inhibition, MSH2, MSH6, GADD45, macrophage migration inhibitory factor (MIF), and p21-rac1 were reduced and a subunit of replication factor C (RFC2), DNA-dependent protein kinase (DNA-PK), PURA, and death receptor 5 (DR5) were increased. In the case of p38 silencing, expressions of cytosolic superoxide dismutase (SOD1) and PURA were down-regulated and MSH2 and death-associated protein 3 (DAP3) were increased. This massive affection of stress factors is consistent with the main function of MAPK – mediation of a cellular response to diverse extracellular stress stimuli. Interestingly, there are deregulated different genes after JNK2 or p38 MAPK inhibition, which suggested distinct functions of their elevation in CML.

p21-rac1 and TNF receptor-associated factor 6 (TRAF6) were down-regulated in the samples treated with JNK2-siRNA. Both genes have been implicated in the activation of NF-kappa-B transcription factor [35,36]. Moreover, both, p21-Rac1 and TRAF6 play a critical role in controlling the JNK signalling pathway [37,38].

Silencing of p38 seems to affect Notch signalling pathway. Notch2 was elevated and Notch4 reduced in transfected KU-812 cells. Notch products are transmembrane receptors that modulate differentiation, proliferation, and apoptotic programs of many precursor cells, including hematopoietic progenitors.

Only one gene showed the same change in the expression – growth factor receptor-bound protein GRB10 was elevated after both, JNK2 and p38 inhibition. GRB10 functions as a negative regulator in the insulin-stimulated MAPK signalling pathway [39].

On the other hand, three genes (epidermal growth factor receptor ERBB-3, PURA, and MSH2) showed opposite expression changes as a result of inhibition of JNK2 or p38 MAPKs. PURA and MSH2 genes, which are connected to DNA repair, were discussed above. Concerning ERBB-3, connections between EGF receptor and JNK or p38 were previously demonstrated [40,41].

In conclusion, the study was aimed for investigation of JNK2 and p38 MAPK over-expressions in CML. In order to look beyond the up-regulation of these MAP kinase genes, array-based expression profiling of CML cell lines with inhibited JNK2 or p38 was performed. We observed several genes, which expressions were elevated/reduced after silencing of the MAPKs genes. As discussed above, deregulation of many mentioned genes is connected to cell transformation processes (as cell cycle, apoptosis, DNA repair). Further, association of some other affected genes with the MAPKs is not so obvious.

In spite of significant up-regulation of JNK2 and p38 MAP kinases in CML cells, specific inhibition of neither JNK2 nor p38 led to the reduction of proliferation of tested cell lines. This observation is not surprising in the case of p38 MAPK since we demonstrated that inhibition of the gene leads to activation of several proliferation and cell cycle promoting factors (*e.g*. CDC2, CDK1, CDC25B, and cyclin H). Moreover, it has been previously found that p38 is essential for the antileukemic effect in BCR/ABL-expressing cells rather than for cell transformation [24,25]. Reasons of p38 over-expression in CML remains unclear; however, it may be expected rather a non-specific impact of CML transformation than a direct effect of p38 activation on cellular processes. Concerning JNK2, we observed activation of several genes connected to inhibition of malignant cell growth after the gene silencing but cell proliferation was not affected. It suggests that there are some additional signalling pathways, which are fundamental for transformation (likely those connected to BCR/ABL oncogene), whose deregulation is independent of the JNK2 MAPK over-expression.

Comparison of gene expression profiles after JNK2 and p38 gene silencing revealed distinct genes, which were affected. It suggests an unequal impact of their deregulation on the CML cells. On the other hand, the huge effect on the expression of DNA repair and stress responsible genes in both experiments illustrates similarities in functions of JNK2 and p38.

Finally, our data demonstrate that combination of two progressive molecular biology methods – siRNA together with microarrays – has a great potential in functional genomics.

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