MOLECULAR CANCER

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Gene expression profiling of chronic myeloid leukemia with variant t(9;22) reveals a different signature from cases with classic translocation

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Abstract

Background: The t(9;22)(q34;q11) generating the *BCR/ABL1* fusion gene represents the cytogenetic hallmark of chronic myeloid leukemia (CML). About 5–10% of CML cases show variant translocations with the involvement of other chromosomes in addition to chromosomes 9 and 22. The molecular bases of biological differences between CML patients with classic and variant t(9;22) have never been clarified.

Findings: In this study, we performed gene expression microarray analysis to compare CML patients bearing variant rearrangements and those with classic t(9;22)(q34;q11). We identified 59 differentially expressed genes significantly associated with the two analyzed groups. The role of specific candidate genes such as *TRIB1* (tribbles homolog 1), *PTK2B* (protein tyrosine kinase 2 beta), and *C5AR1* (complement component 5a receptor 1) is discussed.

Conclusions: Our results reveal that in CML cases with variant t(9;22) there is an enhancement of the MAPK pathway deregulation and show that kinases are a common target of molecular alterations in hematological disorders.

Keywords: Chronic myeloid leukemia, Variant t(9;22) rearrangements, Gene expression profiling, Protein kinases, Cellular pathways

Background

Chronic myeloid leukemia (CML) is a myeloproliferative disorder derived from hematopoietic stem cell transformation and characterized by heterogeneous biological and clinical features. The CML molecular marker is *BCR/ABL1* fusion gene generation as a consequence of a reciprocal t(9;22)(q34;q11) [1,2]. In most cases, the Philadelphia (Ph) chromosome is cytogenetically detectable but about 5–10% of CML patients show variant t(9;22)(q34;q11) rearrangements with the involvement of additional chromosomes [3,4]. In these cases the *BCR/ABL1* fusion gene can be revealed by Fluorescence in situ hybridization (FISH) or reverse transcriptase-polymerase chain reaction [5]. The occurrence of genomic microdeletions proximally to *ABL1* or distally to

BCR has been reported in CML cases with variant translocations with a greater frequency (30-40%) than in cases with classic t(9;22) (10-18%) [6,7]. The prognostic significance of variant t(9;22) was unclear and debated in the pre-imatinib era, whereas recent studies of large CML series have reported that the presence of variant translocations has no impact on the cytogenetic and molecular response or on prognosis [6,8]. However, the molecular bases of biological differences between CML patients with classic and variant t(9;22) have never been elucidated.

In this study, we performed gene expression profiling (GEP) by microarrays to identify a signature discriminating CML patients bearing variant rearrangements from those with classic t(9;22)(q34;q11). A list of 59 genes was found to be significantly associated with the two analyzed groups showing a differential expression. We applied network analysis to evaluate potential pathways

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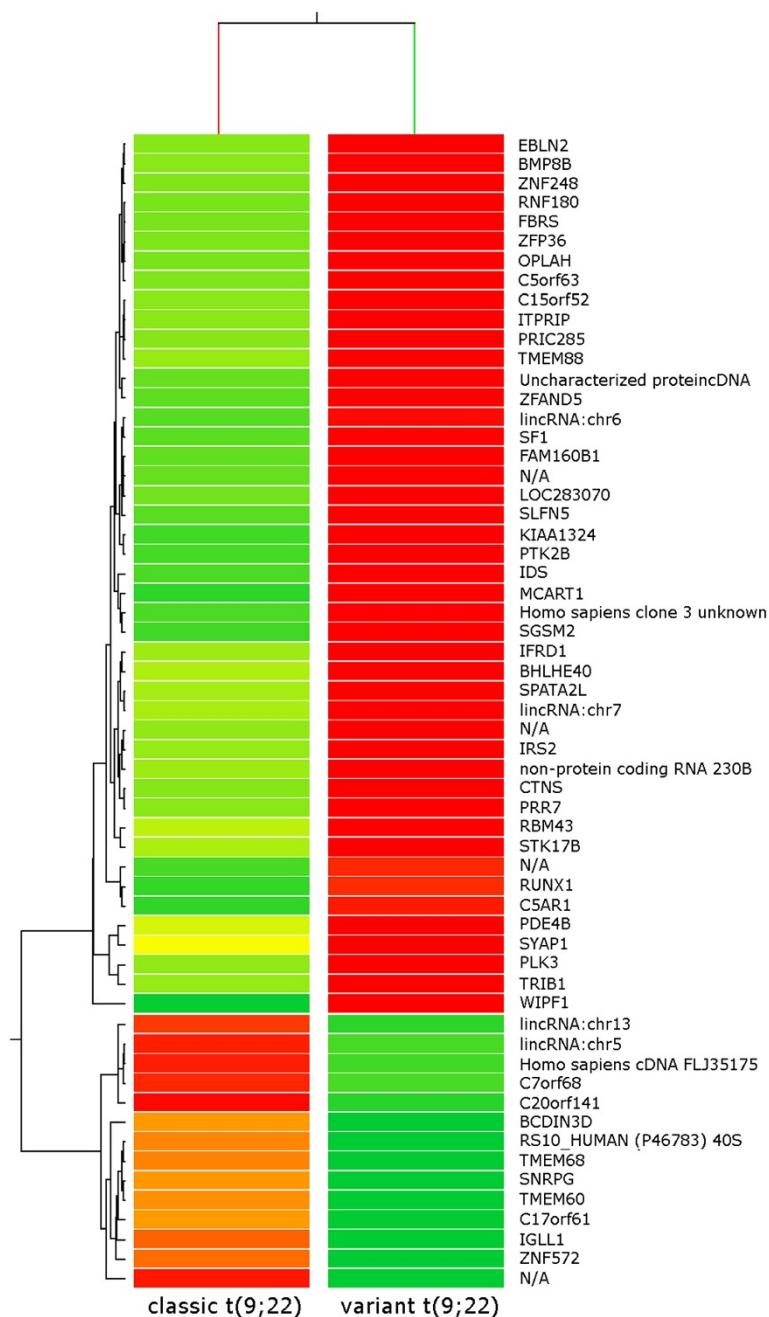
Figure 1 Hierarchical genes clustering in CML with classic (12 cases) and variant (8 cases) t(9;22) showing a 59-gene signature. Each row represents a single gene; green indicates differentially decreased expression of each gene in CML patient samples with variant t(9;22) compared with classic t(9;22) and red indicates differentially increased expression. The more saturated the color, the greater the degree of differential expression. N/A indicates genomic sequences not yet annotated.

involved in CML heterogeneity. An overall deregulation of genes encoding for protein kinases and involved in crucial cellular pathways such as MAPK (mitogen-activated protein kinase) signaling was found, unveiling the biological basis of differences in the CML patients subgroup with variant rearrangements.

Findings

Banding and molecular cytogenetic analyses allowed the identifications of 12 CML cases with classic t(9;22) and 8 cases with variant translocations (Additional file 1, Additional file 2). The *BCR/ABL*1 fusion analysis revealed the occurrence of b2a2 or b3a2 junctions in 10 (7 with classic
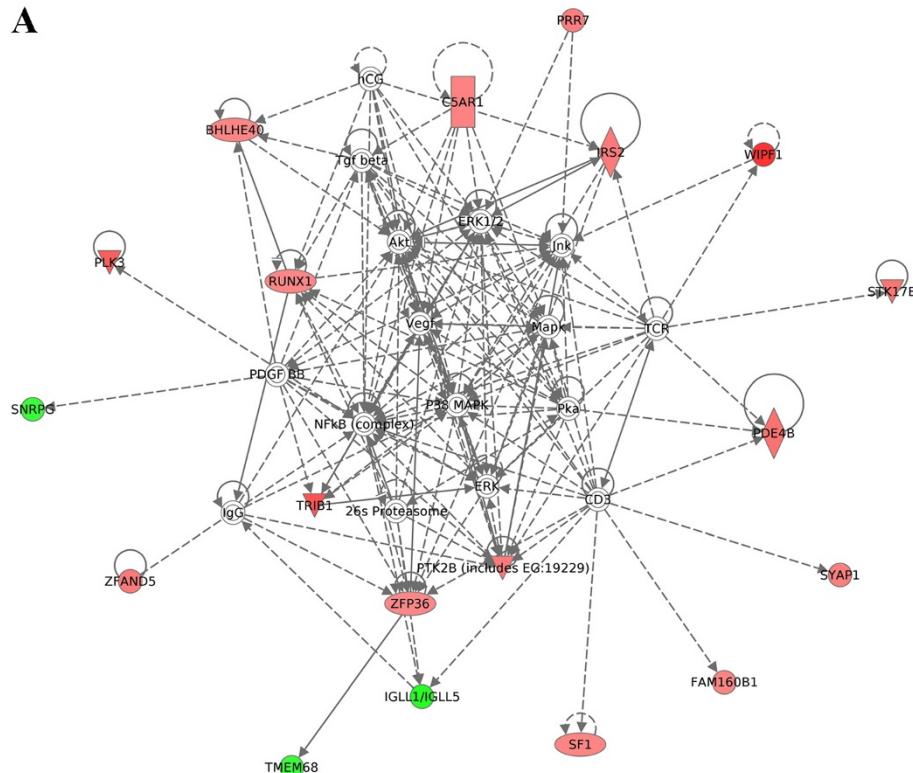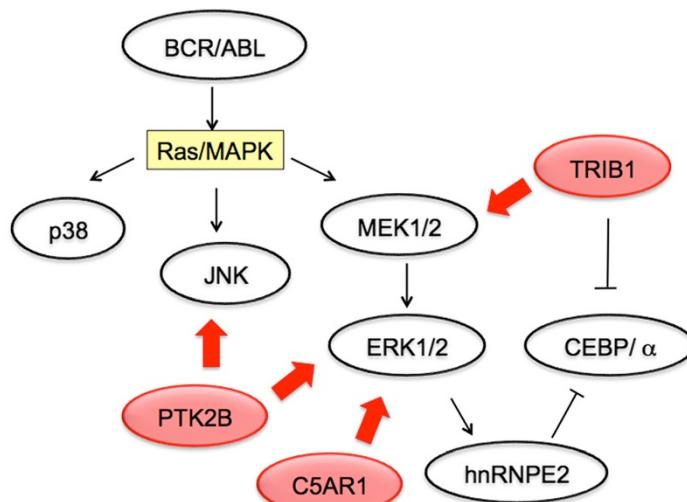
Figure 2 Deregulated genes in CML cases with variant t(9;22). (**A**) "Hematological System Development and Function, Tissue Morphology, Cellular Development" network deriving from GEP in CML cases with variant t(9;22). Both direct (solid lines) and indirect (dashed lines) interactions among genes are shown. Colored symbols correspond to genes included in our set of differentially expressed genes (red = upregulated and green = downregulated). (**B**) The involvement of TRIB1, PTK2B and C5AR1 kinases in the RAS/MAPK pathway downstream to the BCR/ABL oncoprotein.

and 3 with variant rearrangement) and in 10 (5 with classic and 5 with variant rearrangement) cases, respectively. All these patients were selected for further GEP analysis by oligonucleotide microarrays (Additional file 2). A set of 59 genes was identified as differently expressed in CML cases with variant t(9;22) rearrangements. All deregulated genes showed a more than 2 fold expression change; results of gene expression analysis indicated that 45 out of 59 differentially expressed genes were up-regulated whereas 14 were down-regulated (Figure 1; Additional file 3).

Upregulation of kinases genes

Querying the Database for Annotation, Visualization and Integrated Discovery (DAVID) showed that the enhanced biological process in our gene set involved the intracellular protein kinases cascade, a series of reactions in which a signal is passed within the cell by sequential protein phosphorylation. This enhancement was significant (p = 0.003) and associated to a value of 7.6. In detail, the kinases list included 5 genes: *TRIB1* (tribbles homolog 1), *STK17B* (serine/threonine kinase 17b), *PTK2B* (PTK2B protein tyrosine kinase 2 beta), *C5AR1* (complement component 5a receptor 1) and *ZFP36* (zinc finger protein 36, C3H type, homolog). Interestingly, *TRIB1* resulted one of the highest expressed genes showing a 2.9-fold change in GEP experiments. The upregulation of all 5 kinases was confirmed by quantitative real-time polymerase reaction experiments (qRT-PCR) analysis, with statistically significant expression levels ranging from 2.20 to 8.02.

Involvement of kinases in the RAS/MAPK pathway

Further Ingenuity Pathways Analysis (IPA) analysis yielded strong indications that 19 out of 59 dysregulated genes from our dataset are involved in the "Haematological System Development and Function, Tissue Morphology, Cellular Development" network (Figure 2A). A central role in this network is played by several proteins that are known to be activated in *BCR/ABL1* cells, namely ERK1/2 (extracellular signal-regulated kinases), p38MAPK (p38 mitogen-activated protein kinase), JNK (c-Jun N-terminal kinase), and cell cycle regulator AKT (RAC-alpha serine/threonine-protein kinase) that have a key function in multiple cellular processes such as apoptosis and cell proliferation (Figure 2A). In this respect, three main cellular processes are dysregulated by the BCR/ABL oncoprotein: RAS/MAPK which induces activation of proliferation, the PI3K (phosphatidylinositol-3 kinase)/AKT that activates apoptosis, and JAK/STAT which leads to an increased transcriptional activity [9]. Noteworthy, the upregulated kinase genes, previously revealed by DAVID analysis, are also enclosed in the network identified by IPA and establish direct or indirect interactions with other network components (Figure 2A). Moreover, TRIB1, PTK2B and C5AR1 kinases are involved in the regulation of the RAS/MAPK pathway (Figure 2B) [10-12]. TRIB1 is a signaling regulatory protein involved in leukemogenesis by regulating cellular proliferation and myeloid differentiation [10]. This protein binds to the 'middle layer' of kinases in the MAPK network, MAPKK (mitogen activated protein kinase kinase), and acts as an adaptor between the MAPKK pathway and CÆBPα (CCAAT/enhancer binding protein alpha). In fact, TRIB1 interacts with MEK1 (mitogen-activated ERK kinase 1) and MKK4 (MAP kinase kinase 4) and enhances phosphorylation of ERK1⁄2, promoting cell

proliferation and suppressing apoptosis. ERK1/2 phosphorylation is required for CÆBPα degradation and the activation of hnRNP-E2 (poly(rC) binding protein 2), a CÆBPα repressor (Figure 2B) [10]. Moreover, PTK2B is a proline–rich kinase involved in calcium induced regulation of ion channel and activation of the MAPK signaling pathway by stimulating JNK and ERK1/2 activity (Figure 2B) [11]. PTK2B is a member of the FAK tyrosine kinases family that could be activated by BCR-ABL causing an aberrant cell adhesion. C5AR1 is a member of the rhodopsin family of G protein-coupled receptors and is also the receptor of C5a anaphylatoxin, a strong proinflammatory mediator. It has been recently demonstrated that C5a binding to C5aR on human intestinal epithelial cells activates ERK1/2 and AKT phosphorylation [12].

To date several profiling studies in CML have been reported focusing mainly on predicting response to imatinib therapy and identifying a gene-specific signature for different stages of the disease [13,14]. Our GEP analysis performed on CML cases with variant t(9;22) may improve the understanding of the biological mechanisms at the basis of the CML heterogeneity. Overall, our results reveal that in CML cases with variant t(9;22) there is an enhancement of the MAPK pathway deregulation already known to underlie the CML pathogenesis and point out the role of interesting candidate genes, such as *TRIB1*, *PTK2B*, and *C5AR1*. These findings show that kinases are a common target of molecular alterations in hematological disorders and reinforce the idea that a perturbed action of signal transduction pathways is one of the hallmarks of cancer.

Additional files

Additional file 1: Table S1. Molecular cytogenetic characteristics of CML cases with variant t(9;22) translocations.

Additional file 2: Supplementary Materials and Methods.

Additional file 3: Table S2. Differentially expressed genes between CML cases with variant and classic t(9;22). Genes are rank-ordered according to fold change value.

Abbreviations
CML: Chronic myeloid leukemia; Ph: Philadelphia chromosome; FISH: Fluorescence In Situ Hybridization; GEP: Gene expression profiling; MAPK: Mitogen-activated protein kinase; DAVID: Database for Annotation, Visualization and Integrated Discovery; TRIB1: Tribbles homolog 1; STK17: Bserine/threonine kinase 17b; PTK2B: PTK2B protein tyrosine kinase 2 beta; C5AR1: Complement component 5a receptor 1; ZFP36: Zinc finger protein 36, C3H type, homolog; qRT-PCR: Quantitative real-time polymerase chain reaction experiments; IPA: Ingenuity Pathways Analysis; ERK1/2: Extracellular signal-regulated kinases; p38MAPK: p38 mitogen-activated protein kinase; JNK: c-Jun N-terminal kinase; AKT: RAC-alpha serine/threonine-protein kinase; PI3K: Phosphatidylinositol-3 kinase; MAPKK: Mitogen activated protein kinase kinase; C/EBPα: CCAAT/enhancer binding protein alpha; MEK1: Mitogen-activated ERK kinase 1; MKK4: MAP kinase kinase 4; hnRNP-E2: Poly(rC) binding protein 2.

Competing interest
The authors declare that they have no competing interests.

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