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Brief communication

A gene expression signature of primary resistance to imatinib in chronic myeloid leukemia

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ABSTRACT

Using gene expression profiling we show that the expression of 105-probe sets in mononuclear cells collected from chronic myeloid leukemia (CML) chronic phase (CP) patients with raised leukocyte counts who subsequently achieved complete cytogenetic response after 12 months on imatinib, differed substantially from that of patients who failed to achieve any degree of cytogenetic response. In the non-responder cohort, 9 of the 50 overexpressed genes were involved in DNA repair by homologous recombination, whereas 36 genes, including *PTEN*, were downregulated. This pattern of altered gene expression in responders and non-responders was validated in another independent dataset. These findings may prove useful for identifying at the time of diagnosis a subset of CP-CML patients who are likely to be resistant to imatinib and require an alternative treatment.

1. Introduction

Imatinib induces durable cytogenetic responses in the majority of chronic myeloid leukemia (CML) patients who receive the drug early in chronic phase (CP) [1,2] but an appreciable minority of patients fail to achieve any degree of cytogenetic response to imatinib. This is commonly referred as up-front or primary cytogenetic resistance and its incidence seems to be consistent in different patient cohorts [1,2]. The clinical heterogeneity of CML at diagnosis has been recognized for more than 20 years [3], and such heterogeneity could in part explain primary resistance to imatinib. However, because this type of resistance is rare, little is known of its biological basis.

To gain understanding on the molecular mechanisms associated with primary resistance to imatinib, we used microarray technology to compare the gene expression profiles of blood samples from patients who achieved complete cytogenetic responses on imatinib with those of patients who failed to achieve any degree of cytogenetic response (i.e. ≥95% Ph-positive at 12 months). We identified a set of genes whose expression was differentially regulated in patients resistant to imatinib. This set of genes was then tested in publicly available datasets.

2. Materials and methods

2.1. Patient characteristics and response assessment

We selected a total of 125 CP-CML patients treated at our institution with a minimal follow-up of 24 months on imatinib. Among them 12 failed to achieve any degree of cytogenetic response (i.e. ≥95% Ph-positive) during follow-up, of which 5 could eventually be included in the study (Supplementary Table 1). We matched this "non-responder group" with a "responder group" of 8 patients who achieved complete cytogenetic response (CCyR) within the first year of treatment and were still in CCyR at latest follow-up. Two CP patients who could best be classified as having 'acquired resistance' were added. Both had achieved CCyR within 1 year but then lost their responses at 18 and 24 months, respectively.

Cytogenetics was performed on bone marrow (BM) aspirates according to standard protocols. CCyR was defined by the failure to detect any Philadelphia chromosome in 20 metaphases. BCR-ABL transcripts were measured in the blood at 6–12 week intervals using real-time quantitative PCR. All patients signed an informed consent for the use of samples in accordance with the Declaration of Helsinki and with the approval from our Institutional Review Board.

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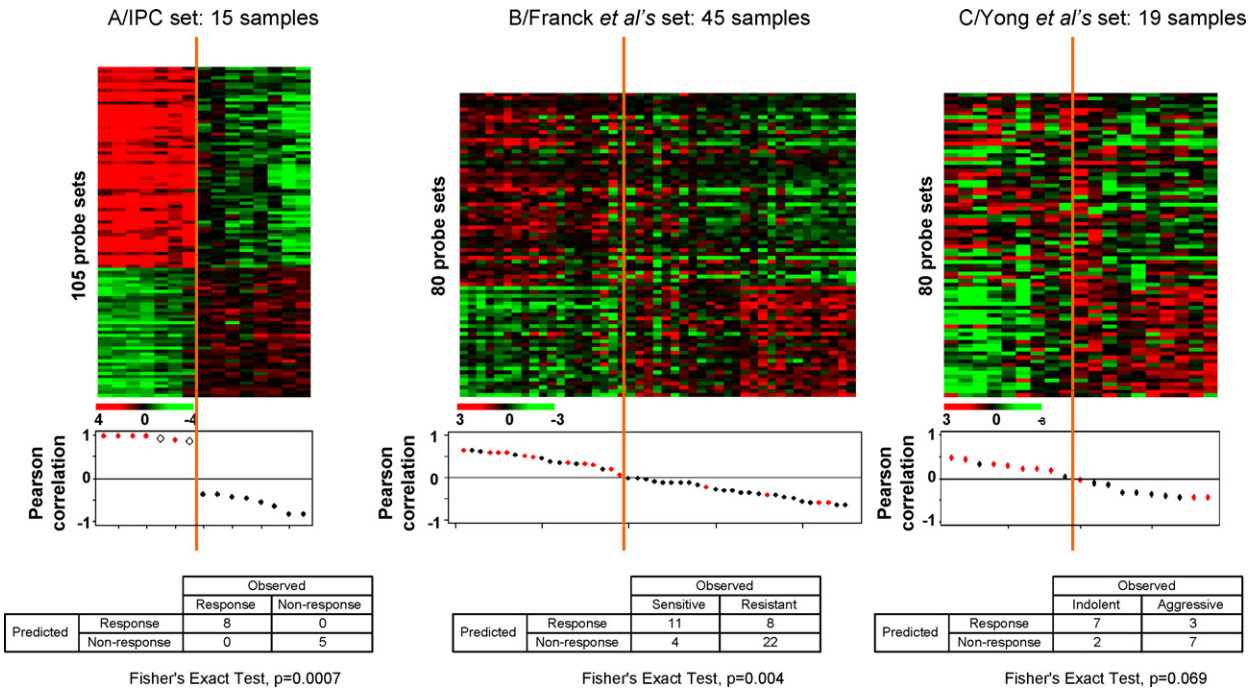

Fig. 1. Supervised classification of CP-CML samples based on gene expression profiling. (*A/*) Classification of 15 CP-CML samples using the 105-probe sets identified as differentially expressed between the 8 "responder" samples and the 5 "non-responder" samples. (*Top panel*) Expression data are depicted as a data matrix where each row represents a gene and each column represents a sample. Expression levels are depicted according to the color scale shown at the bottom. Red and green indicate expression levels, respectively, above and below the median. The magnitude of deviation from the median is represented by the color saturation. Probe sets are ordered from top to bottom by their decreasing signal-to-noise ratio. Samples are ordered from left to right according to the decreasing correlation coefficient of their expression profile with the median profile of the "non-responder" samples (*middle panel*). The solid orange line indicates the threshold 0 that separates the two classes of samples, predicted "non-responder class" (left to the line) and predicted "responder class" (right to the line). The response to imatinib is indicated by colored dots: red, "non-responder"; black, "responder"; open, early secondary resistance. The *bottom panel* displays the cross-table between the observed imatinib response and the response predicted by the probe set signature. (*B/*) Similar to (*A/*), but applied to expression data of the 45 Frank et al.'s samples (30 imatinib-sensitive and 15 imatinib-resistant) and the 80 probe sets common with our list. (*C/*) Similar to (*A/*), but applied to expression data of the 19 Yong et al.'s samples (9 "indolent" and 10 "aggressive") and the 80 probe sets common with our list. The status of samples is indicated by colored dots: red, "indolent"; black, "aggressive".

2.2. Expression data analyses

For each of the 15 patients, peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (Lymphoprep, Nycomed, Oslo, Norway) before starting imatinib. Total RNA was extracted with the RNeasy kit (Qiagen, Hilden, Germany). RNA quality was assayed by Bioanalyser 2100 (Agilent, Palo Alto, CA, USA).

Gene expression profiling was done using Affymetrix (Santa Clara, CA, USA) U133 Plus 2.0 human oligonucleotide microarrays. Experiments were done according to the standard protocols available from the manufacturer. Synthesis of first-strand cDNA was done using 2 μg of total RNA by T7-oligo(dT) priming followed by second-strand cDNA synthesis. After purification, *in vitro* transcription associated with amplification generated cRNA containing biotinylated pseudouridine. Biotinylated cRNA was purified, quantified and chemically fragmented (95 °C for 35 min), then hybridized to microarrays in 200 ml hybridization buffer at 45 °C for 16 h. Automated washes and staining with streptavidin–phycoerythrin were done as recommended. Double signal amplification was done by biotinylated antistreptavidin antibody with goat-IgG blocking antibody.

Data were analyzed by the Robust Multichip Average method in *R* using Bioconductor® and associated packages. Robust multichip average (RMA) did background adjustment, quantile normalization, and summarization of 11 oligonucleotides per gene. Before analysis, a filtering process removed from the data set the genes with low and poorly measured expression as defined by an expression value inferior to 100 units in all 15 CP-CML samples, retaining 24,863 probe sets with expression values ranging from 5 to 30,700 (mean, 156). To identify and rank genes discriminating samples from responder and non-responder patients, supervised analysis [4] was applied to the 24,863 probe sets. The statistical test was the signal-to-noise ratio (SNR), calculated for each gene as $SNR = (M1 - M2)/(S1 + S2)$, where M1 and S1, respectively, represent mean and SD of expression levels of the gene in group 1, and M2 and S2 in group 2. Confidence levels, and, because of multiple hypotheses testing, false discovery rates (*q*-values) were estimated by 1000 random permutations of samples as previously described, with a false positive rate of 1/10.000. The list of discriminator genes was interrogated by Ingenuity Pathway Analysis (version 5.5.1-1002; Ingenuity Systems, Redwood City, CA).

Once identified, the classification power of the gene profiling was illustrated by classifying samples according to the correlation coefficient of their expression profile with the median profile of the "non-responders" samples. A "leave-one-out" (LOO) procedure was applied as cross-validation (CV) of the generated gene expression signature.

2.3. Test of the gene expression signature on two independent datasets

The predictive performance of our signature was tested on two independent gene expression datasets collected from the EBI public repository (http://www.ebi.ac.uk/arrayexpress/), which included pretreatment CP-CML samples profiled using Affymetrix U133A microarrays. These two data sets corresponded to two series treated differently, and permitted us to test the treatment-specificity of our signature. In the first dataset [5] (accession number E-MEXP-433), gene expression profiles from 30 imatinib-responding patients (defined as major cytogenetic response – MCyR – at 12 months) and 15 non-responding patients (defined as lack of MCyR at 12 months) were analyzed. In the second dataset [6] were analyzed 10 patients with an 'aggressive disease' (blastic transformation – BT – within 3 years of diagnosis) and 9 patients with an 'indolent disease' (BT after 7 or more years from diagnosis), all of whom had been treated with interferon but not with imatinib.

3. Results

3.1. Establishment of a gene expression signature of primary resistance to imatinib

We established the gene expression profiles of 15 RNA samples prior imatinib treatment (Fig. 1) Supervised analysis identified 105-probe sets, representing 95 unique sequences (9 ESTs and 86 genes) as significantly differentially expressed (q-value < 0.05) between the 8 responder and 5 non-responder samples (theoretical number of false positives < 5). A total of 64 probe sets (representing 5 ESTs and 50 genes) were overexpressed in the non-responder samples and 41 were underexpressed (4 ESTs and 36 genes) (Table 1 and Supplementary Table 2).