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Identification of chromosomal translocations in leukemias by hybridization with oligonucleotide microarrays

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Background and Objectives. Identification of chromosomal rearrangements is important for a precise risk-stratified diagnosis of hematologic malignancies. As the number of known translocations, specific for different types of leukemia increases, it takes ever more time and increasing amounts of patient's material to screen a single patient with individual polymerase chain reactions (PCR). The aim of this study was to develop a new approach combining specificity with high-throughput sufficient for rapid screening of clinical samples for the presence of numerous translocations.

Design and Methods. We designed an oligonucleotide microarray and used hybridization with microarrays in combination with multiplex reverse transcription-polymerase chain reaction (RT-PCR) assay for accurate and rapid identification of some major leukemias. The following translocations were used as prototypic: t(9;22) p210 and p190, t(4;11), t(12;21), and t(15;17). This approach was tested on five different cell cultures carrying translocations and on 22 clinical samples from leukemic patients.

Results. Distinctive hybridization signals were obtained for all types of chimeric transcripts from cell lines with translocations. Both the type of translocation and the splice variant were determined. The data demonstrated high specificity and reproducibility of the method. Analysis of the 22 clinical samples using the microarray-based approach showed complete agreement with standard PCR analysis.

Interpretation and Conclusions. Our data suggest that oligonucleotide microarrays can be used as an efficient, alternative approach to the traditional post-PCR Southern blot analysis. The oligonucleotide microarray approach appears suitable for clinical screening of major risk-stratifying translocations in patients with leukemia.

Key words: oligonucleotide microarray, RT-PCR, multiplex PCR, leukemia, chromosomal translocations, diagnostics.

At present, more than 50 different translocations specific to particular types of leukemia and lymphoma have been described. Many of these translocations lead to the aberrant expression of transcription factors, i.e. either overexpression or formation of novel fusion (chimeric) proteins with oncogenic properties.[1,2] In the latter case, chimeric RNA or DNA provides a highly specific target for molecular assays. The chimeric RNA in leukemic blasts or residual leukemia cells can be detected using polymerase chain reaction (PCR)-based techniques.

Standard cytogenetic analysis fails to reveal the chromosomal translocations named cryptic. This led to a wider application of PCR for analysis of translocations. PCR is considered to be a very sensitive tool comparable in reliability to fluorescence *in situ* hybridization (FISH).[3,4] Numerous cDNA sequences for an increasing number of chimeric genes are analyzed using the PCR protocols for individual translocations. However, more than 50 separate reactions are necessary to screen a patient using the standard PCR analysis, because of the great number of fusion genes and breakpoint variants already known. To overcome this, multiplex PCR protocols, which can be used for clinical screening of patients, have been developed.[5-9]

In order to obtain a significant reduction in the efforts needed to identify amplification products after multiplex PCR, we designed a microarray containing the immobilized oligonucleotide probes required for detection of various fusion (splice) variants of five well-known translocations: t(9;22) with *BCR/ABL* p210 and *BCR/ABL* p190 fusion transcripts; t(4;11) with the *MLL/AF4* fusion, which is associated with aggressive infant acute lymphoblastic leukemia (ALL); t(15;17) with *PML/RARα* fusion and t(12;21) that produces the *TEL/AML1* chimeric transcript. These chromosomal aberrations are major risk-stratifying translocations[10].

Since the highly specific sequences can be considered as diagnostic markers of this class of molecular abnormalities, the use of microarrays appears to be a promising approach to their parallel hybridization analysis. A number of research groups are working to generate microarrays either by immobilization of DNA and presynthesized oligonucleotides or by oligonucleotide synthesis directly on glass or other surfaces.[11-13] Microarrays of oligonucleotides have proven to be efficient for identification of genetic mutations, detection of gene polymorphism and studies of gene expression.[13-15]Among various applications, DNA microarrays have been used to classify human acute leukemias basing on the monitoring of gene expression.[16] Our group is developing microarrays (microchips) in which oligonucleotides are immobilized within polyacrylamide gel pads.[14,17] The gel provides a stable support with high immobilization capacity and low fluorescence background. In this work, microarrays of gel-immobilized oligonucleotides were used in combination with multiplex PCR as a reliable tool for accurate identification of PCR products and verification of the fusion gene sequence at the splice site. We believe that this procedure can be applied in routine clinical diagnosis of leukemia.

Design and Methods

Cell lines and patient samples

The database references for the genes involved in the translocations are: MLL (L04731), AF4 (NM_005935), BCR (NM_021574), ABL (M14752), TEL (NM_001987), AML1 (X79549), PML (M79462) and RARA (X06538).

Cell lines K-562, MV-4-11, NB4 and REH were used as RNA sources for t(9;22)(q34;q11) with p210 product; t(4;11)(q21;q23); t(15;17)(q21;22), and t(12;21)(p13;q22), respectively.[18] In addition, as a control we used RNA from a patient with acute lymphocytic leukemia (ALL), positive for the translocation t(9;22)(q34;q11) with the p190 product. The leukemic cell line HL-60 served as a negative control. Cells were grown in RPMI supplemented with 10% fetal calf serum.

Bone marrow (BM) aspirates from 21 patients with ALL, chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) were also studied using microarrays.

RNA preparation and reverse transcription

Cultured cells were harvested and washed with phosphate-buffered saline. Lymphocytes from the BM aspirates were isolated after sample hemolysis in 0.8% NH_4Cl. Total RNA was prepared using the RNAqueous Kit (Ambion Inc., USA), according to the manufacturer's recommendations. The RNA solution was subsequently treated with 0.1 U/μL RNase-free DNase I (Ambion Inc., USA) at 37°C for 30 min in 100 μL of the buffer 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$. RNA (2.5 μg) was incubated at 70°C for 5 min with a mixture of the translocation-specific cDNA primers (10 pmol each) and then reverse-transcribed with 200 U of Superscript II (Gibco, BRL) in a total volume of 25 μL containing 25 U of RNase inhibitor (Ambion Inc., USA), 1mM each of dNTPs, 10 mM dithiothreitol, 50 mM Tris-HCl, pH 8.3, 75 mM KCl, and 3 mM $MgCl_2$ at 42°C for 1 h. The set of cDNA-specific primers contained the following oligonucleotides: *ABL:797L12, AF4:1664L12, AML1A:1921L12,* and *RARA:700L12*.[8] The primer's name includes the name of the corresponding gene used to design the primer, and the 5'nucleotide number of the gene sequence at the start of the primer; U or L means upper or lower, and is followed by the length of the primer.

Standard PCR analysis of the ABL gene

The PCR amplification was run in 100 μL of 1XPCR reaction buffer (Perkin-Elmer) containing 2.5 U of Taq DNA polymerase and 50 pmol of each of the following oligonucleotide primers: 5'TTCAGCCGCCAGTAGCATCTGACTT3' (*ABL5'*) and 5'AGATACTCAGCGGCATTG3' (*ALL3'*). Thirty cycles were performed: 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min; followed by the last extension at 72°C for 7 min. A 300-bp segment of c-ABL mRNA was amplified in parallel as an internal positive control for all mRNA samples to identify false-negative results in the RT-PCR assay.[7,19]

Multiplex and standard PCR analysis for the translocations

PCR amplification was performed as a nested two-round multiplex reaction. The RT reaction mixture (1 μL) was added to 25 μL of multiplex mixture containing 11 mM Tris-HCl, pH 8.3, 55 mM KCl,

Table 1. Oligonucleotide probes used in hybridization.

Type of translocation	*Name of gene*	*Splice variants*	*Oligonucleotide**	*Sequence from 5' to 3'†*
normal	ABL		ABL1(−)	TGTAGTTGCTTGGGACCCAGCCTTG
t(9;22	BCR		CML(−)	AGTTTCACACACGAGTTGGTCAGCA
			CML(+)	TGCTGACCAACTCGTGTGTGAAACT
b3a2	BCR-ABL	b3a2	CML-C(−)	gctgaagggcttTTGAACTCTGCTT
b2a2			CML-C(+)	AAGCAGAGTTCAAaagcccttcagc
		b2a2	CML-D(−)	gctgaagggcttCTTCCTTATTCAT
			CML-D(+)	ATCAATAAGGAAGaagcccttcagc
t(9;22)	BCR		ALL (9;22)(−)	AGATCTGCCCGGTCTTGCGGACGCC
		e1a2	ALL (9;22)(+)	GGCGTCCGCAAGACCGGGCAGATC
	BCR-ABL		ALLj(−)	gctgaagggcttCTGCGTCTCCATG
		e1a2	ALLj(+)	CATGGAGACGCAGaagcccttcagc
t(4;11)	AF4		AF4(−)	TAGGCGTATGTATTGCTGTCAAAGG
			AF4(+)	CCTTTGACAGCAATACATACGCCTA
	MLL-AF4	e10e4	AFj1(−)	gggagtggtctgCTTAAAGTCCACT
			AFj1(+)	AGTGGACTTTAAGcagacctactcc
		e9e5	AFj2(−)	atgggtcatttcCTTTTCTTTTGGT
			AFj2(+)	ACCAAAAGAAAAGgaaatgacccat
t(15;17)	RARA		RARA(−)	AAGGCTTGTAGATGCGGGGTAGAGG
			RARA(+)	CCTCTACCCCGCATCTACAAGCCTT
	PML-RARA	S-form	RARAj1(−)	gggtctcaatggCTTTCCCCTGGGT
		(bcr 3)	RARAj1(+)	ACCCAGGGGAAAGccattgagaccc
		L-form	RARAj2(−)	gggtctcaatggCTGCCTCCCCGGC
		(bcr 1)	RARAj2(+)	GCCGGGGAGGCAGccattgagaccc
t(12;21)	AML1		TEL(−)	AACGCCTCGCTCATCTTGCCTGGGC
			TEL(+)	GCCCAGGCAAGATGAGCGAGGCGTT
	TEL-AML1	type 1	TELj1(−)	caagtatgcattCTGCTATTCTCCC
			TELj1(+)	GGGAGAATAGCAGaatgcatacttg
		type 2	TELj2(−)	tcgtggctgcatCTGCTATTCTCCC
			TELj2(+)	GGGAGAATAGCAGaatgcatacttg

**(+) or (-) denotes that the oligonucleotide belongs to the sense (+) or antisense (-) strand. The chimeric oligonucleotides from the splice sites are marked by the letter junction. †Two spliced regions are indicated with capital and small letters.*

1.5 mM $MgCl_2$ (the concentration was optimized for each type of polymerase), 0.2 mM each of dNTPs, a mixture of primers (5 pmol of each primer), and 1.5 U of AmpliTaq-Gold polymerase (Perkin Elmer) or Taq-polymerase (Sileks, Russia). The primers used in the first round were: *BCR:1698U19*, *BCR:3060U23*, *ABL:661L23*, *MLL:3730U20*, *AF4:1636L29*, *PML3:1211U19*, *PML3:861U19*, *RARA:540L19*, *TEL:871U23*, and *AML1A:1891L23*. The mixture volume in the second round was 100 μL and 4 μL of the first round product were added as a template. The following primers were used in the second round (12.5 pmol of each primer in 100 μL of the reaction): *BCR:1777U19*, *BCR:3128U22*, *ABL:642L23*, *MLL:3751U20*, *AF4:1606L25*, *PML3:1370U21*, *PML3:930U20*, *RARA:508L22*, *TEL:944U23*, and *AML1A:1772L21*. All primer sequences have been described elsewhere.[8]

Twenty-five cycles of PCR amplification (per round) were run as follows: 94°C for 1 min, 58°C for 1 min, 72°C for 1 min for the first round and 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and finally 72°C for 10 min for the second round.

Immobilized DNA oligonucleotide probes

Oligonucleotides were synthesized with an Applied Biosystems 394 DNA/RNA synthesizer using the standard phosphoramidite method. The oligonucleotide sequences are presented in Table 1. All oligonucleotides bear an amino group at the 3' end, which reacts with polyacrylamide gel during the immobilization process. The oligonucleotide probes from both sense and antisense DNA strands

were included. For each oligonucleotide probe, a counterpart with two mismatched nucleotides in the central position (*m*) was synthesized and immobilized on the microchip to serve as a reference in the hybridization process (Figure 1 A).

Microarray fabrication

Micromatrices of glass-attached polyacrylamide gel pads of 100×100×20 mm in size spaced 200 mm apart were prepared by photopolymerization of a 4% (v/v) solution of acrylamide:bisacrylamide mixture (19:1), containing 0.5% (v/v) of N-(2,2-diethoxyethylene) acrylamide as an activating monomer in 0.1 M Tris-HCl (pH 8) and 30% glycerol as described previously.[20] Polyacrylamide gel arrays were activated prior to loading oligonucleotides onto gel pads. For activation of the gel, arrays were treated with 2% (v/v) solution of trifluoroacetic acid in water for 10 min, then washed for 1-2 min with Milli-Q water and dried. One nanoliter (1 pmol) of the 3'-amino-oligonucleotide solution in water was loaded on each gel pad of the hybridization microchips using a single pin robot constructed at EIMB.[14] Coupling of oligonucleotides with aldehyde groups within the gel resulted in formation of Shiff bases, which were stabilized by reduction with $NaBH_4$ and oligonucleotides were cross-linked to the activated gel pads.[21,22]

Preparation of the DNA target

The double-stranded (ds) DNA fragment, the product of the second PCR round, was mixed with the 300-bp product of the ABL-gene and fragmented with 0.01 U/μL of DNase I (Gibco, BRL) for 10 min at 37°C in a buffer containing 40 mM Tris, 50 mM KCl, and 2.5 mM $MgCl_2$ to obtain 50-100 bp fragments. They were labeled with tetramethylrhodamine-6-dUTP (New England Nuclear) by reaction with terminal transferase TdT (Promega Corporation) under conditions recommended by the manufacturer. Unincorporated nucleotides were removed by using Qiagen spin columns (Qiagen Nucleotide Removal Kit, Qiagen Inc., USA).

Hybridization

Hybridization was carried out in 40-50 μL of a buffer containing 20% formamide, 6X SSPE (0.06 M phosphate buffer, pH 7.4, 0.9 M NaCl, 3 μM EDTA), and 2 μg of salmon sperm DNA and 1-3 mg of labeled DNA target. The hybridization mixture was completely denatured at 94°C prior to hybridization, briefly cooled on ice and then applied onto a microchip under a hybridization chamber (Cover Well™, Bioworld) and left overnight at room temperature. After that, the chamber was disassembled, the microchip was washed for 20 min with 40 mL of 1X SSPE at 37°C and mounted again in the same buffer for microscope monitoring.

Image analysis

Hybridization signals were monitored with a Research Grade microchip analyzer consisting of a wide-field, high-aperture, long-working-distance fluorescence microscope coupled with a cooled CCD camera.[14,23] Images were obtained with an original WinView software (Princeton Instruments) and processed with customized software.[24] The local denomination of the signal was used. The brightest fluorescent signal was assumed to correspond to the perfectly matched duplex formed by the microchip oligonucleotide and hybridizing DNA target.

Standard PCR and gel electrophoresis analysis of the translocations

Individual PCR reactions used in comparative analysis of clinical samples were performed with individual sets of primers under the same conditions described for Multiplex PCR. The PCR products (10 μL) were analyzed on a 1.5% ethidium bromide-containing agarose gel.[8]

Results

The localization of oligonucleotide probes on the microarray is shown in Figure 1A. One oligonucleotide probe was used to detect the *c-ABL* gene, and fourteen pairs of oligonucleotide probes were used to detect fusion genes with splice variants.

The target DNAs prepared from six control samples (five cell lines and one sample from a patient), were hybridized with microarrays. As can be seen, the signal from the ABL-gene is present in all samples (Figure 1B-G, column 1). Different chimeric transcripts are distinguished by hybridization signals from oligonucleotides specific for each translocation and located in horizontal rows. The first in each row the oligonucleotide, common for all splice variants, is immobilized (Figure 1C-1G, columns 3 and 4); the oligonucleotides, specific for different splice variants of this concrete translocation, are located further away (Figure 1C-1G, columns from 5 to 8). The hybridization signal indicates the presence of *b3a2* splice variant (Figure 1C) for the K-562 cell line carrying t(9;22), and *e1a2* splice variant (Figure 1D) for the patient with ALL diagnosed as having t(9;22). For the cell line MV-4-11, carrying t(4;11), the hybridization pattern also reveals only one splice variant *e9e5* (Figure 1E), as it does for the NB-4 cell line with the *bcr1* type of chimeric transcript (Figure 1F). For the REH cell line with t(12;21) the hybridization signals dis-

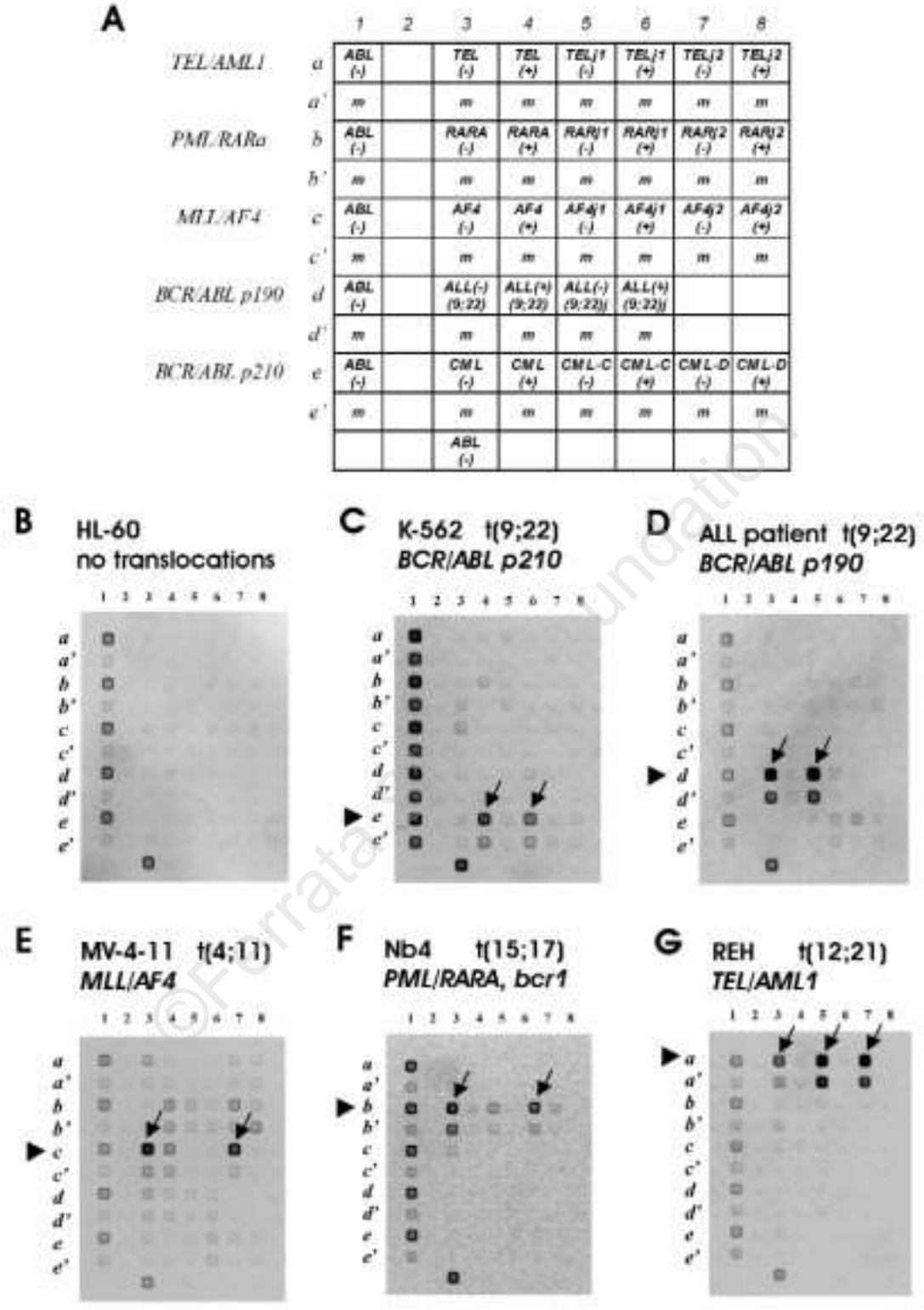

Figure 1. Microarray analysis of control samples carrying chromosomal translocations. Small letters denote the rows with perfect (a-f) and double-mismatched probes *m* (a'-f'). A, the scheme of oligonucleotide location on the microarray; B-G, hybridization patterns obtained for cell lines HL-60, K-562, ALL patient *e1a2*, MV-4-11, NB4 and REH, respectively. Note hybridization signals from the *ABL* gene as an internal positive control (column 1). Arrows show hybridization signals specific for each of five translocations in a respective cell line.

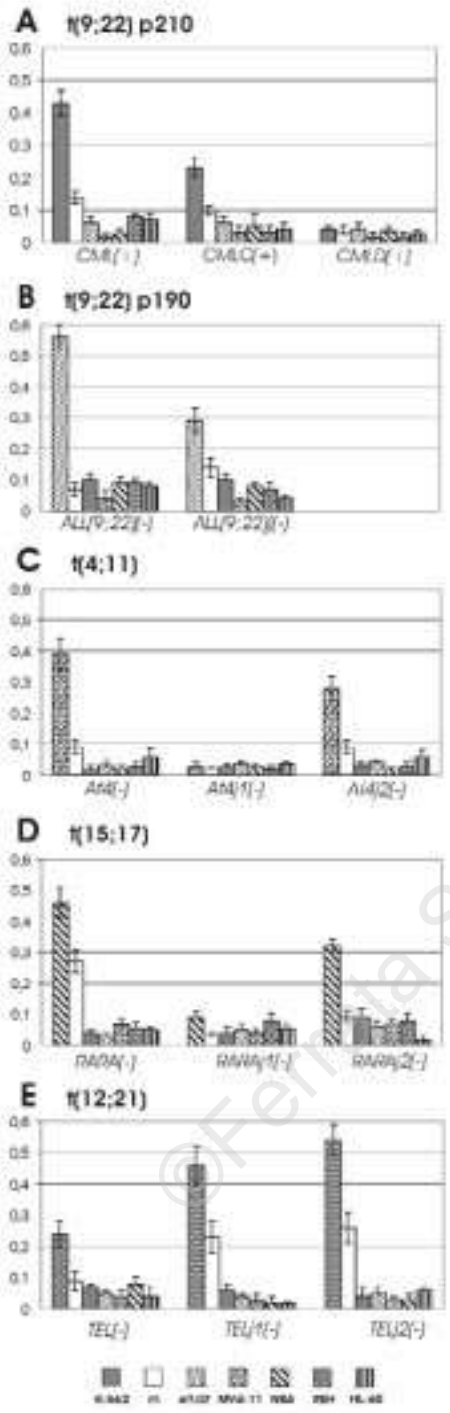

Figure 2. Relative fluorescence, obtained for hybridization of the DNA targets from different cell lines with translocations. The data are averaged from three experiments and mean signal ± s.d. is shown. The fluorescence intensity in a.u. (arbitrary unit) is plotted along the vertical axis. A-D – sets of oligonucleotide probes specific for translocations t(9;22) p210, t(9;22) p190, t(4;11), t(15;17) and t(12;21), respectively.

play two types of chimeric transcripts (Figure 1G). The oligonucleotides complementary to the sense (+) or antisense (-) strands differed in hybridization efficiency with dsDNA. For example, the PCR-amplified dsDNA target from the K-562 cell line was hybridized better with (+) oligonucleotide probes (Figure 1C), whereas dsDNAs from all other cell lines were hybridized more efficiently with (-) oligonucleotide probes (Figure 1 D-G). It seems that the asymmetry of hybridization patterns (better hybridization with either (+) or (-) strand) is connected to the properties of the dsDNA target, resulting from the concrete PCR protocol.

Quantitative analysis of the hybridization patterns was used to validate the sensitivity and specificity of the hybridization method. It was found that about 1 μg of the amplified DNA in the labeling reaction was needed to obtain sufficient intensity of fluorescence on the microarray, allowing visual discrimination between the probes. Three hybridizations for each cell line with the same amount of a sample (2 μg of each PCR product taken for the labeling reaction were mixed with 1-2 μg of the ABL-gene fragment) were carried out in parallel on different clusters of one microchip (each cluster contained a complete set of oligonucleotide probes). The noticeable, rapidly growing signal was registered after 1-2 h of hybridization, but about 20 h were usually required for complete development of the signal. The signal for each positive oligonucleotide probe in a respective cell line was calculated as a mean of three experiments and compared with the signal from its counterpart with two mismatches and with the signals in negative cell lines. The results are shown in Figure 2 A-E. The signals for positive oligonucleotide probes were about 2-10 times stronger than their *m* counterparts. In the presence of a translocation, the hybridization signal for the corresponding oligonucleotide was usually 3 to 20 times stronger than that in its absence. This difference between hybridization signals in positive and negative cell lines is statistically significant (Student t-test, $p<0.001$).

To evaluate the multiplex/microchip assay as a potential diagnostic tool, the BM aspirates from 10 selected patients with known rearrangements were analyzed: two with BCR/ABL p210, three with PML/RARA, one with MLL/AF4, two with TEL/AML1 and two cases negative for all translocations. For each case, the standard PCR analysis with individual primers was run. Some examples are shown in Figure 3 A-D. Retrospectively, the multiplex reaction was performed, followed by hybridization with a microarray. The results of both methods were in

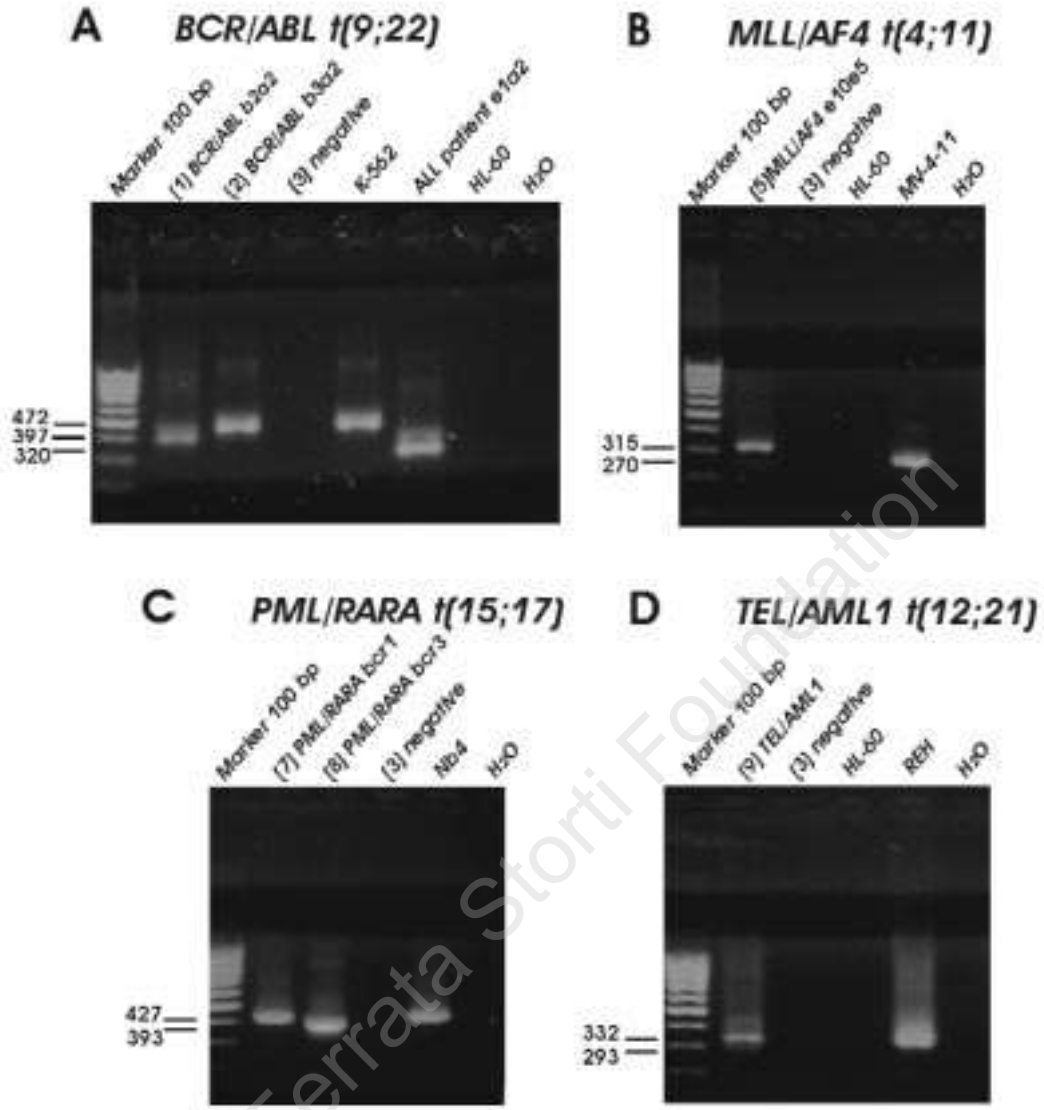

Figure 3. A-D. Chromosomal translocations in cell lines and patients, as determined by standard RT-PCR. The numbers in parentheses above the lanes are index numbers of clinical cases studied by the microarray analysis.

complete agreement.

Finally, we applied the microchip-based assay for the identification of eleven ALL, CML and AML patients in the form of a clinical test. RNA samples were received from the Chicago University Hospital, reverse transcribed with specific cDNA primers and analyzed by multiplex PCR followed by hybridization with a microarray. We revealed five BCR/ABL b3a2, two BCR/ABL b2a2, one BCR/ABL e1a2 in eight CML patients; one PML/RARA bcr 1 in one AML patient; and two negative cases. Single RT-PCR assay of these patients was performed in the Department of Pathology, at the University of Chicago. The results of both methods were almost identical, except for one case in which BCR/ABL e1a2 in a CML patient was found by the microchip approach, despite the fact that initially, at diagnosis, the patient had been determined as carrying both BCR/ABL e1a2 and b2a2 types of chimeric transcript. We performed additional individual PCR reactions for all samples (Figure 4), and for this CML patient the same sample that we had

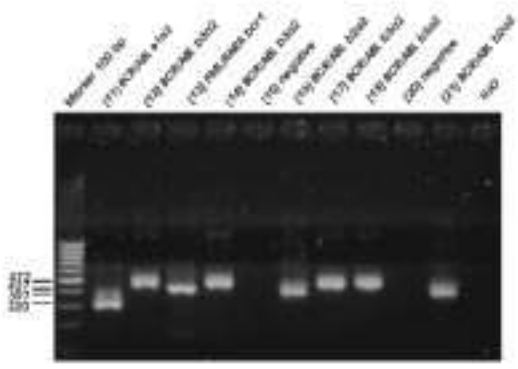

Figure 4. Confirmatory PCR analysis of clinical samples using in blind testing of microarrays. The numbers in parentheses above the lanes are index numbers of clinical cases studied by the microarray analysis.

used in the microarray testing was taken. The presence of only the *e1a2* type of chimeric transcript was confirmed by standard gel electrophoresis analysis (Figure 4, case 11). A shift of chimeric transcript type had probably occurred in the course of disease progression.

Discussion

Multiplex PCR has been previously used to characterize small groups of chromosomal translocations found in leukemic cells.[5-8] A multiplex PCR protocol to detect at least 29 translocations divided into subgroups has been developed[8] and shown to be effective for clinical screening.[9] Since a number of fragments having a similar molecular weight result from each multiplex reaction, additional analysis is required for detection and verification of a fusion gene. Split-out analysis[8,9] and modified post-PCR Southern blot analysis[7] have been suggested for this purpose. The use of microarrays for determinative studies offers several advantages over conventional electrophoretic separation of PCR products and standard blot hybridization. A large number of different oligonucleotides can be immobilized on a single microarray allowing simultaneous detection of a great variety of different targets. Another advantage of using microarrays is miniaturization of the format compared to the format of standard hybridization experiments. Quantification of hybridization signals allows one to estimate the efficiency of the target binding with different oligonucleotides on the array.

To make sure that the amplifiable template RNA/cDNA is present in the sample, control genes are usually amplified and run as an internal standard. One of the genes considered to be an appropriate control in the RT-PCR assay is the *c-ABL* gene, which is expressed at moderate levels and is known to lack processed pseudogenes, unlike, for example, the β-actin gene.[7,19] In our experiments the distinctive hybridization signal of the ABL gene on the microarray ensured adequate quality not only of both mRNA/cDNA, but also of the labeled probe. Proof that all steps of the probe preparation are successfully achieved appears to be important for screening of clinical samples.

All the presented hybridization patterns of fusion transcripts are distinct. This enables one to discriminate not only between different types of translocations, but also between splice variants (Figure 1). Identification of splice variants requires no special analysis and can be done within the screening procedure. The prognostic significance of different splice variants needs to be elucidated in many cases and special effort has been directed toward designing PCR primers that would allow one to distinguish between different types of fusion transcripts.[25]

This may be essential for the CML patients having BCR-ABL with the *b3a2* (55%) or *b2a2* (40%) junctions, and sometimes *e1a2* transcript, usually typical of ALL.[26,27] Two isoforms of the PML-RARA fusion gene were also found: long (L) transcripts (including *bcr 1* and *bcr 2*) and short (S) transcripts (*bcr 3*), which corresponded to different clinical characteristics.[28] So, further accumulation of data on the occurrence of different splice variants may not only provide additional molecular characteristics, but also clarify the significance of different types of chimeric transcripts in the course of the disease, thus allowing a more precise clinical diagnosis.

Quantitative estimation of the hybridization patterns revealed that the normalized fluorescence signals from the corresponding gel pads were 2 to 20 times stronger in the positive cases than in the negative control. The level of the hybridization signal is affected by many factors: hybridization buffer content, time and temperature of hybridization, quantity of the probe and target, washing conditions, etc. However, under strictly uniform conditions of hybridization and measurement, the hybridization patterns appear to be highly reproducible (Figure 2). The sensitivity and specificity of

the assay are sufficient for reliable identification of any of the studied translocations not only in cell cultures, but also in clinical samples, as confirmed by standard PCR analysis (Figures 3, 4). The assay can be integrated into a clinical laboratory using a simple, cheap analyzing system[29] as a screening tool for the initial diagnostic phase of leukemias.

Contributions and Acknowledgments

TN and PD were primarily responsible for this work; PD provided cell lines and the main clinical material, TN designed the study, carried out a substantial part of the experiments and wrote the paper, PD made critical comments. VZ carried out the laboratory experiments, JH was responsible for clinical material to test microarrays, and YL worked with the gene databases. PD and AM initiated the research and AM was the principal investigator. All the authors revised the manuscript and contributed to its intellectual content. The order of the names was a joint decision of all authors.

The criteria for the order of names were the involvement in laboratory research, writing and reviewing the manuscript, and clinical material collection. The order of the names was decided on the basis of each individual contribution to the above criteria.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Cristina Mecucci, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Professor Mecucci and the Editors. Manuscript received October 11, 2001; accepted February 12, 2002.

What is already known on this topic

Microarrays have been proven to be a promising technology in genomic studies of malignancies. RT-PCR is the most sensitive methodology to identify reciprocal translocations in malignancies.

What this study adds

A new approach combining oligonucleotide microarrays plus RT-PCR to detect typical chromosomal translocations in leukemia.

Potential implications for clinical practice

This technology seems a valid and rapid approach to diagnose known genetic subgroups among hematologic malignancies. Results may be helpful to address specific therapeutic protocols.

Cristina Mecucci, Associate Editor