Interphase Fluorescence In Situ Hybridization Assay for the Detection of 3q21 Rearrangements in Myeloid Malignancies

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In myeloid malignancies, chromosome rearrangements involving band 3q21 are associated with a particularly poor prognosis of the disease. Their sensitive and unequivocal detection is therefore of great clinical importance. In this report, we describe the establishment of an interphase fluorescence in situ hybridization (FISH) assay that complements classical cytogenetic analysis in the diagnosis of such aberrations. PACs that map centromeric and telomeric of known 3q21 breakpoints were labeled with different fluorescent dyes, and the separation of the normally colocalizing signals was used as an indicator of the presence of a 3q21 rearrangement. Two cell lines and 10 primary samples from myeloid leukemia and myelodysplastic syndrome (MDS) patients with 3q21 rearrangements were investigated using the newly established method. The rate of false positivity was determined in 27 control samples from patients with various types of myeloid malignancies. In addition to providing a sensitive and rapid test for the detection of 3q21 aberrations, the interphase FISH assay yields preliminary information about the localization of individual breakpoints. Six of the 10 breakpoints in the patient samples map to an only recently described breakpoint cluster region (BCR) 60 kb centromeric of the originally reported 3q21 BCR. These findings may contribute to the understanding of the molecular basis of the clinical features associated with 3q21 rearrangements. © 2001 Wiley-Liss, Inc.

INTRODUCTION

Rearrangements of the long arm of chromosome 3, namely, the inv(3)(q21q26) and the t(3;3)(q21;q26), are found in approximately 2.5% of patients with acute myeloid leukemia (AML), and are not confined to a specific FAB type. They have also been observed in some cases of chronic myeloid leukemia (CML) and myelodysplastic syndrome (MDS) (Fonatsch et al., 1994; Horsman et al., 1995; Secker Walker et al., 1995; Shi et al., 1997; Sancho et al., 1999; Testoni et al., 1999). The clinical features associated with the two aberrations are similar: megakaryocytic dysplasia, normal or elevated platelet counts, and poor responsiveness to conventional and even intensified treatment regimens (Fonatsch et al., 1994; Horsman et al., 1995; Secker Walker et al., 1995; Shi et al., 1997; Sancho et al., 1999; Testoni et al., 1999; Reiter et al., 2000). This may be explained by the fact that the breakpoints in inv(3) and t(3;3) affect the same regions in bands 3q21 and 3q26. However, breakpoints in 3q26 are scattered over several hundred kilobases (kb) (Morishita et al., 1992; Levy et al., 1994). Those in 3q21 seem to be confined to a region of approximately 100 kb, which may actually contain

two distinct breakpoint clusters (Suzukawa et al., 1994; Pekarsky et al., 1995; Rynditch et al., 1997; Wieser et al., 2000a). The more centromeric of these is also affected by the translocation t(1;3)(p36;q21) (Shimizu et al., 2000), in which similar clinical characteristics to those of the inv(3) and the t(3;3) are seen (Horsman et al., 1995). Detection of these chromosome rearrangements by classical cytogenetics (i.e., banding analysis of metaphase chromosomes) may be hampered by insufficient numbers of dividing cells and by low-quality metaphase preparations, making a polymerase chain reaction (PCR) or interphase fluorescence in situ hybridization (FISH) assay highly desirable. Such molecular tests would also provide higher sensitivity than metaphase analysis in the detection of aberrant cells, making them valuable tools for dis-

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ease monitoring. Reverse transcriptase (RT)-PCR assays specific for 3q rearrangements would be difficult to establish because no fusion transcript is consistently encountered as their consequence. Overexpression of the putative oncogene in 3q26, *EVI-1*, may be associated (Fichelson et al., 1992; Morishita et al., 1992; Suzukawa et al., 1994; Testoni et al., 1999), but is not strictly correlated (Russell et al., 1994; Ohyashiki et al., 1995; Ogawa et al., 1996; Fontenay Roupie et al., 1997; Soderholm et al., 1997), with rearrangements of this chromosome band. Furthermore, scattering of breakpoints in 3q26 and 3q21 precludes genomic PCR assays.

In the present study, we established an interphase FISH assay for the detection of 3q rearrangements. The breakpoint region in 3q21 is smaller than that in 3q26, and is affected not only by the inv(3) and the t(3;3) but also by the t(1;3). Thus, we used clones from a previously assembled 3q21 PAC contig and developed two probe sets that allow the efficient, rapid, and sensitive detection of breakpoints in either of the two 3q21 breakpoint cluster regions.

MATERIALS AND METHODS

Cell Culture

All cell lines were cultured in RPMI medium supplemented with 10% FCS at 37°C in a 5% CO_2 atmosphere. For Mutz-3 cells, obtained from Drs. Hans Drexler and Roderick McLeod, 100 U/ml GM-CSF was added to the media. HNT-34 was obtained from Dr Hiroyuki Hamaguchi.

Cytogenetic Analysis

Chromosome banding analyses were performed on short-term cultures of bone marrow and peripheral blood samples. Methods of cell cultivation, chromosome preparation, and staining by a modified Giemsa-banding technique have been reported previously (Stollmann et al., 1985). The karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).

Fluorescence In Situ Hybridization (FISH)

For FISH experiments, fixed nuclei from cell lines or from short-term cultures of bone marrow or peripheral blood samples were processed as described previously (Rieder et al., 1998). 3q21 PAC clones used as probes were derived from the libraries RPCI_1, 3, 4, and 5 (Ioannou et al., 1994), provided by the Resource Centre of the German Human Genome

Project (RZPD), Berlin. All clone names start with 'RPCIP704,' which is omitted here for reasons of clarity. Contig assembly and the establishment of the relative positions of the PACs have been described (Wieser et al., 2000a). Equal amounts of DNA from two or three PAC clones on either side of the breakpoints were mixed and digested with *Eco*RI or *Hin*dIII prior to direct fluorescence labeling by nick translation (Vysis, Downers Grove, IL, USA). In probe set C ('centromeric'), PACs A08194, J06704, and I17506 were labeled with Spectrum Orange, and PACs M14646, L1032, and M091153 with Spectrum Green (Vysis). PAC A08194 was not included in initial experiments, but was used in the majority of the analyses (>80%) in order to obtain stronger signals. In probe set T ('telomeric'), PACs J06704, I17506, and O13961 were labeled with Spectrum Orange, and PACs L1032 and M091153 with Spectrum Green.

One hundred nanograms of Spectrum-Orange labeled and 100 ng of Spectrum-Green labeled probes were blocked with 4 μg human placenta DNA and 2 μg Cot-1 DNA, and hybridized to an 18 × 18 mm area in Hybrisol VII (Oncor, Gaithersburg, MD, USA) at 37°C overnight. After stringent washing, chromosomal DNA was counterstained with 4′ 6-diamidino-2-phenylindole dihydrochloride (DAPI). Results were analyzed on a Zeiss Axiophot microscope (Zeiss, Jena, Germany) using a dual bandpass filter.

Red and green signals were scored as colocalized if they overlapped to yield yellow signals, touched each other, or were less than one signal diameter apart. Signals were scored as separate if they were located more than one signal diameter from each other (Chase et al., 1997). An average of 200 (minimum, 100) interphase nuclei were analyzed for each control sample (i.e., samples without 3q21 rearrangements), resulting in a total of over 5,000 cells analyzed per probe set. Cutoff values for sets C and T were determined for each of the three different aberrant hybridization patterns illustrated in Figure 1. They were defined as the average percentage of nuclei that erroneously exhibited the aberrant pattern, plus three standard deviations.

A minimum of 150 (usually >200) interphase nuclei per probe set was analyzed for samples containing 3q21 rearrangements.

RESULTS

Development of Probes for the Detection of 3q21 Breakpoints by Interphase FISH

We have previously established a PAC contig covering a genomic region of 750 kb that includes the 3q21 breakpoint region (Wieser et al., 2000a).
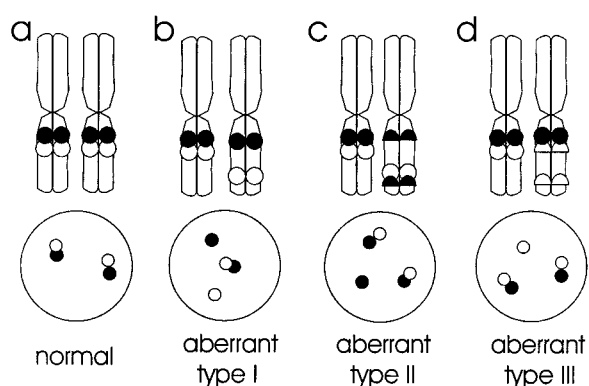
Figure 1. Principle of the interphase FISH assay. PACs centromeric of the 3q21 breakpoint region (closed symbols) were labeled with a red and PACs telomeric of the breakpoint region (open symbols) with a green fluorescent dye. **a**: Expected hybridization pattern on normal chromosomes 3 and corresponding interphase nuclei. **b**: Expected hybridization pattern on chromosomes and in interphases with a 3q21 rearrangement whose breakpoint is located at or near a small region of overlap between the red and green labeled PACs (aberrant pattern type I). An inv(3)(q21q26) is depicted in the scheme, but the same interphase patterns would be expected for other 3q21 rearrangements. **c,d**: Expected hybridization patterns in cells with a more centromeric (**c**) or a more telomeric (**d**) 3q21 breakpoint, which result in split red or green signals, yielding aberrant patterns type II and III, respectively.
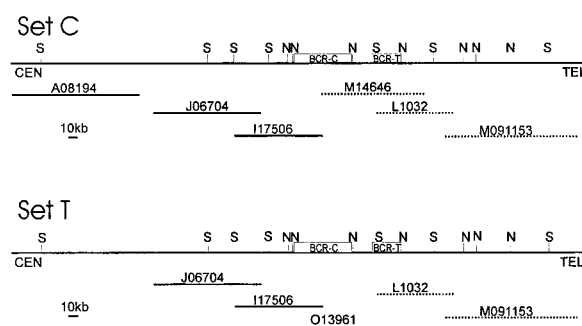

Figure 2. Schematic representation of the 3q21 breakpoint region and the PAC probe sets used for interphase FISH. CEN, centromere; TEL, telomere; N, *Not*I restriction site; S, *Sal*I restriction site. BCR-T, originally proposed, telomeric breakpoint cluster region; BCR-C, recently described, more centromeric, breakpoint region. For probe set C, a PAC contig centromeric of the BCR-C was labeled red (solid lines) and a PAC contig telomeric of the BCR-C was labeled green (dotted lines). Probe set T was designed in a similar manner and centered on the BCR-T.

PACs centromeric of the breakpoint region were labeled with a dye emitting red fluorescence and PACs telomeric of it with a green fluorochrome. In order to achieve adequate signal intensity, two to three PACs on either side of the breakpoint region were employed. The expected hybridization patterns on metaphase chromosomes and in interphase nuclei, without (normal pattern) and with (type I aberrant pattern) 3q21 rearrangements, are illustrated in Figure 1a and b, respectively. Unequivocal colocalization of red and green signals in the majority of normal interphases was only obtained with PACs that overlapped by a few kb, thus precluding the use of probes bracketing all 3q21 breakpoints described so far. Additional types of aberrant hybridization patterns are expected for breakpoints located at some distance from the region of overlap between the red and green probes (aberrant patterns type II and III; Fig. 1c,d). Because these patterns are not necessarily detected with the same efficiency as the type I aberrant pattern, two probe sets were developed: set T was designed for the detection of breakpoints in the originally described, more telomeric 3q21 'breakpoint cluster region' (Suzukawa et al., 1994; Pekarsky et al., 1995; Rynditch et al., 1997), which we term BCR-T in this report; and set C should optimally detect breakpoints in the recently described, more centromeric breakpoint region, BCR-C (Shimizu et al., 2000; Wieser et al., 2000a). The

PACs employed for these two probe combinations and their relative positions are depicted in Figure 2.

Determination of Cutoff Values

Rearrangements of chromosome band 3q21 are not specific for any FAB type of AML and have also been found in patients with CML and MDS (Fonatsch et al., 1994; Horsman et al., 1995; Secker Walker et al., 1995; Shi et al., 1997; Sancho et al., 1999; Testoni et al., 1999). Therefore, background values for the detection of aberrant hybridization patterns were determined on bone marrow (n = 23) and peripheral blood (n = 4) samples from 27 patients, 21 of whom had been diagnosed with AML, three with MDS, and three with CML. Of the AML samples, four were classified as FAB M1, three each as FAB M0, M3, and M5, and two each as FAB M2, M4, M6, and M7. Sixteen of the patients were males and 11 females. The median age was 56 years (range, 20–87). The karyotypes of all samples had been previously determined and no indication for a structural aberration of band 3q21 had been found. The average incidence of the three different types of aberrant hybridization patterns (Fig. 1) and the deduced cutoff values (average plus three SDs) are summarized in Table 1. Percentages of interphase nuclei exhibiting the normal hybridization pattern are also indicated, as well as sum values for the occurrence of various other, erratic hybridization patterns. The latter are largely accounted for by missing red or green signals or signal pairs.

TABLE 1. Average Percentages of Interphase Nuclei Exhibiting Various Types of Hybridization Patterns With Probe Sets C and T in Patient Samples Without 3q21 Aberrations

Hybridization pattern[a]	Set C		Set T	
	Percent of interphases[b] (mean $\pm$ SD[c])	Cutoff value[d] (mean + 3 $\times$ SD[c])	Percent of interphases[b] (mean $\pm$ SD[c])	Cutoff value[d] (mean + 3 $\times$ SD[c])
Type I	0.25 $\pm$ 0.48%	1.7%	0.81 $\pm$ 1.04%	3.9%
Type II	0.12 $\pm$ 0.32%	1.1%	0.13 $\pm$ 0.33%	1.1%
Type III	0.14 $\pm$ 0.45%	1.5%	0.29 $\pm$ 0.56%	2.0%
Normal	97,28 $\pm$ 3,46%	—	96,97 $\pm$ 2,23%	—
Other	2,2 $\pm$ 3,41%	—	1,75 $\pm$ 1,97%	—

[a]Type I, II, and III patterns, hybridization patterns indicative of the presence of a 3q21 rearrangement. Normal, hybridization pattern expected in the absence of a 3q21 rearrangement (Fig. 1). Other, various types of erratic patterns, mainly due to missing signals.
[b]Average percentages of interphase nuclei exhibiting the respective hybridization patterns.
[c]SD, standard deviation.
[d]Cutoff values for false positivity (average percentage of interphase nuclei exhibiting the respective aberrant hybridization pattern, plus three standard deviations).

TABLE 2. Clinical Characteristics and Karyotypes of Patients and Cell Lines With 3q Aberrations

No.	BM/PB[a]	Diagnosis[b]	Sex[c]	Age (years)	Karyotype
1	BM	M1	M	57	46,XY,inv(3)(q21q26) [20]
2	BM	M2	M	12	46,XY,inv(3)(q21q26) [2]/45,idem,−7 [3]/44–50, inv(3)(q21q26), complex aberrations [15]
3	BM	M4	M	65	46,XY,inv(3)(q21q26),t(5;7)(q11;q11) [16]/46,XY [3]
4	BM	CML-AP	F	69	46,XX,inv(3)(q21q26),t(9;22)(q34;q11) [20]
5	PB	MDS?	M	82	45,X,−Y,t(3;3)(q21;q26) [18]
6	BM	MDS	F	56	46,XX,t(3;3)(q21;q26),del(5)(q13q31) [5]/46,idem, del(7)(q22) [15]
7	PB	M0	F	37	45,XX,ins(6;3)(23;q21q26)inv(3)(q21q26),−7 [30]
8	PB	MDS	F	54	47,XX,t(1;3)(p36.1;q21),+21 [15]
9	BM	preB/M1	F	54	46,XX,t(3;3)(q21;q26) [26]
10	PB	AML	F	72	46,XX,inv(3)(q21q26),del(7)(p11)[16]
Mutz-3	cell line	M4	M	29	46,XY,der(1)t(1;3)(q43;q13)inv(3)(q21q26),der(2)t(2;7)(q35;q35),der(3) t(1;3)(q43;q13),der(7)t(2;7)(q35;q35),inv(7)(p14q35),t(12;22)(p13;q11)
HNT34	cell line	CML-BC	F	45	46,XX,t(3;3)(q21;q26),t(9;22)(q34;q11),del(20)(q11q13) [20]

[a]BM, bone marrow, PB, peripheral blood.
[b]M0–M7, French American British (FAB) subtypes of acute myeloid leukemia (AML); preB/M1, mixed type acute leukemia exhibiting characteristics of both a preB ALL and an AML M1; CML-AP, accelerated phase of chronic myeloid leukemia (CML); CML-BC, blast crisis of CML; MDS, myelodysplastic syndrome.
[c]M, male, F, female.

Mixing Experiments With Cell Lines With and Without 3q21 Rearrangements

Next, we determined whether the interphase FISH assay would yield the expected hybridization patterns on two cell lines with 3q21 rearrangements. Mutz-3 cells (Hu et al., 1996; MacLeod et al., 1996) carry a t(1;3)(q43;q13)inv(3)(q21q26), with the 3q21 breakpoint located in the BCR-T (Wieser et al., 2000b). HNT-34 cells harbor a t(3;3)(q21;q26), whose 3q21 breakpoint has been reported to map to the BCR-C (Hamaguchi et al., 1997; Wieser et al., 2000b) (Table 2). As predicted, probe set T yielded a high percentage (76%) of type I aberrant hybridization patterns on interphase nuclei of Mutz-3 cells, whereas with probe set C, 61% of interphases exhibited the type III,

and 23% the type I, aberrant pattern. The reverse situation was observed for HNT-34 cells: 88% of nuclei showed the type I aberrant pattern with probe set C, while probe set T yielded the type I pattern on 38%, and the type II pattern on 52% of the interphase nuclei (Fig. 3A).

Examples for metaphase chromosomes and interphase nuclei with the various types of hybridization patterns are shown in Figure 3B. Unexpectedly, and in contrast to HNT-34 cells, a relatively high proportion of Mutz-3 nuclei (16.4% for probe set C; 23.4% for set T) exhibited the normal hybridization pattern, while no unrearranged metaphases were found in either cell line (see Discussion for possible explanations for this discrepancy).
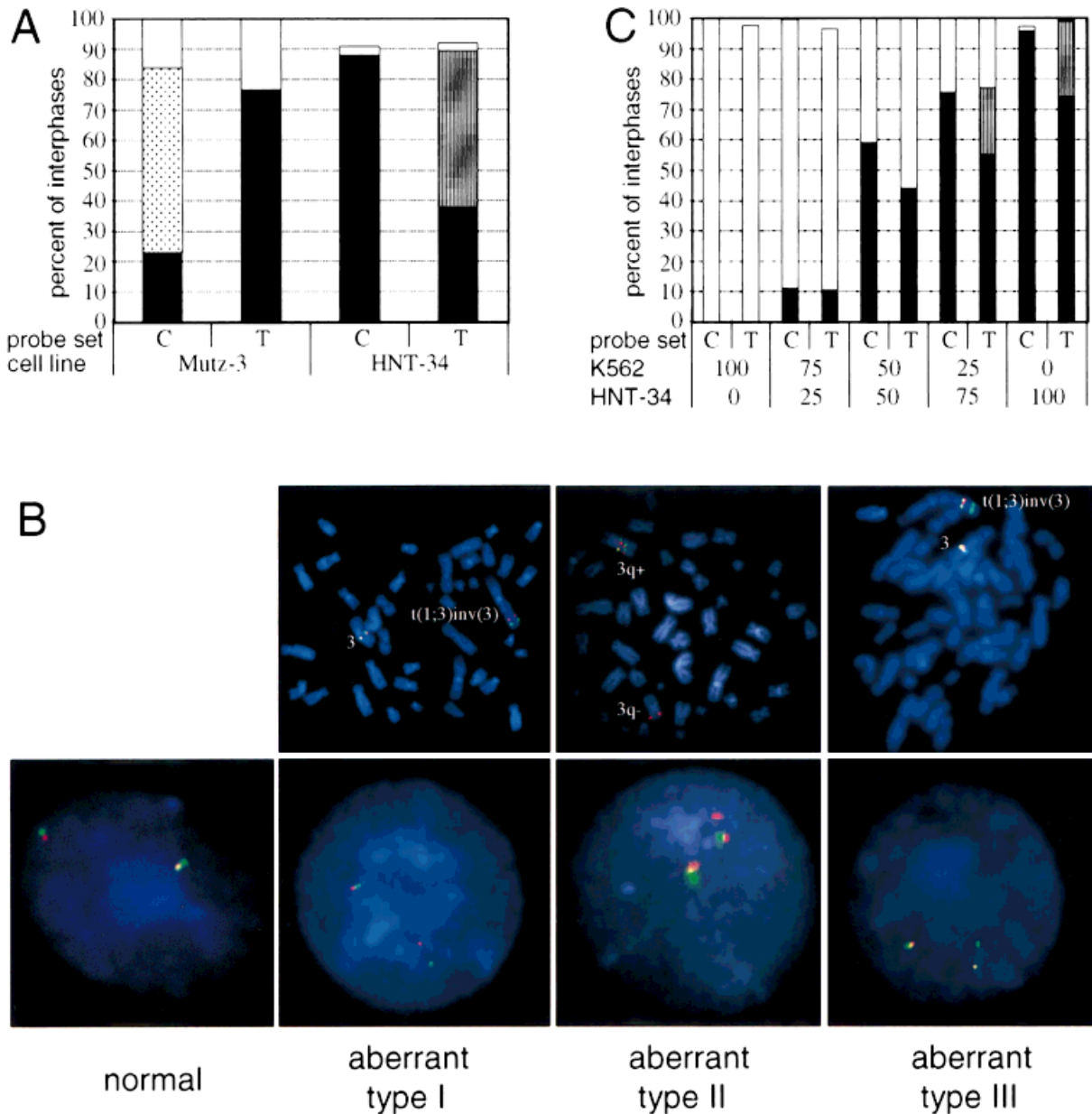
Figure 3. Interphase FISH analysis of cell lines. **A**: 3q21 rearrangements are detected efficiently by the interphase FISH assay, and hybridization patterns observed with the two probe sets reflect breakpoint positions. Mutz-3 cells contain a t(1;3)(q43;q13)inv(3)(q21q26), the 3q21 breakpoint of which maps to the BCR-T. The t(3;3)(q21;q26) breakpoint of the HNT-34 cell line is located in the BCR-C. Black portions of bars indicate the percentage of cells with the type I aberrant pattern; shaded, type II aberrant pattern; dotted, type III aberrant pattern; white, normal hybridization pattern. Total bar length may be less than 100% because a subset of cells exhibited various types of signal patterns that could not be attributed to either of the categories described in Figure 1 (see also Table 1). **B**: Examples of partial metaphases and interphases with the different hybridization patterns. Normal pattern: Mutz-3 cells, set T (interphase only, since no normal metaphases were found in the cell lines); aberrant type I: Mutz-3 cells, set T; aberrant type II: HNT-34 cells, set T; aberrant type III: Mutz-3 cells, set C. Overlapping red and green signals appear yellow. **C**: The percentage of interphases with aberrant hybridization patterns reflects the relative number of cells with 3q21 rearrangements. Fixed nuclei from K562 cells, which are devoid of a 3q21 rearrangement, and from HNT-34 cells, which contain a t(3;3), were mixed in the indicated ratios and hybridized with probe sets C and T. Black portions of bars, percentage of cells with type I aberrant pattern; shaded, type II aberrant pattern; white, normal hybridization pattern.

In order to establish whether the number of interphase cells exhibiting aberrant hybridization patterns in a mixed population actually reflects the percentage of cells containing 3q21 rearrange-

ments, we performed titration experiments. Fixed nuclei from cell line K562, which does not contain a 3q21 rearrangement, were mixed at varying ratios with HNT-34 nuclei. As shown in Figure 3C, a
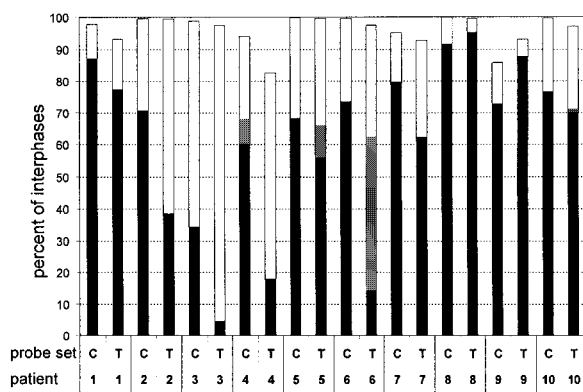
Figure 4. Interphase FISH analysis of patient samples with 3q21 rearrangements. Clinical data and karyotypes of the patients are summarized in Table 2. Black portions of bars, percentage of cells with type I aberrant pattern; shaded, type II aberrant pattern; white, normal hybridization pattern. The type III pattern was not observed in any of the patient samples. Total bar length may be less than 100% because a subset of cells exhibited various types of signal patterns that could not be attributed to either of the categories described in Figure 1 (compare also Table 1).

good correlation between the relative input of cells with the t(3;3) and the number of nuclei scored as aberrant was observed for both probe sets.

Analysis of Patient Samples

Finally, 10 patient samples with rearrangements of chromosome band 3q21, as determined by chromosome banding analysis, were tested in the interphase FISH assay. Clinical data and karyotypes of these patients are summarized in Table 2. If two types of aberrant patterns individually exceeded their respective cutoff values in a given sample, their sum was used as a measure of the percentage of aberrant cells. By this standard, in 7 of 10 samples, both probe sets detected the 3q21 rearrangement with comparable efficiency (Fig. 4). For Patients 2, 3, and 4, probe set C yielded a substantially higher number of aberrant cells than set T, but only in one case (no. 3), the 3q21 aberration would have gone undetected with probe set T, yet could be clearly demonstrated with set C.

In Patient 5, normal hybridization patterns were observed in more than 30% of interphases with either PAC probe set, even though all metaphases exhibited a t(3;3). The leukemic clone in this patient also exhibited loss of the Y chromosome (Table 2), providing a convenient additional marker for interphase FISH. Hybridization of an alpha satellite probe for the Y chromosome along with probe set C revealed that three-quarters of the interphases exhibiting the normal hybridization pattern with the PAC probes were also positive for the

CEN-Y probe, while 224/224 interphases showing the 3q21 rearrangement were devoid of the CEN-Y signal (data not shown). Thus, at least in this sample, normal hybridization patterns with the PAC probes indeed reflected the presence of cytogenetically normal cells in the nondividing fraction.

DISCUSSION

Here we describe an interphase FISH assay that facilitates the rapid, sensitive, and efficient detection of rearrangements of chromosome band 3q21 in samples from patients with myeloid malignancies. This test can be used to confirm or exclude the presence of typical 3q21 aberrations at diagnosis and to monitor response to therapy or disease progression. Experiments with cell lines revealed that the assay is able to detect cells carrying 3q21 rearrangements with high efficiency and that the percentage of interphase nuclei exhibiting aberrant hybridization patterns correlates well with the percentage of aberrant cells in a mixed population (Fig. 3).

However, a relatively high proportion of interphase cells from one of the cell lines (Mutz-3) reproducibly exhibited a normal hybridization pattern. This is unexpected for a supposedly homogeneous cell population, and in disagreement with the fact that all analyzed metaphases carried the t(1;3)inv(3). Our scoring criteria may in part explain this discrepancy: we interpreted red and green signals separated by up to one signal diameter as colocalized. This has been shown to lead to a low false-positive rate, at the possible expense of a relatively high false-negative rate due to accidental colocalization of signals (Chase et al., 1997). But on the other hand, the percentage of cells exhibiting normal hybridization patterns was marginal in the HNT-34 cell line (3% for either probe set), indicating that our probes and scoring criteria do not generally yield a high false-negative rate. The observations made with Mutz-3 cells may therefore also reflect specific aspects of their nuclear architecture (a speculative explanation being the association of the inv(3) with a t(1;3)).

The primary leukemia samples with 3q21 rearrangements also exhibited highly variable percentages of normal as well as aberrant hybridization patterns. The frequency of the normal pattern ranged from 4.2% for Patient 8 and probe set T to 93% for Patient 3 and probe set T. Conversely, 68% or more of the interphases scored aberrant with at least one of the two probe sets in 9 of the 10 patient samples (Fig. 4). In the remaining case (no. 3), some metaphases also lacked the inv(3) (Table

2). However, in only three of the nine samples in which all metaphases exhibited a 3q21 rearrangement did the percentage of aberrant hybridization patterns approach or even exceed 90% (Patients 1, 8, 9; Fig. 4). Because bone marrow and peripheral blood—as opposed to cell lines—represent highly heterogenous cell populations, additional explanations should be considered for these findings: 1) Deletions, as observed in a subset of patients with t(9;22) (Sinclair et al., 2000), could lead to loss of part of a split red or green signal and to the appearance of the normal or the type I pattern instead of type II or III patterns. However, in contrast to what has been observed for the t(9;22), one would have to propose that such deletions were present only in a fraction of the cells with 3q21 aberrations. 2) As outlined below, breakpoints that map too far from the region of overlap between PACs labeled with red and green fluorochromes may be detected less efficiently. 3) Cells harboring 3q21 rearrangements may, at least in some instances, constitute a larger fraction of the proliferating than of the nonproliferating cell population. Such an interpretation is supported by our finding that most cells of Case 5 that appeared normal with the 3q21 PAC probes also did not exhibit loss of the Y chromosome, suggesting that they were cytogenetically normal and not derived from the leukemic stem cell. These possibilities are not mutually exclusive and may account for variable extents of normal hybridization patterns found in patient samples with 3q21 rearrangements. In any case, our observations imply that, for disease monitoring, the percentage of aberrant cells in a given sample should always be compared to a sample from the same patient at diagnosis.

Two probe sets were developed for the interphase FISH assay in order to address the fact that breakpoints in 3q21 have been reported to map to two closely juxtaposed, and possibly contiguous, breakpoint cluster regions (Suzukawa et al., 1994; Pekarsky et al., 1995; Rynditch et al., 1997; Shimizu et al., 2000; Wieser et al., 2000a). Probe sets C and T yielded different percentages of the four hybridization patterns (Fig. 1) in individual patient samples or cell lines, reflecting the positions of the respective breakpoints. For example, in Case 4, 59% of nuclei showed the type I pattern, and 9% the type II pattern with probe set C (Fig. 4). That the red (centromeric) signal of this set is split in some interphase cells most likely indicates that this breakpoint is located towards the centromeric end even of the BCR-C, and therefore should also split the red signal of set T. However,

with this probe only the type I aberrant pattern was observed, in only 18% of the cells. This may be due to inefficient detection of the two parts of the red signal: the portion that colocalizes with the green signal may exhibit insufficient fluorescence intensity in some nuclei due to its small size, leading to the appearance of the type I, instead of the type II, aberrant pattern. The part of the probe that is delocalized from the green signal, on the other hand, contains more repetitive sequences than the gene-rich breakpoint region (Rynditch et al., 1997; Wieser et al., 2000a; R.W., unpubl. obs.), which may lead to reduced hybridization efficiency and the appearance of the normal hybridization pattern in some cells. In Case 6, both probe sets yielded a comparable percentage of aberrant patterns. While only the type I pattern was found with probe set C, the type II pattern was prevalent with set T. This is compatible with the interpretation that this breakpoint is located in the BCR-C, yet further telomeric than that of Patient 4, thus allowing a more efficient hybridization of the part of the red probe that is separated from the green probe in set T. Extensive metaphase FISH analyses of Case 7 had indicated that this breakpoint also maps to the BCR-C (Wieser et al., 2001). In the interphase FISH assay, this is reflected only by a higher percentage of cells exhibiting the type I pattern with probe set C than with probe set T (80% vs. 62%). On the other hand, the breakpoint of Case 8, whose interphase cells exhibited over 90% aberrant patterns with either probe set, was demonstrated to be located in the BCR-T by conventional Southern analysis (data not shown). This suggests that the BCR-T may also be affected in Patient 9, the only case in which probe set T detected a somewhat higher percentage of aberrant cells than set C (88% vs. 73%).

In summary, the positions of individual breakpoints may be reflected by the presence of the type II or III pattern, or by a higher frequency of the type I pattern with one of the two probe sets. Accordingly, the breakpoints of Cases 2–7 are likely to map to the BCR-C, and those of Cases 8 and 9 to the BCR-T. The positions of the breakpoints in Cases 1 and 10 are uncertain based on the results presented here. Clearly, interphase FISH assays cannot define breakpoint positions precisely; more extensive studies have to be carried out before correlations with clinical parameters can be attempted. Notwithstanding, if the data from this and our previous study (Wieser et al., 2000a) are combined, the BCR-C is affected in 9/20 samples analyzed, further stressing the importance of this

region for the understanding of the molecular basis of the clinical characteristics associated with 3q21 rearrangements.

ACKNOWLEDGMENT

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