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Dendrimer FISH detection of single-copy intervals in acute promyelocytic leukemia

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Abstract

Acute promyelocytic leukemia (AML-M3) is characterized by a translocation between chromosomes 15 and 17 [t(15;17)]. The detection of t(15;17) at the single cell level, is commonly done by fluorescence in situ hybridization (FISH) using recombinant locus specific genomic probes greater than 14 kilobases kb in length. To allow a more thorough study of t(15;17), we designed small (0.9–3.6 kb), target-specific, single-copy probes from the human genome sequence. A novel detection approach was evaluated using moieties possessing more fluorophores, DNA dendrimers (up to 375 fluorophores per dendrimer). Two detection approaches were evaluated using the dendrimers: (1) dendrimers modified with anti-biotin antibodies for detection of biotinylated bound probes, and (2) dendrimers modified with 45-base long oligonucleotides designed from the single-copy probes, for direct detection of the target region. The selectivity of the probes was confirmed via indirect labeling with biotin/digoxigenin by nick translation, with detection efficiencies between 50 and 90%. Furthermore, the scFISH probes were successfully detected on metaphase cells with anti-biotin dendrimer conjugates and on interphase cells with 45-base modified dendrimers. Our results bring up the possibility to detect target regions of less than 1 kb, which will be a great contribution to high-resolution analysis of genomic sequences.

Keywords: Acute promyelocytic leukemia; FISH; Single-copy probes; Dendrimers

1. Introduction

Acute promyelocytic leukemia is a subtype of acute myeloid leukemia (AML), designated M3 by the French–American–British classification. The most prominent (50–80%) mutation among patients with AML-M3 is a translocation between the long arms of chromosome 15 (15q22) and chromosome 17 (17q21) [1–4], identified in 1977 by Rowley and referred to as t(15;17). The genes involved in t(15;17) were identified in 1990 as the promyelocytic leukemia gene (PML) on chromosome 15 and retinoic acid receptor alpha gene (RARA) on chromosome 17 [5]. In most cases, the fusion gene consists of the first six exons of PML and the last four exons of RARA [6–10]. There is evidence suggesting that the PML/RARA fusion protein causes repression of the RARA target genes and a blockage in myeloid differentiation [5].

FISH has played an important role in cancer diagnosis, particularly in the identification of subtle chromosomal rearrangements [11,12]; gene mapping [12–14]; study of specific chromosomal regions, or microdeletions [12,15]; and DNA sequence abundance (in the case of repetitive sequences). Conventional FISH commonly uses large recombinant locus specific genomic probes (>14 kilobases (kb) to megabases in length [16]), repetitive sequence probes, and whole chromosome paint probes to achieve specific, robust detection of chromosomal abnormalities. However, the lengths of these probes preclude their use for high-resolution analysis of small deletions and differentiation of heterogeneous chromosomal breakpoints.

Single-copy (sc) FISH probes target shorter chromosomal regions at significantly higher genomic resolution than conventional FISH probes [12]. These shorter sc probes sometimes exhibit lower fluorescence hybridization signals

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Table 1
List of primer sequences and melting temperatures

Primer designation	Sequence[a]	Target region	Coordinates
626-F	GAGACAGGGAAAGGAGCTGAGGCAGGAGGG	RARA, chromosome 15	40654453
626-R	CCGTAGTTGGGGGAGGGGGAGACGCTTATC	RARA, chromosome 15	40657955
660-F	CTGATAAGCGTCTCCCCCTCCCCCAACTAC	RARA, chromosome 15	40651189
660-R	CCTTTCCCCCTTCCTGCTTCTCTCCTCTCC	RARA, chromosome 15	40654485
255-F	TAAAGCCGAGTGGTGTGTG	Centromeric of TOP2A, chromosome 17	40682884
255-R	GCAGACAGACATTTAGGAGGG	Centromeric of TOP2A, chromosome 17	40684141
354-F	ACAGGAGAGCTGAGAAAGAC	Centromeric of TOP2A, chromosome 17	40692298
354-R	GAAAGGAAGGAGACCAGGAC	Centromeric of TOP2A, chromosome 17	40694209
737-F	CTTTCTCCACCATTACTCTCAC	TOP2A, chromosome 17	40731343
737-R	TTCTTTAGCTCTTTGGCTCG	TOP2A, chromosome 17	40732496
827-F	GTCTACCCCACATCTAACCCCCACTGAACC	PML, chromosome 15	67431267
827-R	CAACCTTGTGTGTCCACAGCATCTACTGCC	PML, chromosome 15	67433639
978-F	AGGACAGTGGCTGTGGGATGCAGAGGAGGG	PML, chromosome 15	67411549
978-R	CGGGGAGCATGGGAGTGTAAGGAAGAAGGGG	PML, chromosome 15	67412460

[a] All sequences written from 5′ to 3′.

and as a result this limits their specific activity. Signal amplification methods have been used by others to increase the fluorescent hybridization signal [14,17,18]. We present here a novel approach to signal amplification that utilizes DNA dendrimers [19], branched structures assembled by hybridization of different types of oligonucleotides. Hybridized oligonucleotides form monomers with a double-stranded central region and single-stranded arms, and constitute the building blocks of dendrimer layers. The one-layer dendrimer is formed when the arms of other monomers bind to the arms of the initiating monomer; as more monomers bind the layers grow. Dendrimers labeled with multiple fluorophores have been successfully used to enhance signal intensities in other nucleic acid hybridization assays. For example, several labeling approaches with DNA dendrimer structures were tested and used for detection of RNA/DNA hybrids on synthetic arrays [20], signal amplification of oligonucleotide cassettes [21], and localization of mRNA in tissue [22]. Although dendrimers have been used for detection of probes as part of in vitro hybridization assays, in situ hybridization assays are considerably more challenging, even more when it comes to analysis of methaphase cells. In the present study, we computationally designed single-copy probes within and adjacent to the genes involved in t(15;17), using the method previously described [12] to detect translocations of chromosomes 15 and 17. We also evaluated the ability of DNA dendrimers to enhance hybridization signals of these locus specific scFISH probes hybridized to metaphase and interphase cells of a cell line derived from a patient with AML-M3 and t(15;17). Increasing the scFISH probe's fluorescence will facilitate their introduction into clinical cytogenetics laboratories.

2. Materials and methods

2.1. Cell treatment

HT93A cells [23], kindly provided by Dr K. Toba, Niigata University, Japan, were grown in RPMI 1640 medium (Irvine Scientific, Santa Ana, CA), containing 10% fetal bovine serum (Irvine Scientific), 10 KU/mL penicillin sodium, 10 mg/μL streptomycin sulfate, and 200 mM L-glutamine (Life Technologies, Rockville, MD), at 5% CO_2 and 37 °C in humidified air, at $5–10\times10^5$ cells/mL. Metaphase cells were obtained by treatment with colcemid for 1 h, as previously described [23].

2.2. Characterization of HT93A by conventional FISH

The presence of t(15;17) on HT93A cells was evaluated by FISH with LSI® PML Spectrum Orange™/LSI RARA Spectrum Green™ DNA probe (Vysis, Downers Grove, IL), as described by the vendor. The characterization was done on 21 metaphase cells and 200 interphase nuclei.

2.3. Single-copy FISH probe design of PML and RARA regions from the human genome sequence

The chromosomal regions were selected from the genes involved in the translocation (PML and RARA) and two other regions telomeric of RARA (a region from gene TOP2A and the region between LOC125110 and TOP2A). The location of the breakpoints in PML and RARA was obtained from the literature [1,6–10,24,25]. The PML probes were designed centromeric of the breakpoint. The RARA probes were designed in the breakpoint region as well as telomeric of the breakpoint. The contiguous genome sequences of interest were obtained from the Human Genome Draft (April 2001 and April 2002) available at the National Center for Biotechnology website (http://www.ncbi.nlm.nih.gov). The single-copy regions were identified as previously described [12]. Table 1 shows the PCR primers that were designed according to previously developed procedures [26]. Fig. 1 shows the approximate location of the probes with respect to the breakpoints in the genes.

2.4. Probe generation and characterization on metaphase cells

Amplicons were prepared from high molecular weight human genomic DNA (Promega, Madison, WI) by long PCR

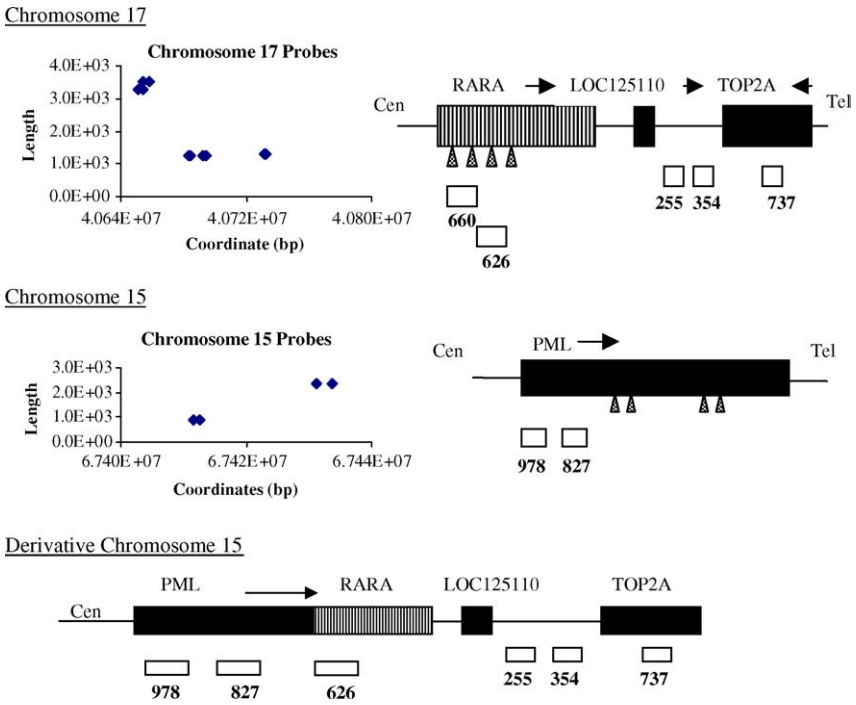

Fig. 1. Partial map of 17q21 and 15q22, and derivative 15q22 showing the location of the breakpoint regions (triangles) and the scFISH probes (white boxes). The distances in the map are not to scale. The arrows indicate direction of transcription. Depending on the location of the breakpoint in chromosome 17 in HT93A cells, probes **660** and **626** might not hybridize to derivative chromosome 15.

amplification with Platinum *Pfx* DNA Polymerase (Invitrogen, Rockville, MD). The reaction products were purified by agarose gel electrophoresis, extracted with a QIAquick kit (Qiagen, Valencia, CA), and labeled by nick translation using digoxigenin-11-dUTP or biotin-16-dUTP (Roche Diagnostics, Indianapolis, IN).

Three probes were characterized on metaphase cells, **827** for PML in chromosome 15, and **354** combined with **737** for the region telomeric of RARA in chromosome 17. The characterization was done by determining the number of cells that had signal on the targeted chromosomal regions. Over 100 HT93A metaphase cells were analyzed for the validation of probe **827** hybridization to PML, and 74 metaphase cells for validation of **354** and **737**. To facilitate identification of chromosome 15, **827** was hybridized in combination with CEP® 15, a chromosome-specific tandem-repeat probe from Vysis, that hybridizes to the band region 15p11.2, locus D15Z1 of human chromosome 15. Due to a translocation, CEP® 15 can hybridize to the centromere region of other acrocentric chromosomes, especially chromosome 14 (bands 14p11.1-q11.1) in HT93A cells.

Denaturing and hybridization conditions were as previously described [27]. The hybridization mixture consisted of labeled probe to give a final concentration of 7–10 ng/μL, 50% formamide, 20% dextran sulfate, and 2× SSC (0.3 M NaCl, 0.03 M Na_3Citrate). After hybridization, the cells were washed in 2× SSC for 2 min, three times in 0.5× SSC for 3 min each and twice in 2×SSC for 3 min each. The probes were detected with avidin conjugated with FITC (Vector Laboratories, Burlingame, CA) or anti-digoxigenin conjugated with Rhodamine (Roche Diagnostics, IN). Cells were counterstained with DAPI (1 μg/mL) and mounted in anti-fade solution (0.95 g/L 1,4-phenylenediamine in 1× PBS, pH 8.0).

2.5. Image acquisition and analysis

Cells were viewed with a Zeiss Axioplan 2ie epifluorescence microscope with the appropriate fluorochrome filter sets. The microscope was equipped with cooled charged coupled device (CCD) camera (Orca ER, Hamamatsu). The camera and microscope were controlled with Openlab and Volocity software (Improvision's, Lexington, MA) and captured images were merged using Adobe Photoshop v.6.0. The intensity profiles of visually detected probe hybridizations were quantitated by Lispix software [28], v.Lx29P.

2.6. Dendimers with specific sequences and hybridization to interphase cells

Segments of 45 bases were selected out of the designed scFISH probes (see Table 2). OligoTech software program from Oligos, etc. (Wilsonville, OR) was used to select stable oligonucleotides and with low or no tendency to form stem loops (secondary structures). The sequence TTT TTT TTT TCG was added to the 3′ end of the oligonucleotide to facilitate attachment to the dendrimer. The 57-oligonucleotides were ordered from TriLink Biotechnologies, Inc. (San Diego, CA) and covalently attached to two-layer and four-layer dendrimers, to make 'specific dendrimers' at Genisphere Inc.

Table 2
List of specific dendrimers target region and melting temperatures

Code	Gene	Coordinates within probe	T_m (°C)
626-943	RARA intron 2	943-987	42
626-1293	RARA intron 2	1293-1337	47
354-245	LOC125110-TOP2A	245-289	46
354-428	LOC125110-TOP2A	428-472	52
737-517	TOP2A exon 22-24	517-561	38
737-937	TOP2A exon 22-24	937-981	34
978-460	PML promoter and exon 1	460-504	46
978-695	PML promoter and exon 1	695-739	44
978-765	PML promoter	765-809	49

T_m, melting temperature calculated for 0.33 M Na^+ and 50% formamide, where $T_m = 81.5 + 16.6 \log[Na^+] + 0.41(\%GC) - 0.63(\%\text{formamide}) - \{300 + 2000[Na^+]\}/N$, and N is the length in bases of the hybrid [29], where %GC is the percentage of guanosines and cytidines in the oligonucleotide or DNA fragment.

(Hatfield, PA). The two-layer dendrimers have approximately 50 fluorophores, while four-layer dendrimers have 375. Dendrimers with specific sequences that target PML were labeled with Cy-3 (Amersham, Piscataway, NJ), while dendrimers designed to hybridize telomeric of RARA were labeled with Alexa-488 (Molecular Probes, Eugene, OR).

Specific dendrimers were hybridized to HT93A cells for characterization, 44 cells hybridized to **978**-765 and **354**-245 (the former labeled with Cy-3 and the latter with Alexa-488) were analyzed for detection of t(15;17), and 87 cells for validation of **354**-245, **354**-428, **626**-943, and **626**-1293 (all labeled with Alexa-488). Approximately 10 ng of dendrimer were mixed with 1 μL of C_ot-1 DNA (1 μg/μL; to prevent crosshybridization of the poly-thymidine sequences to chromosomal targets), 1 μL of salmon sperm DNA (10 μg/μL), 7 μL of LSI hybridization buffer and H_2O to make 10 μL of hybridization mixture. This mixture was denatured and applied to previously denatured target. Slides were incubated at 37 °C overnight. Washings consisted of 40% formamide/2× SSC at 37 °C for 5 min, followed by 10 min in 2× SSC at room temperature. Slides were stained with 50 μL of 1 μg/mL of DAPI in 2× SSC for 5 min, rinsed in 2× SSC and mounted with anti-fade solution (Vectashield, Vector Laboratories, Burlingame, CA).

2.7. Anti-biotin dendrimers and detection of nick translated probes

The manufacturing department at Genisphere conjugated approximately four anti-biotin antibodies to two-layer and four-layer dendrimers labeled with Cy-3 (Amersham, Piscataway, NJ). As the specific dendrimer, the two-layer dendrimers have approximately 50 fluorophores, while the four-layer ones have 375. The antibody has an oligonucleotide covalently attached, and is conjugated to the dendrimer by hybridization of the oligonucleotide to the dendrimer arms. A total of eight experiments were done to test these dendrimers for detection of biotinylated probe **827** labeled by nick translation and hybridized to HT93A cells. A total of 83 metaphase cells were analyzed for experiments where two-layer dendrimers were used for detection, and 38 cells when four-layer dendrimers were used.

To determine if there were biotins still available for binding after detection with two-layer anti-biotin dendrimers, a slide (previously incubated with two-layer dendrimers) was incubated with streptavidin also labeled with Cy-3.

3. Results

3.1. Cell line characterization by conventional FISH

The presence of t(15;17) on HT93A cells was evaluated with LSI® PML/RARA. PML is detected by red fluorescence and RARA is detected by a green hybridization signal. More than 90% (19 out of 21) of the metaphase cells showed one green hybridization signal on chromosome 17, a red signal on chromosome 15 and a yellow fluorescence signal or adjacent red and green signals on the translocated or derivative chromosome 15. The yellow or adjacent red and green hybridization signals are due to the proximity or fusion of

Table 3
Detection efficiencies of scFISH probes characterized on HT93A cells

Probe(s)	Target region	Hapten/tag	Cells analyzed	Detection molecule	Detection efficiency (%)
827, 2.4 kb	PML, chromosome 15	Digoxigenin	102 (metaphase)	Anti-digoxigenin	>90
827, 2.4 kb	PML, chromosome 15	Biotin	45 (metaphase)	Anti-biotin	>50
354, 1.2 kb, and **737**, 1.3 kb	Centromeric of TOP2A, and TOP2A, chromosome 17	Digoxigenin	74 (metaphase)	Anti-digoxigenin	>70
827, **354**, and **737**	t(15;17): chromosome 15, derivative 15 and 17	Digoxigenin and biotin	29 (metaphase)	Anti-digoxigenin	>55
827, 2.4 kb	PML, chromosome 15	Biotin	83 (metaphase)	Anti-biotin two-layer dendrimer	40
827, 2.4 kb	PML, chromosome 15	Biotin	38 (metaphase)	Anti-biotin four-layer dendrimer	>35
978-**765** and 354-**245**	t(15;17): chromosome 15, derivative 15 and 17	No hapten	44 (interphase)	Two-layer dendrimer	<80 [der(15)], >20 [der(15), 15, and 17]

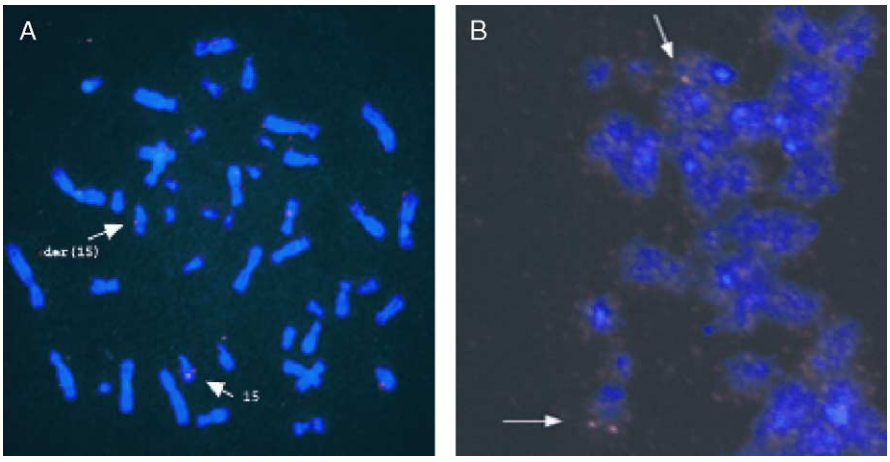

Fig. 2. Detection of probe **827** (2.4 kb, chromosome 15) hybridized to HT93A metaphase cells. (A) The probe was labeled with digoxigenin, detected with Rhodamine anti-digoxigenin (red signal). Both chromosomes and both chromatids show hybridization. (B) Detection of biotinylated probe with Cy-3 anti-biotin dendrimers (red signal). Two group-D chromosomes, indicated by white arrows, were detected with anti-biotin 2-dendrimers, both chromatids for one of the chromosomes and one chromatid for the other chromosome.

PML and RARA on derivative chromosome 15. Almost 90% of the nuclei (177 out of 200 nuclei) had distinct green and red signals and a yellow signal or two very close green and red. Six percent of the interphase cells had signals indicative of inefficient hybridization (e.g. a yellow and a green). Signals with two fused red and green signals (which appeared yellow) were considered artifacts since they constituted less than 1% of the population.

3.2. Probe design and generation (PCR amplification)

A list of the primers designed for the amplification of scFISH probes and their coordinates in chromosomes 15 and 17 was provided in Table 1. Probes **827** and **978** are centromeric of the breakpoint region in PML, chromosome 15; **626**, and **660** are located in the breakpoint region in RARA, while **255**, **354**, and **737** are telomeric of the breakpoint region in RARA, chromosome 17.

3.3. Probe characterization on metaphase cells: indirect detection with fluorescent labeled anti-digoxigenin and anti-biotin antibodies

This was described in terms of detection efficiency. The detection efficiencies are summarized in Table 3. The highest detection efficiency was observed for **827** when it was labeled with digoxigenin-11-dUTP. Fig. 2A shows the signal of **827** when it was detected with anti-digoxigenin Rhodamine conjugate (Roche), both chromosomes and both chromatids showed hybridization. As seen in Table 3, the detection efficiency when biotin-16-dUTP was used as a hapten was lower than when digoxigenin-11-dUTP was used. The binding of **827** to chromosome 15 was further corroborated by the hybridization of the probe in combination with control probe CEP® 15.

3.4. Dendrimers

3.4.1. Specific dendrimers: detection of targeted regions on interphase cells

Analysis of interphase cells hybridized to four-layer dendrimers **978**-765 and **354**-245 showed a fusion (yellow) signal, indicating the presence of t(15;17), in 35 out of 44 nuclei. Only 10 nuclei had the expected number of signals: one green, one red, and one yellow. The other nuclei had 2–4 red signals, and up to three green and two yellow. No specific hybridizations were detected on metaphase cells. The four two-layer dendrimers specific for chromosome 17 evaluated: **354**-245, **354**-428, **626**-943, and **626**-1293 showed 10 nuclei with three green signals, 43 with two green signals, out of 87 cells, the rest of the nuclei had no signal. The detection efficiencies are summarized in Table 3.

3.4.2. Anti-biotin dendrimers: detection of biotinylated probes hybridized to metaphase cells

This was the first dendrimer approach for which signal was consistently observed in both chromatids of a D-group size chromosome (medium sized chromosomes with acrocentric centromeres, includes chromosomes 13–15). Because no control probe was used to identify chromosome 15, it can only be concluded based on the morphology that the dendrimers hybridized a D-group chromosome. Fig. 2B shows the results from an experiment where a two-layer anti-biotin dendrimer was used to detect biotinylated probe **827**. The image shows both chromosomes with signal: one chromosome with signal in both chromatids, and the other chromosome with signal in one chromatid. The detection efficiency was (40 ± 17)% for two-layer dendrimers and (36 ± 15)% for four-layer dendrimers (see summary in Table 3). Dendrimers non-specifically bound throughout the slide. However, specific binding could be discriminated both visually

and by Lispix analysis. The signal-to-noise ratio (S/N) for the four-layer dendrimer signal was only 1.7 compared to 2.4 for the two-layer. The detection efficiency increased from 42 to 67% when a slide previously incubated with two-layer dendrimer was incubated with streptavidin also labeled with Cy-3.

4. Discussion and conclusions

Characterization of **827** on HT93A metaphase cells, demonstrated that the probe was specific for the region centromeric of the breakpoint in PML, chromosome 15. This was further confirmed by co-hybridization with control probe CEP® 15 (15p11.2, locus D15Z1). When labeled with digoxigenin, **827** was detected with an efficiency of >90%, but this number decreased to >50% when the probe was labeled with biotin. This might be attributable to more efficient incorporation and detection of digoxigenin-modified nucleotide than biotinylated dUTP, since endogenous biotin can increase non-specific detection by fluorescently conjugated streptavidin or anti-biotin [30].

Probes **354** and **737** were labeled with digoxigenin, and their detection efficiency was >70%. The lower detection efficiency respect to **827** could be due to their difference in sequence, since the length and chromosomal locations of the probes can affect detection of hybridization [12]. Another factor could be the quality of the metaphase spreads including the presence of excess residual cytoplasm [14], which increases background and decreases hybridization efficiency. Optimization of incorporation of biotin/digoxigenin for maximizing signal intensities may be necessary [31].

ScFISH probes are easy to design and generated by PCR, and they have proven useful for the analysis of chromosomal regions ~100-fold smaller than those possible with conventional recombinant probes [12,26]. Further optimization of the assay conditions for those probes with detection efficiencies lower than 80% is required for the detection of t(15;17). It is very important to detect a high percentage of the target regions, so that their analysis reflects the overall cell population. The difficulty involved in detection of small single-copy targets (1–2 kb) has been previously documented [14,17,18]. Guzzo and colleagues [14] detected no visible signal from digoxigenin-labeled probes by anti-digoxigenin rhodamin/alkaline phosphatase; detection required the use of a cascade of three different antibodies for signal amplification and their detection efficiencies ranged between 50 and 70%. Viegas-Pequignot [17,18] detected biotinylated probes smaller than 1 kb by indirect detection first with an anti-biotin antibody followed by an anti-IgG antibody conjugated to fluorescein, and reported detection efficiencies of at least 60%. Thus, when compared to detection efficiencies reported by others, our results are comparable (>50% detection) or better (>90% detection).

An attempt was made to use DNA dendrimers to amplify the fluorescent signal of shorter scFISH probes. The results of interphase chromosome hybridization, using specific dendrimers, were encouraging, showing almost 80% detection of t(15;17), however, their specificity needs to be determined in metaphase chromosomes. A more exhaustive evaluation of different hybridization conditions could improve the specificity of these dendrimers, another approach would be to use specific sequences longer than 45 bases, but still within single-copy regions. If the conditions for specific binding were found, this approach would constitute a big breakthrough in the identification of chromosomal regions of less than 100 bases, where the probes can be synthetically generated, and no enzymatic amplification is required.

The anti-biotin dendrimers showed the most specific sensitive binding based on detection of both chromatids. However, detection efficiency was low and not very reproducible. This could be caused by steric hindrance, slow dendrimer binding kinetics, and/or low specific activity of dendrimer conjugation with anti-biotin antibodies. The diameter of the dendrimers (90 nm for the two-layer and 190 nm for the four-layer) can potentially affect their ability to bind to biotin moieties in close proximity found on the hybridized chromosomal target. This hypothesis is supported by increased detection efficiency observed when a biotinylated probe detected with an anti-biotin dendrimer conjugate was chased with streptavidin (ca. 5 nm) [32]. Furthermore, Xiao and Barker [33] observed that when detecting hybridized biotinylated total genomic DNA with 15 nm quantum dots conjugated to streptavidin, the centromeric regions lacked signal. The lack of signal suggests that 15 nm quantum dots were not able to bind to biotinylated target bound to highly condensed regions. We have also performed in vitro microscopy studies (data not shown) that suggest that bulky and/or rigid particles are less successful on binding all biotin moieties incorporated into a DNA probe.

Although there are other alternatives available in the market for signal amplification, such as quantum dots [34] and polystyrene nanoparticles [35,36], to our knowledge there are no reports of their use for detection of scFISH probes. DNA dendrimers have the advantage over quantum dots and polystyrene nanoparticles of being less rigid and Genisphere is currently developing fluorescent DNA moieties of smaller diameter. This novel approach for detection of small (less than 3 kb) scFISH probes with dendrimers has the advantage of less steps compared to previous signal amplification methods, where incubation with 2–3 different antibodies was required in order to detect signal [14,17,18]. Furthermore, Viegas-Pequignot et al. [18] required sophisticated hardware for optical image enhancement of the fluorescent signal in order to visualize these short probes. One of the strengths of our method is that the scFISH probes can be visualized with a conventional off the shelf fluorescence microscope, because the dendrimer labeling obviates the need to obtain such expensive hardware. Another advantage is the potential for higher signal amplification, due to the number of fluorophores per dendrimer molecule. Once conditions are optimized for detection of probes of less than 1 kb in length, it should be feasible to perform in situ analysis of chromosomal rearrangements close to the limit of genomic resolution achieved by molecular genetic techniques.

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