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Technical Advance

Use of Nonbreakpoint DNA Probes to Detect the t(X;18) in Interphase Cells from Synovial Sarcoma

Implications for Detection of Diagnostic Tumor Translocations

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Fluorescence *in situ* hybridization studies using nonbreakpoint DNA probes were performed to detect the X;18 translocation on 4-μm sections of synovial sarcoma from paraffin blocks. This was done by using commercially available, large target unique sequence DNA probes for regions of the X chromosome short-arm and the 18 chromosome long-arm together with centromere probes for the alternate chromosomes. We determined that such probe combinations could detect the presence of the diagnostic X;18 translocation in interphase cells. Spatial association of dual color signals from the X centromere and the 18 unique sequence probe, as well as between an 18 centromere and the X unique sequence probe, was seen in a significantly higher percentage of synovial sarcoma cells (81.1% ± 7.7%, confidence interval 95%) than in control nonsynovial soft tissue sarcomas (14.7% ± 8.3%) and control peripheral blood lymphocytes (5.6% ± 0.6%). The observed spatial association supports the use of this strategy to detect the X;18 translocation in synovial sarcoma and suggests that this technique could be applied in the diagnosis of other types of tumors with characteristic translocations when histopathological findings are inconclusive. This study is the first report describing the use of nonbreakpoint unique sequence probes for detecting translocations in tumors on paraffin-embedded slides. *(Am J Pathol 1998, 152:1171–1177)*

The current use of histopathological methods in the diagnosis of solid tumors can give an accurate diagnosis in the majority of cases. However, when histological or cytological criteria are inadequate, other techniques must be used to assure appropriate diagnosis and treatment because many solid tumors have been investigated with cytogenetic methods and have been shown to have diagnostic chromosome changes.[1–4] By using commercial or home-brew DNA probes targeted to sequences near the breakpoints of the translocated chromosomes, fluorescence *in situ* hybridization (FISH) can be used to support or even make the diagnosis in ambiguous cases.[5–7] Recently, commercial suppliers of nonisotopic DNA probes have introduced a number of unique sequence probes. The purpose of the present study was to develop a strategy for using these nonbreakpoint commercial probes to detect specific translocations in nondividing tumor cells using the diagnostic (X;18)(p11.2;q11.2) translocation in synovial sarcoma as an example.

The translocation in synovial sarcoma involves regions close to the centromere of both the X and 18 chromosomes. We assessed the frequency of finding the X centromere probe adjacent to the 18q21.2 probe as well as the frequency of association between the 18 centromere probe and the Xp21.2–21.3 probe using 4-μm slides from tumors diagnosed as synovial sarcoma or other soft tissue sarcomas and also slides of normal lymphocytes from peripheral blood cultures as controls. Comparison of the frequency of probe proximity in cells known to have a specific translocation to those without the translocation can show that the t(X;18) can be diagnosed in interphase cells and that this strategy could also be used to detect characteristic translocations in other tumors in which appropriate probe combinations are available.

Materials and Methods

Study Samples

Twenty specimens of archival paraffin-embedded soft tissue sarcomas from a total of 14 patients were retrieved.

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Eighteen of these specimens were diagnosed as synovial sarcomas by histopathology. The remaining two specimens, diagnosed as malignant fibrous histiocytoma (MFH) and fibrosarcoma respectively, were used as controls. All of the specimens had been formalin fixed, and the paraffin blocks were stored at room temperature for up to 8 years. To assess signal distribution in cells with optimal nuclear morphology, cultured peripheral blood lymphocytes were used from three cytogenetically normal individuals.

Probes and Hybridization Techniques

Paraffin blocks from the 20 cases of soft tissue sarcomas were sectioned at 4 μm and the slides were placed on a 62 to 65°C hot plate for 3 to 24 hours. Deparaffinization was accomplished by soaking the slides first in d-limonene and then in 100% ethanol and followed by air-drying. The slides were then soaked in 45°C sodium thiocyanate for 20 minutes, rinsed with distilled water, and digested with pronase in Hanks' basic salt solution at 45°C for 30 to 40 minutes. The specimens were then dehydrated down through an ethanol series (70, 80, 90, and 100%) and were air-dried.

Fluoroisothiocyanate (FITC)-direct-labeled α-satellite DNA probes for the X chromosome (DXZ1) and the 18 chromosome (D18Z1) and also digoxigenin (DIG)-labeled quintessential DNA unique sequence probes for the same chromosomes (Xp21.2-p21.3 and 18qD18S41) were purchased from Oncor (Gaithersburg, MD).

The denaturation of the DNA in the paraffin-embedded specimens and of the α-satellite (centromere) probes were done by two different methods throughout the study. In the first method, the probes were mixed and applied to the slides, and host DNA and probes co-denatured on a 70°C hot plate for 12 minutes. In the second method, the denaturation of the DNA in the specimens was done by heating slides in 70% formamide in 2× sodium chloride sodium phosphate EDTA (SSPE) at 70°C for 15 minutes and immediately transferring the slides to cold 70% ethanol. The slides were then dehydrated in the graded ethanols as previously described and air-dried. The α-satellite probes were denatured separately in a microwave oven for 40 seconds and promptly cooled down in cold water. The probes were then mixed and applied to the specimens. The second method, separate denaturation of probe and specimen DNA, gave better centromere signals for the paraffin sections and therefore was used for the majority of study specimens.

Slides from all of the paraffin-embedded specimens were probed with each of the probe combinations: DXZ1/18D18S41 and D18Z1/Xp21.2–21.3 (ie, Xcentromere/18q21.2 and 18centromere/Xp21.2–21.3). In addition to the nonsynovial soft tissue sarcomas probed with the X,18 combinations, four randomly selected soft tissue sarcoma slides were also probed with an X,8 combination (DXZ1/8q21.3) as an additional negative control to determine signal association frequencies with a probe combination not related to the synovial sarcoma translocation.

The hybridization of specimen DNA and probes was done in a 37°C humid chamber overnight, after which the slides were washed in 3× SSPE at 68°C for 5 minutes. The unique sequence probes were detected with a rhodamine-labeled anti-DIG detection system (Oncor) and counterstained with diamidino-2-phenyl-indole-dihydrochloride (DAPI). The normal three-step detection procedure (rhodamine-labeled anti-DIG (sheep) antibody, rabbit anti-sheep antibody and rhodamine-labeled anti-rabbit antibody) was extended with a repetition of the last two steps to enhance the rhodamine signals. Of the 20 blocks of soft tissue sarcomas, three cases diagnosed as synovial sarcomas were rejected, one because of insufficient number of nuclei in the specimen and the others because of lack of fluorescent signals after probing and detection.

T-lymphocytes were obtained from standard 72-hour peripheral blood cultures. After air-drying, the slides were soaked in a mixture of 40 ml of 2× SSPE + 200 μl of Nonidet P-40, dehydrated through the ethanol series, and air-dried a second time. Then the FITC-direct-labeled X and 18 α-satellite probes were applied to the slides. The specimen DNA and the α-satellite probes were co-denatured on a 70°C hot plate for 3.5 minutes and hybridized on a humid hot plate at 42 to 44°C for 3 to 5 hours. Then the slides were washed in 3× SSPE at 70°C for 5 minutes, dehydrated down through the ethanols once more, and air-dried. Application of the unique sequence probes were done on the alternate slides so every sample was probed with both probe combinations: Xcentromere/18q21.2 and 18centromere/Xp21.2–21.3 (Xc/18q and 18c/Xp). The slides were then hybridized in a humid chamber at 37°C overnight and the next morning were washed in 3× SSPE at 70°C for 5 minutes. The unique sequence probes were detected in the same manner on lymphocytes as the paraffin-embedded tissues but with three amplification steps only and with shorter detection time necessary for each step. The nuclei were then counterstained with DAPI.

Protocol for Scoring of Signals

To characterize the spatial configurations between the dual colored probes, the positions of the fluorescent signals within the nucleus is divided into three categories labeled random, near, and joined. The random signal position is defined as a large enough separation of the centromere and nonbreakpoint DNA probe signals to preclude spatial association. The signal location defined as near is characterized by at least one red and green signal pair being no farther apart than three centromere signal diameters. The joined signal position is where the two signals are touching or overlapping with no visible space between them (Figure 1).

For the paraffin-embedded soft tissue sarcomas, scoring was done within the tumor area preferably at the periphery of the specimen where the nuclei had a tendency to detach. These isolated nuclei were not overlapping and hence easily scored. Each nucleus was scored and categorized as random, near, or joined. If one red-

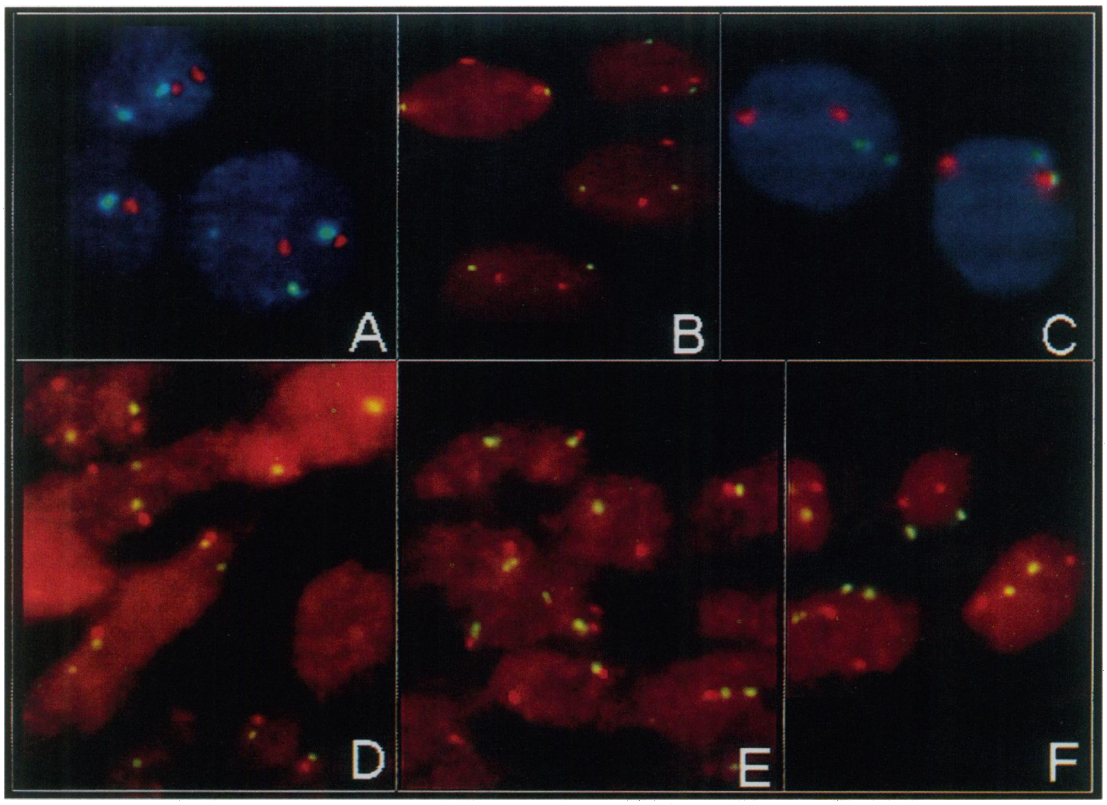

Figure 1. A: Three female lymphocyte nuclei probed with the Xc/18q combination and counterstained with DAPI. The two nuclei with four probes in each are scored into both the near and random probe position category. **B:** Female control lymphocytes probed with Xc/18q. The upper left nucleus has one red-green probe pair that is scored as joined, ie, no visible space in between the two signals. The upper right nucleus has a red-green probe pair scored as near. The remaining six red-green probe pairs are scored as random giving a total count of eight for the four lymphocyte nuclei. **C:** Two lymphocyte nuclei counterstained with DAPI are seen with the Xc/18q probe set. One is scored as joined + random and the other counted twice as random. **D** and **E:** Paraffin-embedded synovial sarcoma tissue from a male patient with detached nuclei from the periphery of the specimen. The nuclei are probed with 18c/Xp. The majority of signal pairs are in the near and joined positions. **F:** Paraffin-embedded nonsynovial sarcoma specimen from a female patient with detached nuclei that were easily scored. Each of the three nuclei containing four signals would all be scored as having random probe position. FITC and rhodamine-labeled detection. DAPI counterstain (**A** and **C**). Magnification, ×60.

green signal pair of near and one pair of joined were found, this nucleus was scored as joined, as was a nucleus with a random and joined red-green pair. An occurrence of a random and a near red-green pair would be scored as near. This priority of joined greater than near greater than random is used because the signals from normal nontranslocated chromosomes would be expected to have a random distribution and thus can be distinguished from their translocated homologues. Figure 1 D to F illustrates the signal position distribution in synovial *versus* nonsynovial sarcomas.

For each of the 17 tumor specimens, 100 to 200 nuclei were scored per probe set (Xc/18q and 18c/Xp) giving a total of 5890 counts. An additional 740 nuclei were evaluated for the four specimens probed with the control Xc/8q21.3 combination.

For the peripheral blood lymphocytes with flatter and broader nuclei than the sarcomas, all of the signals within a nucleus were scored in pairs, so each nucleus was scored twice relative to the signal location categories with the same position priority as for the soft tissue sarcomas (joined > near > random) (Figure 1, A to C). This double count was done because each of these signal pairs is expected to be independent of each other in the absence of translocation. From the three cytogenetically normal individuals, a total of 2000 nuclei were scored for each probe combination (Xc/18q and 18c/Xp) adding up to 4000 scored lymphocyte nuclei and a total count of 8000.

For the entire study sample the scoring was done with a ×60 oil lens. Only nuclei with well-defined and localized fluorescent signals were included in the count. To minimize error caused by truncation, only nuclei with the expected number of signals were scored, ie, if the nuclei in a sample consistently had two X signals then only nuclei containing two green and two red signals were scored. If only one X signal was present per nucleus, as would be expected in a male sample, then only cells with the expected three signals were scored.

Scoring of the slides was done by the same person throughout the study with a second examiner sample checking. During the study the evaluators were blinded to the histopathological diagnosis of the soft tissue sarcomas as well as patient sex. Selected areas were either captured with a VI 470 color CD camera and archived as TIFF images or photographed on 100 ASA Ektachrome slide film using a manual exposure time of 32 seconds with a dual pass red/green filter.

After scoring was completed, the code for the soft tissue sarcomas was broken and the cases were divided into synovial sarcomas, other soft tissue sarcomas, and

Table 1. Probe Association Counts from Synovial Sarcomas

Case number	Probe set	Total count	Probe position (%)		
			Random	Near	Joined
1	Xc/18q	200	10.0	79.5	10.5
	18c/Xp	201	13.9	77.6	8.5
2	Xc/18q	140	11.4	77.9	10.7
	18c/Xp	110	17.3	71.8	10.9
3	Xc/18q	200	23.0	68.0	9.0
	18c/Xp	200	12.5	78.0	9.5
4	Xc/18q	100	10.0	84.0	6.0
	18c/Xp	100	10.0	85.0	5.0
5	Xc/18q	200	2.0	95.5	2.5
	18c/Xp	200	6.0	89.5	4.5
6	Xc/18q	200	11.0	85.5	3.5
	18c/Xp	200	4.5	93.5	2.0
8	Xc/18q	200	6.0	86.5	7.5
	18c/Xp	100	12.0	85.0	3.0
9	Xc/18q	150	69.3	24.7	6.0
	18c/Xp	130	65.4	30.8	3.8
10	Xc/18q	200	9.0	78.5	12.5
	18c/Xp	200	7.5	76.5	16.0
12	Xc/18q	200	10.0	85.5	4.5
	18c/Xp	200	8.5	89.0	2.5
13	Xc/18q	200	4.0	90.5	5.5
	18c/Xp	200	9.5	88.0	2.5
14	Xc/18q	200	10.5	85.5	4.0
	18c/Xp	100	7.0	90.0	3.0
16	Xc/18q	180	27.8	67.2	5.0
	18c/Xp	119	19.3	74.8	5.9
17	Xc/18q	200	12.0	84.5	3.5
	18c/Xp	200	19.0	75.0	6.0
18	Xc/18a	200	59.0	37.5	3.5
	18c/Xp	200	78.5	17.5	4.0

Cases 7, 11, and 15 gave unsatisfactory probe results.

sarcomas with the unrelated probe set: Xc/8q21.3. Within each of the three specimen categories (synovial sarcomas, other sarcomas, and lymphocytes), the results of each of the two probe combinations (Xc/18q and 18c/Xp) relative to position (random, near, and joined) were compared in regard to the difference in their means using a two-tailed *t*-test: paired two sample for means, confidence interval 95%. In addition, the results in the four categories (synovial sarcomas, other soft tissue sarcomas, sarcomas with the unrelated X,8 combination, and lymphocytes) were all compared with each other by two-tailed *t*-tests: two-sample assuming unequal variances, confidence interval 95%.

Results

The results of scoring the synovial sarcoma specimens are listed in Table 1. All but samples 7, 11, and 15 were probed successfully. Two samples, 9 and 18, had a lower frequency of probe association than the others but still had association frequencies five to seven times higher than the controls. Within all of the specimen categories (with the exception of the sarcomas probed with the single unrelated probe combination), the means of the two X,18 probe sets (Xc/18q and 18c/Xp) were all found to be statistically the same. Because of this similarity within each category, it was possible to pool the results of these two probe combinations. The pooled results for the entire study sample are shown in Table 2. Because the frequency of joined signals was low compared with near signals, these two probe position categories were combined into a fourth category labeled associated for comparison purposes.

As additionally shown in Table 2, the results of scoring the four randomly selected sarcoma slides probed with the nondiagnostic X,8 combination (all of which turned out to be synovial sarcomas) and the nonsynovial sarcomas probed with the diagnostic X,18 probe sets are statistically identical within all signal position categories. However, the random and associated positions are statistically different in the lymphocytes compared with the sarcomas probed with the unrelated X,8 probe combination and also compared with the results of the control sarcomas probed with the diagnostic X,18 probe sets. This difference reflects the technical superiority of scoring the flatter, almost two-dimensional lymphocytes.

Most important, the X,18 associated position frequency found in the synovial sarcomas is significantly different from the associated position frequencies found in any of the other specimen categories. The *P* (two-tailed) values ranged from 10×10^{-18} to 10×10^{-12}. This indicates that there is a readily demonstrable difference between the synovial sarcomas probed with the X,18 combinations and the soft tissue sarcomas lacking the translocation and that this large difference of greater than 75% associated or less than 25% random for the

Table 2. Results from Different Specimen Categories

	Total count	Probe position category mean (%) ± C.I. 95%			
		Random	Near	Joined	Associated
Synovial sarcomas					
15 samples Xc/18q	2770	18.3 ± 11.0	75.4 ± 10.9	6.3 ± 1.7	81.7 ± 11.0
15 samples 18c/Xp	2460	19.4 ± 12.2	74.8 ± 12.1	5.8 ± 2.2	80.6 ± 12.2
Pooled data	5230	18.9 ± 7.7	75.1 ± 7.6	6.0 ± 1.3	81.2 ± 7.7
Nonsynovial sarcomas					
2 samples Xc/18q	360	81.9*	15.1*	3.1*	18.1*
2 samples 18c/Xp	300	88.8*	8.3*	3.0*	11.2*
Pooled data	660	85.3 ± 8.3	11.7 ± 7.6	3.0 ± 1.3	14.7 ± 8.3
Synovial sarcomas					
4 samples Xc/8q21.3	740	84.8 ± 7.2	12.1 ± 6.5	3.0 ± 1.3	15.2 ± 7.2
Lymphocytes					
3 samples Xc/18q	4000	94.5 ± 0.9	4.6 ± 0.7	0.9 ± 0.3	5.5 ± 0.9
3 samples 18c/Xp	4002	94.4 ± 0.9	4.7 ± 0.7	1.0 ± 0.3	5.6 ± 0.9
Pooled data	8002	94.4 ± 0.6	4.6 ± 0.4	0.9 ± 0.2	5.6 ± 0.6

*The number of scored events is too small to calculate a valid confidence interval 95%.

synovial sarcomas (Figure 1) would not be obscured by small scoring errors related to differences in the evaluation of the nuclei.

Discussion

This study uses synovial sarcoma with the diagnostic t(X;18) as an example of a tumor in which the use of probes from the growing armamentarium of commercially available probes can be used in making the diagnosis. A variety of centromere and large target unique sequence probes can provide regional probes from which to pick probe sets that are close enough together to produce associated signals in significant numbers of cells with signal association when the translocation is present. Because synovial sarcoma has been used previously by us[8] and others[9–13] to test different FISH probe techniques for detection of the X;18 translocation, this particular tumor is an appropriate choice for assessing a new diagnostic method.

The MHF and fibrosarcoma were used as paraffin-embedded negative control specimens. The MFH is characterized by multiple chromosomal abnormalities rather than by single specific translocations,[14,15] and the fibrosarcoma has been used in a previous study as negative control without showing the t(X;18). Although there are two examples of the t(X;18)(p11;q11) in both MFH and fibrosarcoma in the literature,[16–18] the likelihood of finding the synovial sarcoma translocation in a tumor with a different pathogenesis is remote.

This study demonstrates significant differences between the frequency of probe association in the nuclei of synovial sarcomas compared both with the control sarcoma nuclei and the lymphocyte nuclei, which supports the possibility of using this strategy in future diagnoses of histopathologically ambiguous cases that resemble a tumor characterized by a diagnostic translocation.

The fact that all of the results obtained are statistically identical within the two X,18 probe sets, as shown in Table 2, indicates that it is not important which of these two probe pairs is used in this diagnostic method. Therefore, for tumors with a questionable diagnosis, it is possible to use any combination of commercially available nonbreakpoint probes adjacent to the suspected translocation breakpoints even if such a probe pair is available for detecting translocation in only one of the chromosomes involved.

Because the joined probe frequencies were very low compared with the near position frequencies and the appearance of joined signals could be caused by superimposed probes in a three-dimensional matrix (as suggested by the results of the lymphocytes that reflect the low percentage of signals joined by chance), we pooled the near and joined categories into the associated probe position category as shown in Table 2. Three categories were initially scored because these results were not anticipated before the study, but the results clearly demonstrate that it is only necessary to score two categories, namely random *versus* associated.

Differences between the results from the peripheral blood lymphocytes and the paraffin-embedded control specimens, including the X,8 probed slides, can be explained by the origin and preparation of the two sets of specimens. Nuclei in the paraffin-embedded specimens are smaller than hypotonic swelled lymphocyte nuclei, which limits probe distance and increases the random chance of two probes being in near or joined positions. Paraffin-embedded nuclei are also three dimensional compared with the flat lymphocytes. The more realistic baseline for the paraffin-embedded specimens is probably given by comparing the results found in the control sarcomas and in the sarcomas probed with Xc/8q21.3. The results of the lymphocyte scoring indicate what percentages can be expected under optimal morphological conditions and show how unlikely it would be to obtain signal associations mimicking a translocation by chance. Similar results for lymphocytes have been previously described.[19]

This is the first report in which paraffin-embedded sections of synovial sarcoma have been probed with commercially available X and 18 unique sequence DNA probes together with centromere probes. In other studies

Table 3. Examples of Tumors with Specific Translocations

Tumor	Chromosomal abnormality
Myxoid liposarcoma	t(12;16)(q13;p11)
Rhabdomyosarcoma (alveolar)	t(2;3)(q35;q14) or t(1;13)(p13;q14)
Leiomyoma	t(12;14)(q14-15;q23-24)
Lipoma	t(12;V*)(q14;V*)
Ewing's sarcoma	t(V*;22)(V;q12)
Synovial sarcoma	t(X;18)(p11;q11)
Pleomorphic adenoma	t(3;8)(p21;q12) or t(9;12)(p13;q13)
Clear cell sarcoma	t(12;22)(q13;q12-13)
Desmoplastic small round cell tumor	t(11;22)(p13;q11.2-12)

*Variable chromosomes.

using commercial FISH probes in the diagnosis of synovial sarcoma, probing has been done on isolated nuclei using painting probes together with the centromere probes for both the X and 18 chromosomes.[20] The clear advantage of probing the 4-μm slides as opposed to extracted nuclei is that the sections essentially maintain the morphology of the tissue and make it possible to score cells within the tumor area. A disadvantage of using sections *versus* isolated nuclei is that nuclear overlap can make scoring a more time-consuming process because of having to reject more cells than would be the case with isolated nuclei.

For most of the paraffin-embedded sarcoma slides, a 40-minute pronase digestion was optimal, but with this treatment there was a tendency for loss of nuclear borders. However, although a 30-minute pronase digestion appeared to give more distinct nuclei, it also yielded less signal amplification in the detection process making scoring more problematic.

In the evaluation of the distance between the probes used in this study, we compared probe distance between the X centromere and *XIST* (Xq13.2), which has an estimated physical distance of about 16 megabases (J Dietz-Band, Oncor, personal communication), with that of the X centromere and the 18q21.2 probe in a translocated chromosome. Rough visual comparison suggests a distance of about 32 to 40 megabases between the X centromere and the 18 18q21.2 probe in the translocation. Although one cannot determine the maximal interphase span for possible probes in all other suspected translocations, as the difference between the associated and random position frequencies for the synovial sarcomas is so significant when compared with the controls, it may be possible to use probes for chromosome regions with relatively larger distances between them. Such a strategy would probably produce lower association frequencies, but the difference between tumors with the translocation and negative controls would still be significant.

The main problem arising with this type of diagnostic strategy is that the scoring category near *versus* random is somewhat arbitrarily defined and is partially dependent on the evaluator. As a guideline, it can be said that in cases in which probes were large because of signal amplification, the distinction between near and random would be at approximately three signal diameters (Figure 1). With smaller fluorescent signals, near would involve a smaller separation distance between signals. However, the observer can rapidly acquire assessment skills and learn to approximate the distances to expect between random and associated signals by comparing tumors with known translocations to tumor nuclei with nontranslocated chromosomes as controls. More importantly, the results obtained in this study leave much room for broad interinvestigator differences in the scoring procedure without jeopardizing the correct diagnosis. Although there is a possibility that the diagnosis could be missed in early or atypical tumors containing many necrotic or stromal cells resulting in more false negative cells as in cases 9 and 18 (Table 1), the likelihood of a false positive would be very low if strict scoring criteria are followed. Future studies will have to establish limits for diagnostic detection distances between probes.

Other types of malignancies in which this diagnostic procedure might be applicable would be in tumors with known diagnostic translocations as listed in Table 3.[21–25] For example, in addition to the synovial sarcoma specimens, we have used this approach to detect the t(8;21) in a nondividing acute myeloid leukemia bone marrow specimen using the 8q21.3 and 21q22.1 unique sequence probes and obtained very similar results. Other possible combinations that could be applied but have not been used so far by us would be the 8q21.3 unique sequence and 14 telomere probes to reveal t(8;14) in Burkitt's lymphoma.

Relative simplicity and ease of diagnosis makes the use of FISH with readily available unique sequence DNA probes a useful adjunct to standard histopathological diagnosis and should be routinely used in larger surgical pathology laboratories when appropriate. In fact, our study should encourage laboratories to maintain a full chromosomal complement of commercially available DNA probes to enable detection of diagnostic translocations even in cases in which only approximate breakpoints are known.

Conclusion

This study introduces the strategy of using FISH with nonbreakpoint DNA sequences that will consistently associate spatially when a translocation is present. This can become a significant aid in the diagnosis of paraffin-embedded solid tumors with a potential diagnostic trans-

location especially in cases in which the histopathology or tumor location raises doubt about the diagnosis. In addition, for solid tumors in which breakpoints are not well established, this diagnostic strategy could assist in the detection of specific diagnostic translocations.

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