Instructional lecture

Advantages and limitations of cytogenetic, molecular cytogenetic, and molecular diagnostic testing in mesenchymal neoplasms

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Introduction

Elucidation of the pathogenesis of bone and soft tissue tumors has been challenging because the genetic events are unique for the different mesenchymal tumor subtypes. However, enormous progress has been achieved with the advancement of cytogenetic and molecular genetic techniques. As a result, relevant oncogenes and tumor suppressor genes have been identified and localized, and new gene constructs and their protein products that result from translocations during sarcoma genesis have been determined. The identification of tumor-specific genetic markers for bone and soft tissue tumors, such as Ewing’s sarcoma, has added a new dimension to the formulation of a diagnosis and the resolution of cellular origin. Many of the genetic markers appear to have prognostic value, and studies to determine their potential applications as specific therapeutic targets are in progress. Three common genetic approaches used to identify mesenchymal tumor-specific abnormalities are conventional cytogenetic, molecular cytogenetic (fluorescence in situ hybridization, or FISH) and reverse transcription-polymerase chain reaction (RT-PCR) analyses. In this instructional lecture, emphasis is placed on the practical applications of each of these techniques, including their advantages and limitations. Certain case presentations are included to illustrate the integration of traditional histopathological and genetic approaches and serve as useful paradigms.

Genetic approaches commonly used as diagnostic aids

Cytogenetic analysis

Tissue submitted for cytogenetic analysis must be fresh (not frozen or fixed in formalin) because living, dividing cells are required. This specimen requirement is distinctly limiting because for some hospitals, specimens are received in the cutting room already fixed in formalin or the pathologist may prematurely place it in formalin. Thus, to use the karyotyping approach, the pathologist must plan ahead and submit a viable portion of the specimen prior to fixation. A mesenchymal tumor sample submitted for cytogenetic analysis should be representative of the neoplastic process and preferably be part of the specimen submitted for pathological study. Ideally, a 1- to 2-cm³ (approximately 0.5–1.0 g) fresh sample is provided for analysis. Also, small biopsy specimens or fine-needle aspirates (<500 mg) can be analyzed successfully (although prolonged culture may be needed to produce enough cells for examination). Efforts to perform cytogenetic analysis are worthwhile even when material is limited, as the presence of a single cell exhibiting a tumor-specific chromosomal abnormality provides strong diagnostic support.

The basic process of cell culturing and karyotypic analysis is the same for all mesenchymal neoplasms. The length of time that a bone or soft tissue tumor is cultured to attain satisfactory karyotypic findings is variable, though, and may be dependent on the histopathological type, grade of tumor, tumor cellularity, and size of specimen submitted for analysis. A short-term culture usually results in a sufficient number of mitoses within 10 days or less. Lengthy culture times should be avoided because undesired overgrowth by normal fibroblasts is more likely to occur.

An alternative to tissue culture is direct or same-day harvest. With this technique, endemic dividing cells are arrested after a 1- to 12-h incubation in colchicine and...
culture medium. This method is useful for obtaining fast or preliminary results but is constrained by the in vivo mitotic index. Thus, direct harvest may be most useful for high-grade tumors. Also, for best success, it is imperative that the laboratory receive the tissue sample within 1 h after biopsy.

G-banding is the most common form of banding for karyotyping. This is attributable to the relative ease of performing the technique, the reliability of the results, and the permanence of the preparations. G-bands can be obtained with Giemsa or Wright stains pretreated with trypsin or phosphate buffer, respectively. The number of alternating light and dark bands detectable with G-banding in the haploid genome varies with the level of chromosomal contraction in each metaphase cell, but it is in the range of 350–550 bands per haploid set. Because one band represents approximately 5–10 × 10^6 base pairs (bp) of DNA and therefore could potentially contain hundreds of genes, cytogenetic analysis is not considered a high-resolution technique.

Simplistically, chromosomal abnormalities can be divided into two major categories: numerical and structural abnormalities. Numerical abnormalities manifest as changes in complete sets of chromosomes [i.e., triploid (3N) or tetraploid (4N) complements] or in the number of individual chromosomes [i.e., loss of a single chromosome (monosomy) or gain of a single chromosome (trisomy)]. Structural abnormalities of chromosomes result from chromosomal breakage and rejoining of the broken ends to form new combinations. A frequently observed structural abnormality in mesenchymal neoplasms is translocation. In a reciprocal translocation, chromosomal material is exchanged between two or more nonhomologous chromosomes. An example of the shorthand system used to describe numerical and structural aberrations is 47,XY,+8,t(11;22)(q24;q12), in which 47 indicates the total chromosome number. XY indicates the sex constitution, and +8 indicates an extra copy, trisomy, of chromosome 8. The “t” is an abbreviation for translocation and in this example specifies an exchange of chromosomal material between the long arms of chromosomes 11 and 22 at bands q24 and q12, respectively. (A comprehensive compendium of cytogenetic nomenclature can be found in the 2005 An International System for Cytogenetic Nomenclature.) The 11;22 translocation is a characteristic rearrangement in Ewing’s sarcoma, and trisomy 8 is a frequent secondary anomaly in this neoplasm. A significant strength of cytogenetic analysis is that it provides a global assessment of both numerical and structural abnormalities in a single assay, including both primary and secondary anomalies. Moreover, in contrast to FISH or RT-PCR, knowledge of the anticipated anomaly or histological diagnosis is not necessary, as illustrated in the following case.

An 88-year-old woman presented with a large (7 cm), palpable, painless lumbar region mass that projected into the retroperitoneal space (Fig. 1). A computed tomography (CT)-guided needle biopsy was performed. The lesion appeared to be composed of stellate- or spindle-shaped cells in a relatively dense collagenous stroma, and a diagnostic interpretation of desmoid tumor was rendered (Fig. 2). Karyotypic studies of desmoid tumors have revealed two cytogenetic subgroups: one group characterized by loss or deletion of 5q21–22 and one group characterized by extra copies of chromosomes 8 and/or 20. Chromosomal analysis of the current case, however, showed one to three supernumerary ring chromosomes (Fig. 3A). Subsequent FISH studies performed on unstained metaphase cells with a chromosome 12 paint probe demonstrated that the supernumerary ring chromosomes were composed of chromosome 12 material (Fig. 3B). The finding of supernumerary ring chromosomes composed of chromosome 12 material and accompanied by few or no other abnormalities is characteristic of well-differentiated liposarcoma, in particular involving the 12q14–15 region resulting in low-level amplification of this chromosomal region and the genes mapped within, such as MDM2 (Fig. 3C) and CDK4.

Equipped with these unexpected genetic findings, the surgical pathology slides were retrieved for rereview and on closer examination, rare atypical cells were discovered. It was thought that this biopsy probably represented a nonlipogenic zone in a well-differentiated (sclerosing) liposarcoma. This is a recognized pitfall of small-needle biopsies; that is, sampling specimens from such areas (nonlipogenic sclerosing areas) can lead to an erroneous conclusion that the tumor is not a liposarcoma. Subsequently, the patient’s tumor was resected, and lipogenic areas with lipoblasts were detected in addition to the highly atypical stromal cells consistent with a diagnosis of sclerosing liposarcoma.

This case illustrates the value of cytogenetic analysis as a diagnostic aid in the classification of mesenchymal neoplasms, particularly in the setting of limited tissue
Molecular cytogenetics

A revolutionary tool in the analysis and characterization of chromosomes and chromosomal abnormalities has been the development of in situ hybridization (ISH) techniques. Hybridization refers to the binding or annealing of complementary DNA or RNA sequences that serve as probes. With this approach, specific nucleic acid sequences can be detected in morphologically preserved chromosomes, cells, or tissue sections.

Molecular cytogenetic assays typically are performed with chromosome-specific probes labeled with fluorescent dyes such as fluorescein and detected with fluorescence microscopy (FISH). Probe detection or labeling with fluorescent molecules with different excitation and emission characteristics allows simultaneous analysis of several probes. Alternatively, hybridization signals can be detected with peroxidase or alkaline phosphatase, but these approaches are generally less sensitive.

A distinct advantage of FISH in contrast to conventional cytogenetic analysis is that this technique can be performed on nondividing (interphase) cells from fresh or aged samples (e.g., blood smears, touch imprint cytological preparations, cytospin preparations), paraffin-embedded tissue sections, and disaggregated cells retrieved from fresh, frozen, or paraffin-embedded material. Importantly, this procedure can provide results (e.g., identification of a tumor-specific translocation or loss of a tumor suppressor gene locus) when the tissue is insufficient or unsatisfactory for cytogenetic analysis, when conventional cytogenetic analysis has failed to yield results, or when cryptic rearrangements are present.12

Blood smears, touch imprint cytological preparations, and cytospin preparations are air-dried and subsequently fixed in methanol–glacial acetic acid (3:1) for 5 min. To visualize an anomaly in a specific region of a tumor or in a specific cell type, a 4- to 6-μm thick

Fig. 2. Computed tomography (CT)-guided needle biopsy specimen exhibiting spindle- and stellate-shaped cells in a collagenous background.

Fig. 3. A Multiple ring chromosomes (arrows) in a G-banded metaphase cell of a sclerosing (well-differentiated) liposarcoma. B Ring chromosomes (arrows) are composed of chromosome 12 material, as demonstrated by fluorescence in situ hybridization (FISH) analysis with a whole chromosome 12-paint probe. This image is of a partial metaphase cell also showing two normal chromosome 12 homologues. C Bicolor FISH analysis with a chromosome 12 centromere-specific probe (green) and MDM2 locus-specific probe (red) demonstrates MDM2 amplification in the ring chromosomes (arrows). This image is of a partial metaphase cell also showing two normal chromosome 12 homologues (red and green signals).
paraffin-embedded tissue section can be used. Analysis of thin sections, however, is limited because portions of most nuclei are lost during sectioning, which may lead to false-positive results in the evaluation of chromosomal deletions or losses. For the most accurate assessment of subtle aneuploidy changes, the preferred approach is to obtain whole or intact nuclei by disaggregating and releasing cells from a much thicker (50–60 μm) section. FISH is a same-day or overnight procedure, depending on the probes used or the type of specimen analyzed (or both).

Chromosomal probes (complementary DNA sequences) frequently used to examine bone and soft tissue tumors can be divided into three categories (Fig. 4): (1) Centromere-specific probes are composed of tandemly repeated monomers or α-satellite sequences (171 bp) that are unique for each chromosome. These probes are useful for chromosome enumeration. (2) Single copy probes that are homologous to specific targets ranging from 15 to more than 500 kilobases (kb) in size are referred to as “locus-specific” probes. Locus-specific probes are often used for assessing oncogenes or tumor suppressor genes. Many tumor-specific translocations can be identified with locus-specific probes. (3) “Paint,” or whole chromosome, probes are comprised of probe mixtures with homology at multiple sites along the target chromosomes. Interphase cell analysis is impractical with whole chromosome probes because the decondensed chromosomes are spread over too large an area for proper signal interpretation. Whole chromosome probes are most useful for characterizing structural chromosomal anomalies in metaphase cells.

Conventional karyotyping is limited by its inability to identify cryptic abnormalities, complex aberrations, and marker chromosomes accurately. To facilitate karyotyping — particularly analysis of complex cases with multiple chromosomal rearrangements and marker chromosomes — universal chromosome painting and multicolor banding techniques [e.g., multilow-FISH (M-FISH), spectral karyotyping (SKY), spectral color banding (SCAN)] — have been developed. These approaches are well suited to solid tumors because the complexity of the karyotypes may often mask the presence of recurrent chromosomal aberrations. Consider the following case.

An 81-year-old woman presented with a painless mass in the proximal anterior right thigh. Six years earlier, the patient had
a “spindle cell hemangioendothelioma” resected with adequate margins at a site distal to the more recent lesion. Magnetic resonance imaging (MRI) of the current lesion indicated a large nonhomogeneous mass pushing the femoral vessels laterally. A biopsy performed by her local physician was interpreted as a “spindle cell sarcoma.” The patient was subsequently referred to the University of Nebraska Medical Center.

At operation, a 14 × 11 × 10 cm mass was resected in continuity with the superficial inguinal nodes, preserving the femoral vessels and nerves. The cut surface of the lobulated, firm mass was pink-red with focal punctate calcifications. Histopathological examination revealed an admixture of thin-walled, variably dilated cavernous spaces and cellular zones of moderately pleomorphic spindle-shaped cells and rounded epithelioid-like cells (Fig. 5). Flattened endothelial cells lined the cavernous vessels, some of which contained organized and calcified thrombi. Mitoses were rare. The spindle-shaped and epithelioid-like cells were immunoreactive for vimentin but negative for factor VIII-related antigen, lectin *Ulex europaeus* agglutinin, desmin, muscle-specific actin, CD31, CD34, and S-100 protein. Focal immunoreactivity for CD31, factor VIII-related antigen, and lectin UEA-1 was present in the endothelial cells lining the vasculature. A definitive diagnosis based on the histological and immunohistochemical findings could not be rendered even following expert consultation.

A representative portion of the sample submitted for conventional cytogenetic analysis demonstrated the following abnormal primary clone: 48,XX,+r1,+r2. In an effort to determine the composition of the ring chromosomes identified by G-banding, M-FISH studies were employed and revealed that the larger ring was composed of material from chromosomes 7 and 16 [der(7)r(7;16)(?p22q33;?p11.2p13.3)], and the smaller ring was composed of chromosome 13 [r(13)?p11.2q21] (Fig. 6A). These findings prompted further FISH investigation with locus-specific probes to assess for the presence of the (7;16)(q33;p11.2) rearrangement or *FUS/CREB3L2* fusion. Fusion of the *FUS* and *CREB3L2* gene loci, characteristic of low-grade fibromyxoid sarcoma, was identified (Fig. 6B) and played a central role in establishing the diagnosis of this clinically unusual (uncommon age) and histopathologically atypical (foci of epithelioid cytomorphology and cavernous vascular spaces) case.

Advantages of FISH as illustrated in this case is that it can (1) be informative in both metaphase and interphase cell preparations, the latter to include formalin-fixed, paraffin-embedded tissue; (2) assist in deciphering the origin of marker chromosomes, ring chromosomes, and cryptic or complex chromosomal rearrangements; and (3) reveal tumor-specific anomalies that are diagnostically useful, especially for classifying mesenchymal neoplasms with atypical clinical or histopathological features. Similar to other mesenchymal tumor types (e.g., Ewing’s sarcoma, peripheral primitive neuroectodermal tumor), the morphological spectrum of low-grade fibromyxoid sarcoma has recently expanded following genotypic characterization.

A summary of advantages and limitations of molecular cytogenetic approaches is provided in Table 2.

**Reverse transcription-polymerase chain reaction analysis**

Translocations, or exchange of chromosomal material between two or more nonhomologous chromosomes, are encountered frequently as tumor-specific anomalies in mesenchymal neoplasms. These tumor-specific translocations serve as important guides for molecular biologists conducting positional cloning studies of the genes at the translocation breakpoints. The most common genetic consequence of these translocation events is the fusion of two genes, one from each translocation partner, resulting in the formation of a chimeric gene. The fusion proteins encoded by these chimeric genes are not found in normal cells and are frequently tumor-specific (Table 3).

### Table 2. Advantages and limitations of molecular cytogenetic analysis

| **Advantages** | **Limitations** |
|----------------|-----------------|
| Can be performed on metaphase or interphase cell preparations (fresh, frozen, or paraffin-embedded material) | More targeted approach; not screening tool (generally requires prior knowledge of anomaly of interest) |
| Can localize anomaly in specific cells or tissue types | Exceptions are CGH and SKY |
| Can provide results when tissue is insufficient or unsatisfactory for cytogenetic analysis, when conventional cytogenetics has failed to yield results, or when cryptic rearrangements are present | Still a relatively gross approach when contrasting other molecular approaches capable of detecting single base mutations |
| Diagnostically useful, Sensitive and specific | Number of commercially available probes is limited |
| Rapid turn-around time | Requires fluorescence microscopy (signal fading) |
| **Limitations** | Interpretation may be challenging when analyzing suboptimal specimens (i.e., background fluorescence or autofluorescence, particularly with formalin-fixed, paraffin-embedded material) |
| **FISH nomenclature not consistent among laboratories** |

**CGH**, comparative genomic hybridization; **SKY**, spectral karyotyping
In sarcomas, the fusion genes most often code for aberrant transcription factors that result in inhibition of normal cellular differentiation, cell cycle activation, and loss of responsiveness to extracellular signals. Note that new cytogenetic and molecular genetic variants continue to be defined. Cytogenetic variants are defined as differing chromosomal translocation partners [i.e., t(2;17) and t(2;2) in inflammatory myofibroblastic tumor (Fig. 7)]; and molecular variants are often the result of genomic breakpoint differences that lead to distinct fusion product exon combinations (Fig. 8). For example, the two most frequent exon combinations in Ewing’s sarcoma-associated EWS/FLI1 fusion transcripts include fusion of EWS exon 7 to FLI1 exon 6 (type I) and fusion of EWS exon 7 to FLI1 exon 5 (type II). These molecular variants can be detected by their unique RT-PCR product band size. The identity of less common or unexpected product band sizes should be confirmed utilizing...
additional approaches, such as direct sequencing or digestion with specific restriction endonucleases.

The highly specific gene rearrangements that result from chromosomal translocations in bone and soft tissue tumors can be identified with RT-PCR analysis. The PCR technique uses specific synthetic primers to amplify a section of a gene in vitro. PCR can be carried out on RNA following reverse transcription (mRNA → cDNA). Snap frozen tissue is preferred for RNA extraction and RT-PCR analysis, but this procedure can also be performed on archival (paraffin-embedded) material if the RNA is of sufficient quality, as illustrated in the following case.

A 39 year-old-man presented with an intermittently painful swelling on the ulnar aspect of his right wrist. He also complained of occasional numbness and tingling in the hand. T2-weighted MRI demonstrated an enhancing lesion originating at the distal radioulnar joint and extending across the wrist in the volar aspect deep to the flexor tendon. An open biopsy of the lesion revealed a monomorphic population of spindle cells arranged in interlacing fascicles and sheets (Fig. 9). The neoplastic cells were focally immunoreactive for epithelial mem-

### Table 3. Characteristic and variant chromosomal translocations and associated fusion genes in bone and soft tissue sarcomas

| Neoplasm                          | Translocation                                      | Fusion gene(s) |
|-----------------------------------|-----------------------------------------------------|----------------|
| Alveolar soft part sarcoma        | der(17)t(X;17)(p11.2;q25.3)                         | ASPL/TFE3      |
| Alveolar rhabdomyosarcoma         | t(2;13)(q35;q14)                                   | PAX3/FOXO1b    |
|                                   | t(1;13)(p36;q14)                                   | PAX3/FOXO1     |
|                                   | t(X;2)(q13;q35)                                    | PAX3/AFX       |
|                                   | t(2;2)(q35;p23)                                    | PAX3/NCOA1     |
| Angiomatoid fibrous histiocytoma  | t(7;22)(q34;q12)                                   | EWS/CREBI      |
|                                   | t(12;22)(q13;q12)                                  | EWS/CREBI      |
|                                   | t(12;16)(q13;p11)                                  | EWS/CREBI      |
| Clear cell sarcoma                | t(12;22)(q13;q12)                                  | EWS/ATF1       |
|                                   | t(2;22)(q34;q12)                                   | EWS/ATF1       |
| Congenital fibrosarcoma           | t(12;15)(p13;q25)                                  | ETV/6/NTRK3    |
| Dermatofibrosarcoma protuberans   | t(17;22)(q22;q13)                                  | COL1A1/PDGFB   |
| Desmoplastic small round cell tumor| t(11;22)(p13;q12)                                  | EWS/WT1        |
| Epithelioid hemangioendothelioma   | t(1;3)(p36;q25)                                    |               |
| Ewing sarcoma/pPNET               | t(11;22)(q24;q12)                                  | EWS/FLI1       |
|                                   | t(21;22)(q22;q12)                                  | EWS/ERG        |
|                                   | t(7;22)(q22;q12)                                   | EWS/ETV1       |
| Extradural myxoid chondrosarcoma  | t(9;22)(q22;q12)                                   | EWS/NRA43f     |
|                                   | t(9;17)(q22;q11)                                   | RBP56/NRA43f   |
|                                   | t(9;15)(q22;q11)                                   | TCF12/NRA43f   |
|                                   | t(3;9)(q11–12;q22)                                 | TFG/NRA43f     |
| Inflammatory myofibroblastic tumor| t(1;2)(p22;p23)                                    | TPM3/ALK       |
|                                   | t(2;19)(p23;p13)                                   | TPM4/ALK       |
|                                   | t(2;17)(p23;q23)                                   | CLT/ALK        |
|                                   | t(2;2)(p23;q13)                                    | RANDP2/ALK     |
| Low-grade fibromyxoid sarcoma     | t(7;16)(q33;p11)                                   | FUS/CHEB3L2    |
|                                   | t(11;16)(p11;p11)                                  | FUS/CHEB3L1    |
| Myxoid/round cell liposarcoma     | t(12;16)(p13;p11)                                  | FUS/CHOP6f     |
|                                   | t(12;22)(q13;q12)                                  | EWS/CHOP6f     |
| Pericytoma                        | t(7;12)(p22;q13)                                   | AGTB/GLI       |
| Synovial sarcoma                  | t(X;18)(p11.2;q11.2)                               | SS18/SSX1      |
|                                   | t(X;20)(p11.2;q13.3)                               | SS18/SSX4      |
|                                   |                                                     | SS18L1/SSX1    |

pPNET, peripheral primitive neuroectodermal tumor

* A balanced form of this translocation is seen also in a subset of pediatric renal neoplasms
* Also referred to as *FKHR*
* Also referred to as *TEC, MINOR, CHN*, and *NOR-1*
* Also referred to as *TAF2N*
* Also referred to as *TLS*
* Also referred to as *DDIT3*
* Also referred to as *SYT*
brane antigen and S-100 protein and negative for MAK6 (cytokeratin). Consequent RT-PCR studies performed on the formalin-fixed, paraffin-embedded biopsy specimen revealed the presence of an \( SS18/SSX2 \) fusion transcript, thereby confirming the favored diagnosis of monophasic synovial sarcoma (Fig. 10). Subsequently, a below-elbow amputation was performed (Fig. 11). Monophasic spindle cell synovial sarcoma may be confused with, or misdiagnosed as, fibrosarcoma, leiomyosarcoma, malignant peripheral nerve sheath tumor, or hemangiopericytoma. Identification of the synovial sarcoma-specific X:18 translocation and/or its associated fusion transcripts (\( SS18/SSX1 \) or \( SS18/SSX2 \)) is useful for establishing the diagnosis.

RT-PCR analysis is remarkably sensitive. It may allow detection of abnormalities present in cells too few to be identified with traditional cytogenetic or FISH methods. It may be suitable for the detection or monitoring of minimal residual disease. Also, RT-PCR analysis is not dependent on successful cell culture; and, similar to FISH, it is rapid, with a short turnaround time. Compared with cytogenetic analysis, the greatest disadvantage of RT-PCR analysis is the inability to detect chromosomal anomalies other than those for which the test was designed. With conventional cytogenetic analysis, all major chromosomal abnormalities,
including those not initially anticipated by the clinician or the laboratory staff may be uncovered. Additional advantages and limitations of RT-PCR analysis are listed in Table 4.

**Conclusion**

Dramatic advances in cytogenetic and molecular biological techniques have furthered our understanding of sarcomagenesis. Cytogenetic and molecular genetic assays are used routinely for diagnostic and prognostic purposes in molecular pathology laboratories and represent a powerful adjunct to conventional microscopy and radiographic assessment for formulating an accurate diagnosis. Care should be taken, however, to recognize the limitations of these approaches. Ideally, more than one technical approach should be available to a diagnostic laboratory to compensate for

**Fig. 9.** Neoplasm composed of vague fascicles of uniform, spindle-shaped cells with ovoid, pale-staining nuclei. **Inset** Neoplastic cells are immunohistochemically negative for MAK6 (cytokeratin)

**Table 4.**

|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| M | 1 | 2 | 3 | 4 | M | 5 |

**Fig. 10.** Ethidium-stained gel of RT-PCR results for a patient with suspected synovial sarcoma. Total RNA was extracted from formalin-fixed, paraffin-embedded tissue and subjected to RT-PCR. M, A DNA molecular weight marker (100-bp ladder); lanes 1 and 2, patient sample run in duplicate is negative for a SS18/SSX1 fusion transcript; lane 3, SS18/SSX1 fusion transcript 110-bp positive control; lanes 4 and 8, negative no template control; lanes 5 and 6, patient sample run in duplicate is positive for a 110-bp SS18/SSX2 fusion transcript; lane 7, SS18/SSX2 fusion transcript 200-bp variant positive control

**Fig. 11.** Flesh-toned synovial sarcoma mass measuring 4.4 cm in greatest dimension was deep to the flexor muscles and advanced along the intermuscular planes proximally
Table 4. RT-PCR analysis

Advantages
- Can be performed on fresh, frozen, or paraffin-embedded material
  - Tissue quantity requirement is small
- Can provide results when tissue is insufficient or unsatisfactory for cytogenetic analysis, when conventional cytogenetics has failed to yield results, or when cryptic rearrangements are present
- Diagnostically useful
  - Sensitive and specific
  - Rapid turn-around time
- Because of its remarkable sensitivity, RT-PCR may be useful for detecting minimal residual disease or early relapsed disease

Limitations
- Not all sarcomas exhibit characteristic fusion gene transcripts
- Targeted approach; not a screening tool
  - Requires prior knowledge of fusion transcript
  - RNA quality may be inadequate secondary to RNA degradation
  - Reagent contamination is a potential hazard in small laboratories
  - Revisited primer sets may not detect unusual molecular variants (false negative)
  - Identification of some product bands may require validation by additional approaches such as direct sequencing, transfer and hybridization with internal oligonucleotide probes, digestion with specific restriction endonucleases, or reamplification with internal primers (nested RT-PCR)

the shortcomings of another in different clinical situations.

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