Reproducibility of MDM2 and CDK4 Staining in Soft Tissue Tumors

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Abstract

MDM2 and CDK4 immunostaining can be useful adjuncts in diagnosing liposarcoma among soft tissue neoplasms. We examined the reproducibility of MDM2 and CDK4 staining between 2 laboratories and between tissue microarrays and whole tissue sections. Sixty-two soft tissue tumors were immunostained at the Bergonié Institute, Bordeaux, France, and the Curie Institute, Paris, France. We also examined 203 soft tissue neoplasms on standard tissue sections and tissue microarrays.

There was high concordance of results obtained from the 2 laboratories (with 2 different pathologists) for MDM2 (κ, 0.93) and CDK4 (κ, 0.8) staining. There also was excellent concordance between results on tissue microarray and on whole tissue sections for MDM2 (κ, 0.80) and CDK4 (κ, 0.93). Immunostaining for MDM2 and CDK4 is a reproducible technique that may be exported to different laboratories for routine use. Tissue microarray is indicated for studying large series.

Owing to the high prevalence of lipomas, adipocytic tumors represent the most common type of mesenchymal tumors. Three categories of liposarcoma (LPS) have been identified: atypical lipomatous tumor (ALT)–well-differentiated LPS/dedifferentiated LPS, myxoid/round cell LPS, and pleomorphic LPS. For ALT–well-differentiated LPS/dedifferentiated LPS cases, which represent the most common type of LPS, prognosis is conditioned mainly on the local recurrence rate. Metastases, which occur only in dedifferentiated LPS, remain a rare event (about 15% of cases of dedifferentiated LPS).1

Histologically, ALT–well-differentiated LPS cases easily might be confused with lipoma, especially when they show secondary changes in the form of fibrosis or liponecrosis, and, less frequently, with spindle cell/pleomorphic lipoma and angiomyolipoma. Conversely, dedifferentiated LPSs, which occur mainly in the retroperitoneum or along the spermatic cord but also in other sites, may be confused with other spindle cell/pleomorphic undifferentiated tumors (eg, fibrosarcomas, malignant peripheral nerve sheath tumors, and so-called malignant fibrous histiocytomas). When a heterologous component is present (5%-10% of cases), dedifferentiated LPS may mimic leiomyosarcoma, rhabdomyosarcoma, chondrosarcoma, or osteosarcoma.

At the cytogenetic level, ALT–well-differentiated LPS/dedifferentiated LPS cases characteristically show supernumerary ring and/or giant chromosomes.2 Fluorescent in situ hybridization (FISH), comparative genomic hybridization, and Southern blot studies demonstrated that these long and/or ring chromosome markers are composed of 12q13-15 amplifications, which consistently include the MDM2 gene and frequently the CDK43-5 gene, resulting in protein overexpression.
In a large series of soft tissue neoplasms, we previously showed that immunohistochemical detection of MDM2 and CDK4 gene products was a useful adjunct in the diagnosis of ALT–well-differentiated LPS/dedifferentiated LPS; positive staining correlated with gene amplification.6 To evaluate the reproducibility of this technique, stains from 2 laboratories were compared. Moreover, stains performed on tissue microarray (TMA) also were compared with those performed on whole tissue sections (WTS).

Materials and Methods

Cases and Materials

All cases were retrieved from the files of the Bergonié Institute, Bordeaux, France. Pathologic specimens were fixed in Holland Bouin fluid. Sixty-two WTS were studied at the Bergonié Institute and at the Curie Institute, Paris, France. The pathologic diagnoses, based on World Health Organization criteria,7 included dedifferentiated LPS (n = 19), ALT–well-differentiated LPS (n = 5), leiomyosarcoma (n = 9), so-called malignant fibrous histiocytoma (n = 7), myxoid/round cell LPS (n = 5), gastrointestinal stromal tumor (n = 3), spindle cell lipoma (n = 1), and pleomorphic LPS (n = 1).

For 205 cases, MDM2 and CDK4 immunostaining was performed on TMA and WTS. The pathologic diagnoses in this second series included so-called malignant fibrous histiocytoma (n = 43), ALT–well-differentiated LPS/dedifferentiated LPS (n = 41), leiomyosarcoma (n = 29), rhabdomyosarcoma (n = 14), synovial sarcoma (n = 10), myxoid LPS (n = 9), angiosarcoma (n = 8), dermatofibrosarcoma protubersans (n = 8), gastrointestinal stromal tumor (n = 8), malignant peripheral nerve sheath tumor (n = 6), clear cell sarcoma (n = 5), pleomorphic LPS (n = 4), primitive neuroectodermal tumor (n = 4), extraskeletal myxoid chondrosarcoma (n = 3), lipoma (n = 1), alveolar soft part sarcoma (n = 2), and desmoplastic small round cell tumor (n = 1).

Tissue Microarray

A manual microarrayer (Beecher Instruments, Sun Prairie, WI; distributed by Alphelys, Plaisir, France) was used to prepare the TMAs. For each case, well-preserved tissue areas (ie, nonnecrotic) were selected. Two biopsy procedures were used: in 149 cases, paraffin blocks were sampled 4 times using a 0.6-mm-diameter needle, and in 54 cases, two 1-mm cylinders were taken. A database was designed for each tissue microarray.8

Immunohistochemical Analysis

For MDM2 and CDK4 immunostaining, 4-µm-thick paraffin sections were cut and mounted on glass slides. Preparations were dried for 1 hour at 58°C and then overnight at 37°C. Sections were deparaffinized with toluene and rehydrated with ethanol. Preparations were pretreated with citrate buffer (0.01 mol/L of citric acid, pH 6.0), and a heat-based antigen retrieval method was used before incubation. Endogenous peroxidase was blocked with a 3% hydrogen peroxidase solution for 5 minutes. Sections were incubated for 1 hour at 22°C with the primary antibodies followed by staining with a streptavidin-biotin peroxidase kit (Elite ABC, Vector, Birmingham, England). Sections then were immersed in a diaminobenzidine solution for 15 minutes and stained with hematoxylin for 1 minute. Immunohistochemical staining was calibrated with cases for which the amplification status of MDM2 and CDK4 was known, using comparative genomic hybridization, as described previously for comparing HER-2 immunostaining and FISH in breast cancer.9 Data on the primary antibodies are shown in Table 1.

Immunostains were evaluated independently by 2 pathologists (M.B.N.B. and J.-M.C.), and discordant cases were reevaluated collegially. A tumor was considered as MDM2+ or CDK4+ when at least 1 cell nucleus was stained per high-power field on WTS and when at least 1 nucleus was positive on a TMA biopsy sample.

Statistical Analysis

Interobserver and interlaboratory (ie, comparison between the Bergonie Institute and the Curie Institute) and intermethod (ie, TMA vs WTS) reproducibility was studied using a κ test.

Results

Details of immunostaining on TMA and WTS are shown in Table 2. Image 1A and Image 1B show, respectively, MDM2+ and CDK4+ staining obtained on a TMA slide. In a first step, the reproducibility between the 2 institutions (with 2

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Bergonie Institute, Bordeaux, France</th>
<th>Curie Institute, Paris, France</th>
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<tbody>
<tr>
<td>Anti-MDM2 (clone IF2), Zymed Laboratories, South San Francisco, CA</td>
<td>1:100</td>
<td>1:50</td>
</tr>
<tr>
<td>Anti-CDK4 (clone DCS-31), Biosource International, Camarillo, CA</td>
<td>1:200</td>
<td>1:100</td>
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different pathologists) was examined. Sixty-two tumors were tested at both institutions. Table 3 shows the results of immunohistochemical analysis in both laboratories. MDM2 stains were more often positive at Curie (37 cases) than at Bergonié (35 cases), but there was a high interlaboratory concordance with a $\kappa$ value of 0.93. CDK4 stains were positive in 31 cases at Bergonié but in only 29 cases at Curie ($\kappa$, 0.8).

In a second step, we compared WTS and TMA stains as shown in Table 4. A TMA case was considered positive when at least 1 of the biopsy cylinders was stained. For 205 cases, immunohistochemical analysis was performed on WTS and on TMA preparations. Data were available for 202 cases with MDM2 stains (98.5%) and for 197 cases with CDK4 stains (96.1%). There was excellent concordance between WTS (75 positive cases) and TMA (57 positive cases) stains for MDM2 ($\kappa$, 0.80). CDK4 staining reproducibility between WTS (47 positive cases) and TMA (42 positive cases) was better than for MDM2 ($\kappa$, 0.93).

Discussion

MDM2 is an oncogene whose product links to p53 and inhibits p53 function in the cell cycle in 3 ways: blockage of p53 interaction with DNA, inhibition of p53 transfer from the cytoplasm to nucleus, and ubiquitination of p53. The action of MDM2 results in p53 inactivation without mutation or deletion of p53, resulting in inhibition of apoptosis. CDK4 also is an oncogene whose product inhibits RB1. CDK4 protein phosphorylates RB1, which links to E2F and, thus, is inactive, resulting in loss of the G1-S checkpoint. MDM2 and CDK4 are amplified genes in ALT–well-differentiated LPS/dedifferentiated LPS. Amplicons of MDM2 and CDK4 genes form ring chromosomes and/or giant chromosome markers. Gene amplification is responsible for MDM2 and CDK4 protein overexpression that may be detected using immunohistochemical analysis with good sensitivity and specificity.6

We previously showed that staining for MDM2 and CDK4 was a useful adjunct in the diagnostic approach for LPS in 2 situations: ALT–well-differentiated LPS vs benign adipose tumor and dedifferentiated LPS vs pleomorphic or
spindle cell sarcoma.\textsuperscript{6} MDM2 and CDK4 amplification have been demonstrated to have a role in the oncogenesis of numerous malignant neoplasms, including carcinomas, sarcomas,\textsuperscript{10} and hematopoietic malignant neoplasms.\textsuperscript{11} As such, they seem to be potentially interesting targets for developing new drugs.\textsuperscript{12,13} More than an adjunct in diagnosis, the detection of MDM2 and CDK4 amplification might be needed to guide treatment. Holland Bouin fluid is a formalin-based fixative that is not used routinely in many institutions. According to our experience (J.-M.C. consultation files), immunostaining on formalin-fixed tissue samples gives results similar to those observed with Holland Bouin fluid.

When we examined the reproducibility of MDM2 and CDK4 immunostaining between the 2 institutions (with 2 different pathologists), $\kappa$ values showed a good concordance between results. These $\kappa$ values were higher than those reported in the literature concerning immunohistochemical reproducibility for estrogen receptor in breast cancer ($\kappa$, 0.42-0.72)\textsuperscript{14} and were comparable to those for Ki-67 in breast cancer ($\kappa$, 0.6-0.82),\textsuperscript{15} in astrocytoma ($\kappa$, 0.68-1),\textsuperscript{16} and HER-2/neu in breast cancer ($\kappa$, 0.73-1).\textsuperscript{17} This confirms that MDM2 and CDK4 immunohistochemical analysis is an exportable technique and can be used as a routine test. However, it is important to accurately calibrate the immunohistochemical technique by using genetic data (obtained with FISH, quantitative polymerase chain reaction, or comparative genomic hybridization), as already described for calibrating HER-2 immunostaining.\textsuperscript{9}

We also compared results of staining on TMA and WTS. The $\kappa$ values showed that stains obtained on TMA were concordant with those on WTS, with $\kappa$ values of more than 0.8. These results are similar to those described in other studies of tissue microarray concordance with WTS.\textsuperscript{18,19} Hoos et al\textsuperscript{18} found $\kappa$ values ranging from 0.85 to 0.87 in human fibroblastic tumors, and Camp et al\textsuperscript{19} showed a 95% comparable result in HER-2/neu evaluation in breast cancer. MDM2 staining reproducibility was slightly lower than for CDK4 because MDM2 staining sometimes is scattered and may be missed with biopsy sampling during TMA building. Therefore, TMA may be an alternative method for studying MDM2 and CDK4 immunodetection in a large series of soft tissue tumors. Immunohistochemical analysis of MDM2 and CDK4 is a useful adjunct in the diagnosis of ALT–well-differentiated LPS/dedifferentiated LPS. The present study showed that it is a reproducible technique that may be used in different laboratories and interpreted by different pathologists with similar results and that TMA also might be used for retrospective large-scale analyses.

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References