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INTRANODAL PALISADED MYOFIBROBLASTOMA: ANOTHER MESENCHYMAL NEOPLASM WITH *CTNNB1* (BETA-CATENIN GENE) MUTATIONS. CLINICOPATHOLOGIC, IMMUNOHISTOCHEMICAL, AND MOLECULAR GENETIC STUDY OF 18 CASES

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Abstract

Intranodal palisaded myofibroblastoma is a benign, lymph node-based myofibroblastic tumor of unknown pathogenesis. We report the clinicopathological, immunohistochemical, and genetic molecular features of this rare entity. The study cohort consisted of 14 males and 4 females ranging in age from 31 to 65 (mean, 47; median 49) years with tumors arising in inguinal lymph nodes (n=15), a neck lymph node (n=1), and undesignated lymph nodes (n=2). Most individuals presented with a painless mass or lump. Possible trauma/injury to the inguinal region was documented in four cases. Tumors ranged in size from 1.0 to 4.2 (mean, 3.1; median; 3.0) cm. Microscopically, the process presented as a well-circumscribed, often times pseudoencapsulated nodule (n=17) or nodules (n=1). Tumors consisted of a cellular proliferation of cytologically bland, spindled cells arranged in short fascicles and whorls within a finely collagenous(n=11) or myxocollagenous(n=7) matrix. In 12 tumors, scattered fibromatosis-like fascicles of spindled cells were noted. Histological features characteristic of the process included nuclear palisades (n=16 cases), collagenous bodies (n=15), and perinuclear intracytoplasmic hyaline globules (n=10). Mitotic activity ranged from 0 to 8 (mean,2; median, 1) mitotic figures/50 high-powered fields with no atypical division figures identified. Immunohistochemically, all tumors tested expressed (vimentin (n=3), smooth-muscle actin and/or muscle-specific actin (n=5, each), and nuclear beta-catenin and cyclin D1 (n=8, each). The latter two results prompted a screening for mutations in the beta-catenin gene glycogen synthase kinase-3 beta phosphorylation mutational “hotspot” region in exon 3 using PCR amplification and Sanger sequencing. Single nucleotide substitutions leading to

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missense mutations at the protein level were identified in 7 of 8 (88%) analyzed tumors and are responsible for the abnormal expression of beta-catenin and cyclin D1. These results demonstrate that mutational activation of the beta-catenin gene is likely a pivotal event in the pathogenesis of intranodal palisaded myofibroblastoma.

Keywords

intranodal palisaded myofibroblastoma; beta-catenin; cyclin D1, PCR; Sanger sequencing

INTRODUCTION

Among the mere handful of spindle cell mesenchymal tumors primary to lymph nodes, the intranodal palisaded myofibroblastoma (IPM), alternatively termed intranodal hemorrhagic spindle-cell tumor with amianthoid fibers¹ and solitary spindle cell tumor with myoid differentiation of the lymph node², is currently considered a histologically distinctive entity³. Occurring almost exclusively within inguinal lymph nodes, this benign tumor is composed of spindled cells with palisaded nuclei and characteristic stellate, rounded, and elongated hyalinized bundles of eosinophilic (amianthoid-like) collagen fibers that blend imperceptibly with the lesional cells.

In 1989, three separate groups provided evidence of myofibroblastic differentiation in IPM^{1,2,4} and elucidated its clinicopathologic features^{1,4}. While many of the subsequent 39 studies of IPM in the English language literature [PUBMED] essentially reaffirmed these initial findings and the tumor's predictably uneventful clinical course, others documented novel findings including occurrence of the lesion in lymph nodes outside the inguinal region^{3,5-11} and in soft tissue¹², ultrastructural evidence showing that the collagenous bodies are composed on type I collagen and not abnormally thickened amianthoid collagen fibers^{13,14}, and the addition of cyclin D1^{9,15-18}, D2-40¹¹, and factor XIIIa¹⁵ immunoexpression to the lesion's established immunohistochemical profile of muscle specific actin, smooth muscle actin, muscle myosin, and vimentin positivity^{1,4,7,8}.

Despite our present-day understanding of the salient clinicopathologic features of this process, the etiopathogenesis of the tumor remains unknown. Investigators have targeted two mesenchymal elements of the lymph node as possible cellular origins for the tumor: (1.) stromal cells with myoid features^{19,20}, which are found in greater concentration in inguinal lymph nodes compared with control lymph nodes²¹, and (2.) modified smooth muscle cells comprising the walls of hilar blood vessels^{1,7,19}. In addition, the finding that Epstein-Barr virus and herpesvirus-8 have the potential to disrupt myofibroblast proliferation pathways raised suspicion of a viral etiology for the tumor^{22,23}, but investigators have failed to unequivocally identify a viral genome^{15,24,25} or immunohistochemical evidence of a virus within lesional cells^{15,26,27}.

In this clinicopathologic study of 18 classic examples of IPM including molecular interrogation of the beta-catenin gene, *CTNNB1*, our finding of gain-of-function mutations in exon 3 of *CTNNB1* has relevance regarding the pathogenesis of this rare neoplasm.

MATERIALS AND METHODS

Mesenchymal tumors occurring within lymph nodes accessioned to the Armed Forces Institute of Pathology/Joint Pathology Center between 1970 and 2011 and coded as intranodal schwannoma/neurilemmoma, intranodal peripheral nerve sheath tumor, intranodal leiomyoma, or intranodal palisaded myofibroblastoma were retrieved. All clinical data accompanying the case and histologic slides were reviewed. Eighteen tumors with salient histological features previously delineated for IPM^{1,4}; namely (1.) light microscopic confirmation of an intranodal process, (2.) fascicular growth of cytologically bland spindled cells with myofibroblastic features exhibiting nuclear palisading, and (3.) presence of rounded, ovoid, stellate, or elongated collagenous structures, formed the core of the study. The following histological parameters were recorded for each case: (1.) location of tumor in relation to the hilum of the lymph node, (2.) characteristics of the interface between the tumor and the residual lymph node, (3.) focality of the process, (4.) cellularity, (5.) presence of nuclear palisading, (6.) presence of intracytoplasmic hyaline globules, (7.) presence of cytologic atypia, nuclear inclusions or grooves, (8.) mitotic activity (per 50 high-powered fields), (9.) stromal composition and presence of collagenous bodies, (10.) presence of intralesional hemorrhage, fibrin, or hemosiderin, and (11.) type and quantity of intralesional inflammatory cells.

Immunohistochemical Analysis and Scoring

In eight cases, where formalin-fixed, paraffin-embedded (FFPE) material was available, beta-catenin immunohistochemical testing was performed with 1:150 diluted mouse monoclonal antibody Novocastra™ clone 17c2, provided as Bond™ Ready-To-Use Primary Antibody (Leica PA0083) by Leica Biosystem (Newcastle, UK). Cyclin D1 testing was performed on the same eight tumors with 1:100 diluted rabbit monoclonal antibody, clone SP4, provided by Thermo Fisher Scientific Inc. (Rockford, IL). Immunohistochemical staining was performed on a Leica Bond-Max automatic immunostainer (Bannockburn, IL).

Remaining immunohistochemical results reported in this study were available for 11 tumors at time of slide review. As these immunohistochemical tests were performed at the former Armed Forces Institute of Pathology or at contributing outside hospitals over a time period spanning close to 30 years, the antibody clones, their sources and dilutions, and retrieval methods undoubtedly varied and are not readily traceable.

Nuclear (beta-catenin, cyclin D1, S-100 protein) and cytoplasmic staining (remaining immunomarkers) was scored as negative or positive, and semiquantitative estimates of the percentage of positive cells were recorded.

DNA extraction

The eight cases with FFPE tumor tissue were subjected to DNA extraction. One to ten 5 μ m thick sections (depending on the sample size) were deparaffinized with xylene, washed twice in ethanol, lyophilized, and incubated with 10 μ g/ml proteinase K (Roche Diagnostics, Indianapolis, IN) in Hirt-Buffer at 55°C for at least 24 hours. DNA was recovered using the

Maxwell®16 robotic system and DNA IQ™ Casework Pro Kit for Maxwell® 16 following manufacturer's protocol (Promega, Madison, WI).

PCR amplification for Sanger sequencing

CTNNB1 exon 3 was PCR amplified using AmpliTaq Gold® DNA polymerase (Applied Biosystems, Roche, Branchburg, NJ) and previously published forward 5'-TTTGATGGAGTTGGACATGG and reverse 5'-CTGAGAAAATCCCTGTTCCC primer²⁸ following standard three-temperature PCR protocol with denaturing at 95°C for 30s, annealing at 48°C for 45 seconds, and extension at 72°C for 45 seconds. 50µl PCR reactions were evaluated on 2% agarose gels. PCR products (199-bp fragments) were extracted and purified using QIAquick Gel Extraction Kit (www.qiagen.com). Sanger sequencing of these products was performed with forward and reverse primers by MacroGenUSA (www.macrogenusa.com) Obtained sequences were analyzed and aligned with *CTNNB1* reference sequence, NG-013302.1 (www.ncbi.nlm.nih.gov). All PCR and sequencing experiments were repeated at least two times.

CLINICAL RESULTS

The study cohort consisted of 14 males and 4 females ranging from 31 to 65 (mean, 47; median 49) years of age. Fifteen tumors were located in the inguinal region (9 on the right side and 6 of the left), one tumor arose in a left neck lymph node, and tumor location was not documented in the remaining two cases. Thirteen patients had clinical signs and symptoms ranging from 1 to 48 (median, 12) months before surgical intervention. Six individuals presented with a painless mass or lump in the inguinal region, while two patients experienced a painful or tender inguinal lesion. Three individuals, including a 38-year-old male with a cervical neck mass, reported gradual enlargement of the mass prior to presentation. Three patients were clinically diagnosed as having inguinal lymphadenopathy, including a 55-year-old male who presented with "matted" lymph nodes. A history of direct trauma or injury to the inguinal region was documented for four study members. Two patients, a 58-year-old male and a 45-year-old male, had an inguinal hernia repair one year or more prior to presentation with an inguinal tumor. However, there was no documentation of tumor laterality in either case. The third patient, a 61-year-old male, had a cardiac catheterization 1 month prior to presenting with a right inguinal mass, but the site of the catheter insertion was not documented. The fourth patient, a 52-year-old male with a remote history of left testicular seminoma, was clinically staged with a bipedal lymphangiogram 15 years before presenting with a right inguinal tumor. The involved lymph node was surgically excised in 16 patients, while one patient had a surgical biopsy only and the 55-year-old male with matted lymph nodes underwent core needle biopsy only.

PATHOLOGIC RESULTS

The tumors ranged from 1.0 to 4.2 cm. in greatest dimension with a mean and median size of 3.1 cm. and 3.0 cm., respectively. At scanning magnification, a single, well-circumscribed, cellular spindle cell nodule was observed within the lymph node and surrounded by a mantle of lymphocytes (Fig. 1A). In one case with a 3.5 cm. tumor, a separate 0.8 cm. nodule of tumor was found external to the lymph node capsule. The tumor

nodules had a rounded, or less often, lobulated contour. Most tumor nodules were separated from the lymph node tissue by a thin fibrous pseudocapsule (Figs. 1A,B), which in one case, had deposits of calcium. Seven tumors were in close proximity to groups of veins and arteries indicating their location near the hilum of the lymph node.

The tumor nodules were composed of a moderate to highly cellular population of spindled cells with scant, lightly eosinophilic, fibrillary cytoplasm arranged in short fascicles and whorls (Figs. 2A,B). Perinuclear vacuoles were frequently identified. The elongated cell nuclei were cytologically bland (Fig. 2B) with nuclear grooves observed in 17 tumors and intranuclear inclusions in 13. A minority of cases exhibited minimal to mild nuclear atypia with some hyperchromasia and slight variation in nuclear size. Mitotic activity ranged from 0 to 8 (mean, 2, median 1) mitotic figures per 50 high-powered fields with no atypical division figures identified. The stromal matrix was finely collagenous (n=11) or myxocollagenous (n=7) with areas of hemorrhage, extravasated red blood cells, and/or hemosiderin deposition identified in all cases (Figs. 1 and 2).

Three salient histological features typified the process and were analyzed separately. First, nuclear palisades within spindled cell fascicles were identified in 16 cases and were a prominent feature in all but three cases (Fig. 3A). Second, in 15 cases the uniform fascicular and gentle swirled arrangement of spindled cells was interrupted by a variable number of round, stellate, or elongated eosinophilic bodies composed of fine, fibrillary and centrally hyalinized collagen (Fig. 3B) and harboring calcifications in two examples. In five tumors, conglomerates of small open vessels mantled by fibrillary eosinophilic collagen blended imperceptibly with, or were incorporated into these collagenous bodies (Fig. 3C). The characteristic collagenous bodies were not identified in three cases where only one histologic slide of the tumor was available for review. Finally, small, rounded, salmon-colored, perinuclear intracytoplasmic inclusions were observed in 10 cases (Fig. 3D,E). Their presence was highlighted in one case with the Masson trichrome histochemical stain, where the inclusions appeared bright red.

Additional stromal changes included the presence of scattered thin fascicles with more evenly dispersed spindled cells within a collagenous stroma vaguely reminiscent of conventional desmoid-type fibromatosis in 12 cases (Fig. 3F), foci of keloid-like fibrosis in two tumors with metaplastic bone observed in one of the lesions, and foci of hemangiopericytomatous vessels in two cases. Intermingled with lesional tissue were mast cells (almost all cases), lymphocytes and/or plasma cells (n=11), and eosinophils (n=7). Microscopic foci of extramedullary hematopoiesis were noted in four cases.

IMMUNOHISTOCHEMICAL RESULTS

Smooth muscle actin (n=5) and muscle-specific actin (n=5) expression was identified in all eight tumors tested (Fig. 4A). Four tumors showed >50% expression in lesional cells and between 10% and 30% of cells were positive in the remaining three examples. A perimembranous accentuation of reactivity ("tram-track" pattern) was observed in three cases. The one tumor tested with calponin showed expression in approximately 80% of cells. One of eight tumors tested expressed desmin (clone DER-11) in 20% of cells. Expression of

vimentin was observed in between 10% and 90% of the three of the four tumors tested. Rare cell expressed KP1 in two of the three tumors examined. No expression was found for S-100 protein(n=10), keratin(s)(n=6), CD34(n=5), epithelial membrane antigen(n=3); CD10, CD35 and CD21(n=2, each), or collagen type IV, CD117, and CD23 (n=1, each).

Nuclear beta-catenin (Fig. 4B) and cyclin D1 (Fig. 4C) expression was observed in all eight cases tested. Beta-catenin immunoexpression was observed in nearly all tumor cells in four cases, 80% of tumor cells in one example, and 2% to 3% in the remaining three tumors. Cyclin D1 expression ranged from 10% to 80% of tumor cells with at least 50% of cells positive in three examples.

MOLECULAR RESULTS

CTNNB1 mutations were identified in 7 of 8 (88%) analyzed tumors and are listed in Table 1 along with their predicted amino acid substitutions. In codon 32, a G to C transversion (c.94G>C) was found in two tumors. A T to C transition (c.97T>C) was detected in codon 33 in one case. In codon 34, G to C and G to T transversions (c.100G>C, c.101G>T) and G to A transition (c.101G>A) were identified in three separate tumors. Codon 37 was affected by a C to G transversion (c.110C>G) in one lesion. Wild-type (WT) *CTNNB1* sequences were identified in one tumor. The results were reproducible in three independent PCR experiments. Representative example of Sanger sequencing is shown in Figure 5.

COMPARISON OF MOLECULAR AND IMMUNOHISTOCHEMICAL RESULTS

The results of molecular genetic and immunohistochemical studies are summarized in Table 1. Five of seven tumors with *CTNNB1* mutation revealed beta-catenin immunoexpression in a majority of tumor cells. Four of these cases also showed nuclear expression of cyclin D1 in at least 30% of tumor cells. Two *CTNNB1* mutants and the one *CTNNB1* WT tumor showed beta-catenin expression only in 2% to 3% of tumor cells and a concomitant reduction in cyclin D1 expression to 10%(n=2) and 20%(n=1) of tumor cells.

DISCUSSION

Although previous studies have provided a comprehensive analysis of the characteristic clinicopathologic features of IPM²⁹, the etiopathogenesis of this rare lesion remains unknown. We contend that our finding of beta-catenin gene gain-of-function mutations in exon 3 is the key to the tumor's pathogenesis.

Our clinical findings compare favorably to those presented in series reports. IPM mostly affects adults, shows a male bias, and typically presents as a painless and less often tender, slow-growing inguinal mass^{1,4,7,8,29}. The process is benign with no reported cases exhibiting destructive invasion or metastasis to date.

All but two study members with known tumor location had a single inguinal lymph node-based mass. One individual diagnosed on core needle biopsy presented with matted inguinal lymph nodes suggesting the possibility of multicentric tumors, a phenomenon only rarely documented in the literature^{30,31}. The other patient had a lesion involving a cervical neck

lymph node. This case represents only the third reported example of an IPM arising in this location^{7,11}.

Our histological findings in IPM confirm and extend the work of others. Similar to previous reported microscopic findings^{1,4,5,7,8,10,16,24–27,32–34}, tumors in this study grew as small, well-circumscribed, often times pseudoencapsulated intraparenchymal nodules composed of benign-appearing spindled cells that frequently possess perinuclear vacuoles and exhibit nuclear palisading within tumor fascicles. One histological feature not previously documented is the focal presence of thin fascicles of rather evenly dispersed tumor cells within a collagenous stroma imparting a growth pattern vaguely reminiscent of desmoid-type fibromatosis.

In seven cases in our study, lesional tissue was juxtaposed to thick-walled veins suggesting a relationship of the tumor to hilar vessels. Michal et al.⁷ detected tumor cells blending with veins located outside the pseudocapsule of the lesion, but they did not specifically localize the veins to the hilum. Suster et al.¹ postulated a hilar origin for IPM based on the compressive effect on the peripheral nodal cortex by the expansile tumor nodule in their series.

We observed hyalinized collagenous bodies in all but three cases, where limited material for review could have resulted in a falsely negative result. As documented by others^{1,25,35}, we found aggregates of small vessels with thick mantles of hyalinized collagen merging with these structures raising the possibility that their origin is from condensation of perivascular collagen.

Intracytoplasmic hyaline globules, first described by Weiss et al.⁴ in IPM, were identified in 56% of our tumors. Studies report that trichrome histochemical staining^{4,5,7,8,36} and anti-actin immunostaining^{5,7,8,16,37} highlight the often times inconspicuous inclusion. Not restricted to IPM, the hyaline globule is a well-recognized hallmark of another myofibroblastic process, the infantile digital fibroma/fibromatosis³⁸, and has also been described in a number of pathologically diverse myofibroblastic and smooth muscle proliferations³⁹. The inclusion is believed to result from perturbations in the assemblage of actin filaments within the cytoplasm of myoid cells³⁹. Michal et al.³⁶ showed ultrastructurally that the fully developed globule in IPM consists of tightly packed actin microfilaments and, like Weiss et al.⁴, noted a higher concentration of these inclusions adjacent to the collagenous bodies.

The immunohistochemical expression of muscle actin(s) in our series is similar to previously reported results^{1,4,7,8}. We did find focal desmin expression in one tumor. Although typically not expressed in IPM, Hisaoka et al.³³ reported focal desmin expression in one example of IPM. Not surprisingly, calponin was strongly expressed in the one of our tumors tested but, to our knowledge, this result has heretofore not been reported.

In this study, nuclear expression of beta-catenin and cyclin D1 was detected immunohistochemically in all analyzed IPMs. Beta-catenin represents the terminal component of the canonical Wntless/Wnt signaling pathway. Several different mechanisms including mutational activation of *CTNNB1* can lead to aberrant Wntless/Wnt

activation^{40,41}. Beta-catenin gene activating mutations cluster in exon 3 and target codons 33, 37, and 45 affecting serine phosphorylation sites, codon 41 affecting a threonine phosphorylation site, or flanking codons creating “missense” mutations⁴². These altered amino acid residues are not recognized by the serine/threonine kinase, glycogen synthase kinase-3 beta (GSK3 β), and therefore phosphorylation of these key sites necessary for targeting the protein for ubiquitin-dependent, proteasome-mediated degradation does not occur^{42,43}. The stabilized beta-catenin protein accumulates in the cytoplasm and then translocates to the cell nucleus, where it forms bipartite complexes with, and activates T cell factor/lymphoid-enhancing factor (TCF/LEF) leading to up-regulation of TCF/LEF-responsive target genes, including *CCND1*^{40,41,44,45}. This sequence of events results in Immunohistochemical overexpression of cyclin D1, which has been documented in a number of neoplastic processes exhibiting *CTNNB1* mutations and nuclear expression of beta-catenin^{46–56}.

CTNNB1 exon 3 mutations were found in seven of our cases. In two tumors, mutations leading to substitution of serine (p.S33L and p.S37C) were identified. In five other cases, “missense” mutations were found at neighboring codons, 32 (p.D32H) and 34 (p.G34A, p.G34E, p.G34V), which potentially could alter protein structure and block its degradation. The mutations reported in this study are similar to those identified in a number of epithelial and neuroectodermal tumors (a complete list of references are available through the COSMIC, the catalogue of somatic mutations in cancer, at <http://cancer.sanger.ac.uk>), but appear rare in mesenchymal neoplasms.

Nuclear expression of beta-catenin has been reported in various mesenchymal tumors⁵⁷, but only in desmoid-type fibromatosis and rare cases of synovial sarcoma and endometrial stromal tumor has this phenomenon been linked to gain-of-function mutations in the *CTNNB1* GSK3 β region^{28,46,58}. To date, mutations affecting *CTNNB1* exon 3 codons 33 and 37 and flanking codon 32 as identified in this study have only been reported in an endometrial stromal nodule⁵⁸, synovial sarcoma⁴⁶, and in a case of malignant fibrous histiocytoma (undifferentiated pleomorphic sarcoma)⁵⁹. In contrast, desmoid-type fibromatosis is characterized by molecular changes in codon 41 and codon 45, whereas mutations affecting codons 32, 33, 34 and 37 are extremely rare^{28,60,61}.

Only one tumor coexpressing beta-catenin and cyclin D1 was *CTNNB1* exon 3 WT. Besides the possibility of a false-negative molecular result, this discordance could be explained by a *CTNNB1* deletion that extends from the GSK3 β region into intron 2⁶². Such a deletion could potentially eliminate 5' priming sequence of PCR assay designed to amplify short products from FFPE tissues and thereby prevent PCR amplification of the mutant allele.

Beta-catenin was diffusely expressed in all but two of the tumors tested. As these two cases also showed reduced cyclin D1 expression, technical limitations related to antigen preservation of FFPE tissue could provide an explanation. Alternatively, *CTNNB1* mutants have been identified that lack nuclear beta-catenin expression²⁸.

The reason IPM predilects to inguinal lymph nodes remains an enigma. Unidentified mutagenic factors triggered by trauma, vascular stasis, or inflammation affecting inguinal

lymph nodes may very well contribute to the tumor's etiopathogenesis. Indeed, four study patients experienced inguinal region trauma and one case of IPM with antecedent inguinal trauma appears in the literature³². Additionally, it is well established that trauma stimulates growth of sporadic and syndromal desmoid-type fibromatosis^{63,64}.

In conclusion, our finding of *CTNNB1* mutations in tumor cells of IPM sheds new light on the pathogenesis of this rare neoplasm. Based on this study, the IPM can now be added to the ever-expanding list of mesenchymal and epithelial neoplasms driven by gain-of-function *CTNNB1* mutations.

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Figure 1.

A, Scanning magnification of intranodal palisaded myofibroblastoma demonstrates a well-circumscribed, spindle cell nodule surrounded by a mantle of lymphoid tissue. B, Intranodal palisaded myofibroblastoma (bottom) is separated from the surrounding lymph node tissue (top) by a fibroconnective tissue pseudocapsule.

Figure 2.

A, Low-power magnification of the lesion exhibits a cellular proliferation of cytologically bland spindled cells arranged in loose whorls and fascicles with extravasated red blood cells.

B, Cells of intranodal palisaded myofibroblastoma have scant, fibrillary, eosinophilic cytoplasm and elongated, cytologically bland nuclei.

Figure 3.

Histological features that typify intranodal palisaded myofibroblastoma (A–E). A, Fascicles of spindled cells exhibiting nuclear palisading. B, Elongated and stellate, centrally hyalinized collagenous bodies. Inset shows high-power image of collagenous bodies. C, Numerous small vessels with abundant perivascular collagen are in close proximity to, and focally merge with an elongated collagenous body. D, Spindled tumor cells harboring pale paranuclear hyaline globules (arrowheads). E, High-power of rounded, salmon-colored

intracytoplasmic globules. F, Desmoid fibromatosis-like fascicle (identified by arrowheads) composed of more evenly distributed spindled cells within a collagenous stroma.

Figure 4.

Immunoprofile of intranodal palisaded myofibroblastoma. A, Smooth muscle actin expression in a perimembranous (“tram-track”) pattern. Smooth muscle or muscle-specific actin immunoexpression was observed in all tumors tested. B, Nuclear expression of *CTNNB1* encoded protein, beta-catenin, and Wnt pathway downstream target protein, (C) cyclin D1, was present in all eight tumors analyzed.

Figure 5.

Examples of *CTNNB1* exon 3 sequences obtained by PCR amplification of DNA from intranodal palisaded myofibroblastoma and direct sequencing of PCR products. Arrows indicate single nucleotide substitutions: c.94G>C in codon 32 (A), c.97T>C in codon 33 (B), c.100G>C in codon 34 (C), and c.110C>G in codon 37 (D). At the protein level, these substitutions will result in the following mutations: p.D32H, p.S33L, p.G34A and p.S37C.

Table 1

CTNNB1 mutations and Beta-catenin and Cyclin D1 expression in intranodal palisaded myofibroblastoma.

Case	Age(yrs)/Sex/ Lymph node site	<i>CTNNB1</i> DNA mutation ⁺⁺ #	Predicted <i>CTNNB1</i> amino acid substitution ⁺⁺ #	Beta-catenin expression (%) [*]	Cyclin D1 expression (%) [*]
1	35M/NA	c.94G>C	p.Asp32His	100	60
2	50M/Ing	c.94G>C	p.Asp32His	100	30
3	35M/Ing	c.97T>C	p.Ser33Leu	100	50
4	52M/Ing	c.100G>C	p.Gly34Ala	3	10
5	58M/Ing	c.101G>A	p.Gly34Glu	2	10
6	38F/Ing	c.101G>T	p.Gly34Val	80	80
7	38F/Neck	c.110C>G	p.Ser37Cys	100	10
8	56F/Ing	WT	WT	3	20

Abbreviations:

⁺ - number refers to first nucleotide affected;

[#] - *CTNNB1* mutation and predicted *CTNNB1* mutant sequences described as recommended by Human Genome Variation Society (www.hgvs.org);

⁺⁺ - number refers to exon 3 codon affected;

^{*} - percentage of cells with nuclear staining (positive); M-male; NA-data not available; Ing-inguinal region; F-female; WT-wild-type sequence