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Original contribution

Characterization of the leiomyomatous variant of myofibroblastoma: a rare subset distinct from other smooth muscle tumors of the breast☆,☆☆

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Summary Mammary myofibroblastoma is a benign spindle cell tumor that can show variable morphologic patterns and lines of differentiation. Myofibroblastoma belongs to a family of CD34-positive tumors with similar morphology that show a deletion of 13q14, which includes *RB1* and *FOXO1A* genes. A subset of these tumors demonstrates distinct smooth muscle differentiation. We aimed to characterize 4 cases of the leiomyomatous variant of myofibroblastoma arising in the breast by clinicopathological, immunohistochemical, and molecular means. All 4 examples arose in women aged 41 to 62 years (median, 46.5 years). Tumors ranged in size from 1.7 to 2.5 cm (median, 2.2 cm). Morphologically, all tumors were characterized by bundles of smooth muscle cells with elongated cigar-shaped nuclei and eosinophilic cytoplasm. All 4 tumors showed diffuse positive staining with desmin, caldesmon, smooth muscle actin, estrogen receptor, and Bcl-2. CD34 staining was diffusely positive in 2 cases, was weak and patchy in 1 case, and was negative in 1 case. Two (50%) of 4 tumors showed deletion of *RB1* by fluorescence in situ hybridization. Loss of Rb staining was seen in 1 tumor with *RB1* deletion by fluorescence in situ hybridization, whereas intact Rb staining was observed in 1 nondeleted case studied. In conclusion, this rare variant of myofibroblastoma is a distinct subgroup of tumors among an already uncommon category of (smooth muscle) breast tumors. Some reported examples of "parenchymal leiomyoma" may represent the leiomyomatous variant of myofibroblastoma.

1. Introduction

Tumors of smooth muscle derivation are exceedingly rare in the breast, largely in part due to the absence of indigenous smooth muscle in this anatomic site, with the exception of the nipple. Still, reports of benign smooth muscle dominant tumors such as leiomyomas and myoid (or "muscular") hamartomas have been described in the literature [1-11]. In addition,

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other benign mammary entities are known to occasionally demonstrate smooth muscle differentiation to varying degrees such as fibroadenomas, phyllodes tumors, and myofibroblastomas. Because of the rarity of these tumors, a critical review of benign mammary smooth muscle tumors has not been performed. This invariably perpetuates the ambiguity of not only classifying them in practice but also understanding them as part of distinct subgroup possessing specific clinical, morphologic, immunohistochemical, and/or molecular features.

Mammary myofibroblastoma is a benign stromal tumor characterized by bland spindle cells growing in fascicles with intervening bands of collagen and variable amounts of fat. Various morphologic variants have been described, including cellular, infiltrating, epithelioid, and myxoid, among others, and some show heterologous differentiation in the form of mature chondroid, osseous, or leiomyomatous components [12,13]. In recent years, myofibroblastoma and morphologically similar nonmammary CD34-positive tumors including (extra) mammary-type myofibroblastoma, spindle cell lipoma, and cellular angiofibroma of vulva have been found to share a deletion of 13q14, which includes *RB1* and *FOX01A* genes [14-18]. In reported cases of mammary myofibroblastomas specifically, deletion of 13q14 has been found in 78.5% (11/14) of examples studied [14,16,18-21]. Identifying this common deletion in CD34-positive tumors motivated us to better characterize a rare variant of myofibroblastoma that can be nonimmunoreactive for CD34—considered a hallmark of mammary myofibroblastomas, which in its absence may pose a diagnostic pitfall. Moreover, we aimed to better understand how the leiomyomatous variant of myofibroblastomas fits in the overall context of benign smooth muscle tumors in the breast.

2. Materials and methods

2.1. Case selection

This study was conducted under an institutional review board—approved protocol. Four cases of mammary myofibroblastoma with leiomyomatous differentiation were identified from our surgical pathology and breast consultation files. Slides were reviewed by 2 breast pathologists (T. D., S. S.). Clinical data were obtained from electronic patient records and from submitting pathologists and clinicians.

2.2. Immunohistochemistry

Immunohistochemical staining was accomplished using the Bond III Autostainer (Leica Microsystems, Buffalo Grove, IL) (Table 1). Formalin-fixed, paraffin-embedded whole-tissue sections were first baked and deparaffinized. For antigen retrieval, slides were heated on the Bond III Autostainer at 99°C to 100°C. Sections were then subjected to sequential incubation with the endogenous peroxidase block, primary antibody, postprimary (equivalent to secondary antibody), polymer (equivalent to tertiary antibody), 3,3′-diaminobenzidine, and hematoxylin. Finally, the sections were dehydrated in 100% ethanol and mounted in Cytoseal XYL (Richard-Allan Scientific, Kalamazoo, MI). Appropriate positive and negative controls were included.

Table 1 Antibodies, retrieval methods, dilutions, and sources of immunohistochemical stains used in this study

Antibody	Clone	Retrieval	Dilution	Source
CD34	QBEND/10	H2: 20 min	RTU	Leica, Buffalo Grove, IL
Desmin	D33	H1: 10 min	1:30	Dako, Carpinteria, CA
ER	6F11	H2: 30 min	1:50	Novocastra, Buffalo Grove, IL
SMA	1A4	NT	1:200	Dako, Carpinteria, CA
Caldesmon	h-CD	H2: 20 min	1:100	Dako, Carpinteria, CA
Bcl-2	124	H2: 20 min	1:50	Dako, Carpinteria, CA
Rb	4H1	H2: 20 min	1:100	Cell Signaling, Danvers, MA

Abbreviations: H1, heated in Bond Epitope Retrieval 1; H2, heated in Bond Epitope Retrieval 2; NT, no treatment; RTU, ready to use.

2.3. Fluorescence in situ hybridization analysis

Dual-color interphase fluorescence in situ hybridization (FISH) was performed on 5-μm-thick paraffin whole-tissue sections from selected blocks using the following probes: *RB1* (red) and a control probe on 10q (green). The *RB1* probe was made from BAC RP11-305D15 DNA, and the control probe was made from BAC RP11-431P18 (BACPAC Resource Center, Children's Hospital Oakland Research Institute, Oakland, CA) as per standard methods. Cutoff value for *RB1* deletion was determined by analyzing 5 normal formalin-fixed, paraffin-embedded tonsil samples. The cutoff values were calculated using the beta inverse function formula (=BETAINV[CONFIDENCE LEVEL,#FALSE POSITIVE+1, #CELLS SCORED]) on an Excel spreadsheet. A sample was considered to demonstrate *RB1* deletion if at least 18.5% cells showed allelic deletion of the *RB1* gene. A minimum of 100 nonoverlapped intact (uniform DAPI staining with intact nuclear contours) interphase nuclei of consecutive cells in at least 2 different areas of the section were scored. A hematoxylin and eosin (H&E)–stained section was used to verify the presence of tumor. Spindle cell lipomas of soft tissue served as positive controls for *RB1* deletion.

3. Results

A summary of the clinicopathological, immunohistochemical, and cytogenetic features of studied cases is outlined in Table 2.