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SHORT COMMUNICATION

VIA MEDICA

Role of immunohistochemical and histochemical profiling in H&E-based diagnosis of scrotal leiomyosarcoma of dartos muscle

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Abstract: Histochemical and immunohistochemical methods should often but not always complement standardized histopathologic procedures. Here, we illustrate use of these ancillary techniques in a report of scrotal leiomyosarcoma. 62-year-old male patient presented with a palpable, subcutaneous 2,5 cm wide tumor arising from dartos muscle. The tumor was diagnosed leiomyosarcoma G2 pT1b. Interestingly, the sarcomatous mass was focally strictly attached to convoluted, benign bundles of smooth muscles that were intermingled with tumor mass at peripheral, lateral and superior sides of the lesion. We have used immune- and histochemical methods to confirm histopathological findings based on H&E staining. As expected, in tumor cells smooth muscle actin and desmin were strongly immunopositive similarly as Masson trichrome staining, while S100 and CD34 antigens were immunonegative except for sustained positivity for CD34 in vessels. The auxiliary staining methods can provide additional information on the tumorigenesis of leiomyosarcoma. They can also serve to determine additional features of prognostic significance, since *e.g.* immunoreactivity of CD34 accurately maps vascular density of tumor and enables a careful assessment of vascular invasion in course of leiomyosarcoma as well. *(Folia Histochemica et Cytobiologica 2013, Vol. 51, No. 4, 339–342)*

Key words: leiomyosarcoma; dartos muscle; Masson trichrome staining; αSMA, desmin; CD34; origin of tumor

Introduction

Leiomyosarcomas were not only reported in a paratesticular region, but also inside testes [1]. Although they are usually found in an inguinal area without coexistent infectious diseases, they can also develop in the background of unspecified, bilateral epididymo-orchitis or even scrotal filariasis [2, 3]. In opposition to rhabdomyosarcomas that comprise almost half of all paratesticular malignancies, leiomyosarcoma is a rare entity in this location. This neoplasm can arise from any smooth muscle layers that are normally found in this region, *e.g.* cremasteric muscle of spermatic cord [4, 5]. Another site of potential origin for leiomyosarcoma is a dartos muscle layer, which closely

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adheres to the skin of scrotum [6]. If this tumor grows in such a superficial location, it is classified as a cutaneous leiomyosarcoma. Leiomyosarcoma of dartos muscle was reported to have a very good prognosis with no recurrence after 36 months from the date of surgical excision [6].

Here, we report an educational example of scrotal leiomyosarcoma, arising from a dartos muscle of scrotum with a special impact on histochemical and immunohistochemical profiles as well as basic histopathological traits that turned out to be sufficient clues for defining tumor's origin.

Material and methods

A 62-year old man presented with a palpable 2,5 cm-in-diameter tumor of scrotal sac that grew for a year and a half and produced an intermittent pain in the last three months before tumorectomy. In gross post operative examination the ovoid solid tumor was connected with a band of an overlying skin that was 0,4 cm in width. On cross section, a grayish, whorled appearance of the tumor was discerned.

The postoperative material was immediately fixed in 10% neutral buffered formalin and routinely processed for histology. Formalin-fixed and paraffin-embedded tissues were sectioned into 5 mm-thick sections and then stained according to the standard procedure with hematoxylin and eosin (H&E) and with Masson trichrome method. After examination of H&E microscopic slides, chosen specimens were subjected to routine immunohistochemical staining. After examination of H&E slides, chosen specimens were subjected to routine immunohistochemical staining for SMA, desmin, CD34 and S100. For evaluation of smooth muscle actin (SMA), monoclonal mouse, anti-human, anti-SMA, primary antibody was applied at dilution 1:50 for incubation at room temperature for 30 min. (Clone 1A4, Code M0851, Dako Denmark, Glostrup, Denmark). The unspecific tissue peroxidase activity was inhibited by preincubation for 5 min. with 3% hydrogen peroxide. Heat-induced epitope retrieval was achieved within 20 minutes after exposing the slides to 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0 for 20 minutes at sub-boiling temperature up to 100°C. Dako LSAB™ +/HRP kit (code K0679) was used to visualize antigen-antibody complexes. S100 protein was detected with a primary, lyophilized, rabbit, polyclonal antibody (product code: NCL-S100p, Leica Biosystems, Newcastle Upon Tyne, UK) at dilution of 1:400 during incubation for 1 hour at room temperature. For evaluation of expression of others markers we used primary antibodies at dilution of 1:50 for 60 minutes at room temperature: desmin (Novocastra™ liquid mouse monoclonal antibody, product code: NCL-L-DES-DERII, clone DE-R-11, Leica Biosystems) and CD34 (product code: NCL-END, clone QBEnd/10, Novocastra™ lyophilized mouse monoclonal antibody, Leica Biosystems). In these cases trypsin digestion of paraffin sections was applied at 37°C for 30 minutes and visualization followed with avidin/biotin complex-horseradish peroxidase (ABC-HRP method). In all immunohistochemical protocols slides were exposed to 3,3'-diaminobenzidine tetrahydrochloride (DAB, Dako) used as chromogen for 5 minutes to produce final color reaction. All slides were counterstained with hematoxylin. Positive controls for presented stainings comprised wall of large intestine (muscularis propria for smooth muscle markers and Auerbach's and Meissner's plexuses for S100) and tonsil tissue (for CD34 expression in vascular elements).

Results

The tumor was classified as a high grade sarcoma, diagnosed leiomyosarcoma G2 and staged as pT1b according to 7th edition of pTNM, issued by the American Joint Committee on Cancer (AJCC).

The tumor was removed with a very thin, discontinuous margin of an uninvolved tissue and the tumor tissue was focally present in an inked line of a surgical cutting surface. Thus, the excision was reported to be incomplete. The sarcomatous texture was focally strictly attached to convoluted bundles of smooth muscle that were intermingled with tumor mass at lateral and superior side of the lesion (Figure 1A). At the interface between surroundings and tumor periphery there was gradual increase in atypia of cells from normal histological appearance of myocytes to malignant spindle cells of the sarcoma. It gave an undoubted impression that leiomyosarcoma truly grew out of dartos muscle layer. The tumor was composed of highly atypical, spindle cells that were streamed into intersecting long fascicles (Figure 1B and 1D). Necrosis comprised 8% of a sectioned tumor surface in all examined microscopic specimens. A mitotic index was at the level of 10 mitoses per 10 high power fields (Figure 1C) with the presence of atypical figures.

Although in this case histochemistry and immunohistochemistry was not necessary for definition of histopathological type of the tumor, it was applied to confirm basic findings in H&E slides. Namely, Masson's trichrome stain produced mostly red color both in the tumor and neighboring smooth muscle bundles (Figure 1A). CD34 (Figure 2B) was immunonegative except for a sustained positivity for CD34 in a network of vessels. Smooth muscle actin (SMA) and desmin stainings (Figure 2C and 2D, respectively) were strongly positive while S100 (not shown) and CD34 (Figure 2B) were immunonegative except for a sustained positivity for CD34 in a network of vessels.

Discussion

Cutaneous leiomyosarcomas are believed to originate from the dartos or arrector pili smooth muscles [7]. An

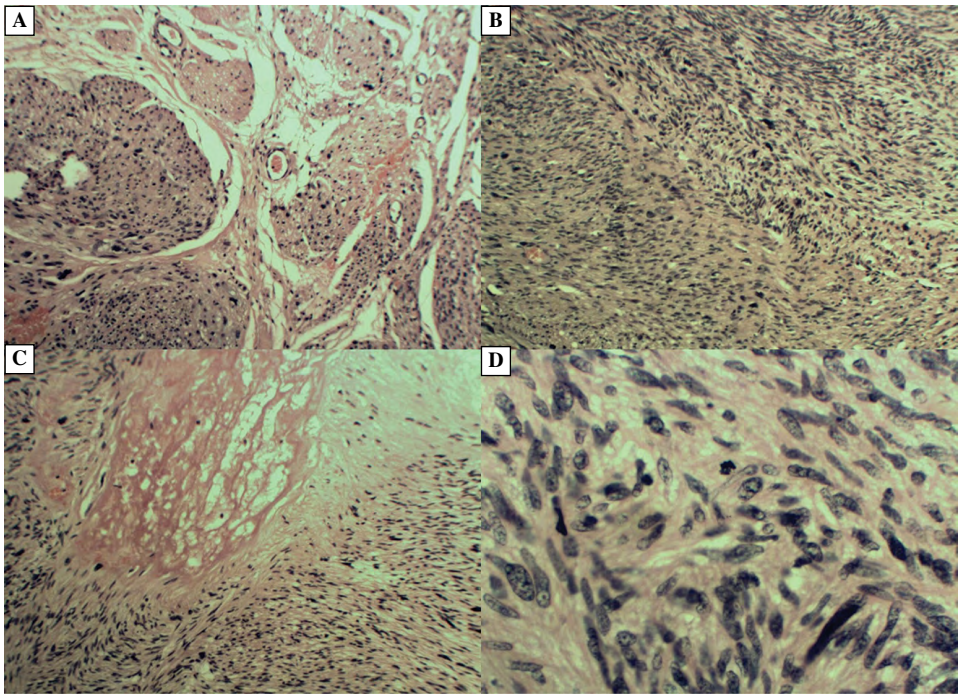

Figure 1. Basic histopathological findings in scrotal leiomyosarcoma (H&E staining). **A.** The outgrowth of leiomyosarcoma from bundles of dartos smooth muscle fibers (× 100). **B.** Intersecting fascicles of leiomyosarcoma (× 100). **C.** Presence of necrosis within malignant texture of the tumor (× 100). **D.** Highly atypical sarcoma cells arranged in fascicles (× 400)

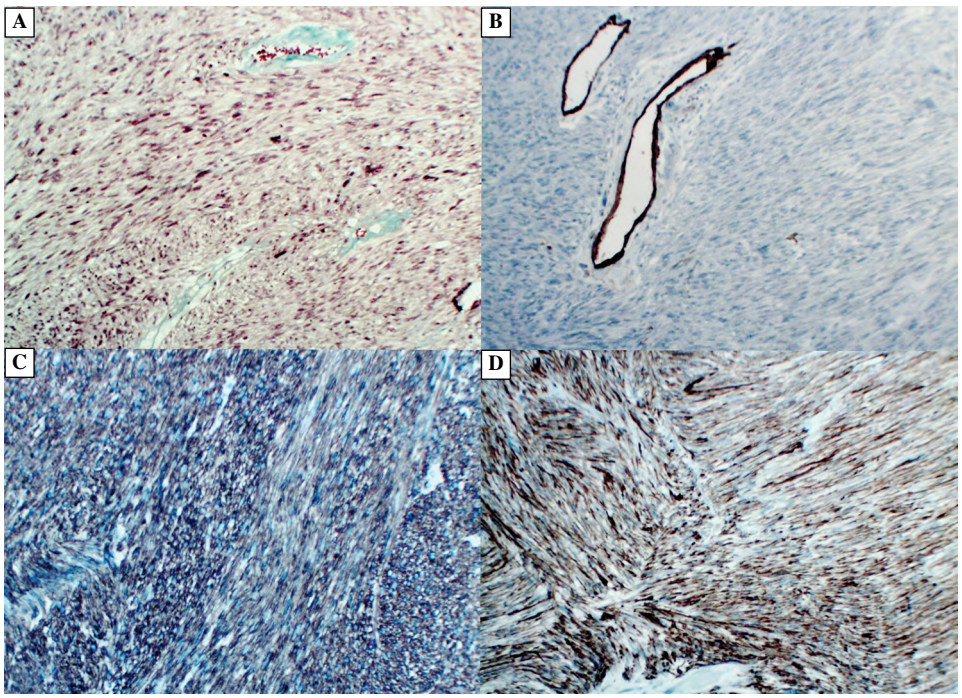

Figure 2. The histochemical and immunohistochemical (IHC) profile of leiomyosarcoma. **A.** Masson's trichrome staining of the tumor texture (× 100). **B.** CD34 immunohistochemistry (IHC): lack of immunoreactivity in leiomyosarcoma cells except for network of vessels (× 100). **C.** Smooth muscle actin IHC: very strong immunoreactivity (× 200). **D.** Desmin IHC: strong immunoreactivity (× 100)

origin for such a primary leiomyosarcoma is limited to sites of dermal occurrence of smooth muscle tissue that include also tunica muscularis of skin vessels [8]. Whenever completely removed, these superficial lesions are characterized with such a good prognosis that an inguinal lymphadenectomy usually is not recommended [7]. However, long-term follow-up is very important because it is not possible to exclude a late, local recurrence or metastasis [7]. Atypical smooth muscle tumors with high mitotic index and necrosis are obviously classified to leiomyosarcoma group, but sometimes smooth muscle tumors with bizarre cells are found in a scrotal region. They are an equivalent of symplastic or atypical leiomyoma of the uterine corpus and, identically, they are of exclusively benign nature [9]. Scrotal leiomyosarcoma could grow in various histopathological variants (*e.g.* epithelioid leiomyosarcoma), but it is believed that it does not affect prognosis of these tumors more than grading or staging [10]. Tumor grading strictly affects prognosis since well differentiated, low grade (G1) leiomyosarcomas are completely curable by local excision, while all poorly differentiated (G3) leiomyosarcomas resulted in death of the patients [11].

Additional methods are sometimes necessary for definition of tumor's histopathological type. In spite of the fact, that origin of the tumor from an adjacent muscle was clearly visible, we performed additional morphologic evaluation by applying histo- and immunohistochemistry in the illustrated case. As expected, smooth muscle markers were immunopositive and other markers immunonegative in tumor cells. However, in one study muscle-specific actin was negative in 1 case of 14 examined tumors, SMA was detected in all stained neoplasms, desmin failed to be expressed in 1 case of 17 tumors, while CD34 was shown to be confusingly positive in 3 out of 9 examined tumors [11]. Moreover, the same authors described even a focal positivity to cytokeratin in 3 of 8 tumors and detected S100 protein in one neoplasm [11]. Therefore, immunohistochemistry should be applied with a considerable caution, but this technique is still of a great value in formulating the final diagnosis. It is also worth to mention that SMA positive immunoreactivity is often not sufficient to confirm that tumor is leiomyosarcoma, as other sarcomas have been also described to be SMA-positive, *e.g.* paratesticular myxofibrosarcoma [12]. In comparison, desmin appears to be much more specific for leiomyosarcoma and is probably the most helpful in the immunohistochemical evaluation of muscle differentiation in case of leiomyosarcoma and rhabdomyosarcoma. Besides utility in definition of tumor's histopathological type, the immunohistochemical methods provide additional information on histogenesis of neoplasm that could substantially contribute in further studies on tumorigenesis of leiomyosarcomas. They can also serve to determine additional features of prognostic significance. For instance, CD34 maps accurately vascular density of tumor and enables a careful inspection of a possible vascular invasion in the course of leiomyosarcoma, as well.

To sum up, the demonstrated case shows that classical H&E staining may be sufficient to reveal the origin of the tumor, if its outgrowth is clearly visible from closely adhering, adjacent tissues. The use of auxillary histochemical and immunohistochemical methods can be recommended to obtain a detailed and precise histopathological report.

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