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Teaching cases

Complex karyotype in a case of cutaneous lymphangiosarcoma associated with chronic lymphedema of the lower limb

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ABSTRACT

Lymphangiosarcoma is a rare malignant neoplasm of endothelial cells. The term is used to describe an angiosarcoma associated with chronic lymphedema. The skin of the head and neck region is the most common site of origin. Rather few cytogenetic studies on lymphangiosarcoma are reported in the literature. We here describe a case of an 87-year-old woman, with a history of recurring lymphangitis and with an ulcerated nodular lesion of the leg. The histological diagnosis was a malignant neoplasm of vascular origin, with the morphological and immunohistochemical features of a lymphangiosarcoma. A series of antibodies (CD31, CD34, vimentin, podoplanin and HHV-8), conventional and molecular cytogenetic and Spectral Karyotyping (SKY-FISH) analyses were used to study this case.

The immunohistochemical evaluation revealed that the neoplasm was positive for vimentin, CD31, CD34 and podoplanin and negative for HHV-8. The proliferation rate (Ki-67) was about 70%. Karyotype was defined using conventional cytogenetic and SKY-FISH. In addition, high-level of amplification was observed with MYC split signal probe.

The morphological and immunohistochemical evaluations supported the diagnosis of lymphangiosarcoma. Moreover, the cytogenetic and molecular findings contributed towards accurately defining the karyotypic aberrations of this rare sarcoma.

Introduction

Cutaneous lymphangiosarcoma is a rare malignant neoplasm of endothelial cells that accounts for less than 1% of all sarcomas [4]. A reliable distinction between lymphangiosarcoma and sarcoma with blood vascular differentiation is currently not feasible, however, lymphangiosarcoma is the term applied to an angiosarcoma associated with chronic lymphedema [4,19]. This latter entity has been more frequently described in association with chronic lymphedema of the upper limb after mastectomy and radiotherapy for breast carcinoma [8,23,24]. In addition, it can arise in chronic, congenital or acquired, lymphedema of the lower limb [1]. These forms are considered as secondary angiosarcomas in contrast with de novo or primary forms.

Lymphangiosarcoma usually arises in elderly individuals and the prognosis is generally poor, with only 15% of patients being alive five years after diagnosis and treatment [19].

In order to differentiate lymphangiosarcoma from hemangiosarcoma, a number of lymphatic markers for immunohistochemistry have been recently proposed [13]. These include antibodies directed against Prox-1, vascular endothelial growth factor-3 (VEGFR-3), lymphatic vessel hyaluronan receptor-1 (LYVE-1) and podoplanin (recognized by the D2-40 clone). In particular, in the distinction between lymphatic and hematic endothelia podoplanin showed high specificity [2,11,13]. Furthermore, Mankey et al. [13] observed an association between D2-40 immunoreactivity and a hobnail pattern of malignant cells, suggesting that the concept of lymphangiosarcoma, originally based upon clinical findings, may evolve into a pathological definition based on morphological ground.

Here we report the morphological and immunohistochemical features of a rare case of cutaneous angiosarcoma of the leg. In addition, we performed on this case both a conventional and a molecular

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cytogenetic analysis and compared our results with a review of the previously reported data.

Materials and methods

Histopathology and immunohistochemistry

We fixed tumor samples in buffered formalin (formaldehyde 4% w/v and acetate buffer 0.05 M) and routinely processed them to paraffin wax. Serial sections were stained with hematoxylin and eosin (H&E) for the histopathological evaluation.

For immunohistochemistry, 3 μm-thick sections were mounted on poly-L-lysine coated slides, deparaffinized, followed by the avidin–biotin complex (ABC) procedure according to Hsu et al. [9]. Immunoreactions were counterstained with Harris' hematoxylin. The antibodies used were CD31 (Dako, Glostrup, Denmark, JC/70A, 1:20), CD34 (Novocastra, Newcastle, UK, QBE nd/10, 1:1), vimentin (Dako, Glostrup, Denmark, V9, 1:80), podoplanin (Abcam, Cambridge, UK, D2-40, 1:80), HHV-8 (Novocastra, Newcastle, UK, 13B10, 1:50) and Ki-67 (Dako, Glostrup, Denmark, MIB-1, 1:100).

Cytogenetic analysis

A fresh tumor sample of the angiosarcoma was minced into small pieces, incubated and processed according to the method described by Tibiletti et al. [25]. Conventional chromosome analysis was performed on direct preparations incubated for 24 h. Slides were QFQ-banded and analyzed according to the 2013 ISCN recommendations [21]. Spectral karyotyping FISH (SKY-FISH) was performed as previously described by Calabrese et al. [3]. In addition fluorescence in situ hybridization (FISH) with MYC split signal probe (Dako Italia S.p.a., Milano) on paraffin embedded tissue following the procedure described by Tibiletti et al. [26] was performed.

Results

Clinical case report

An 87-year-old woman, with a history of hypertension, phlebitis of the lower limbs, recurring lymphangitis and a pelvic fracture, presented with an ulcerated nodular lesion of the right leg, with a greatest diameter equal to 1.3 cm, which was biopsied and diagnosed as angiosarcoma. After a few days, a secondary lesion appeared on the skin of the right thigh, associated with a septic state, so the patient was treated with amputation of the lower limb; she did not receive chemotherapy or radiotherapy, due to her age and her general condition. After two months, she was hospitalized again because she had developed lung and pleural metastases and, after a few days, she died. The patient did not receive any therapeutic irradiation. There was no post-mortem examination.

Histopathology and immunohistochemistry

At microscopic evaluation, we observed a proliferation of anastomizing and dilated vascular structures with irregular lumina lined by crowded atypical endothelial cells (Fig. 1A), focally arranged in papillary processes and with an evident hobnail pattern. Solid areas were also present. Neoplastic cells were spindle and epithelioid, with enlarged hypercromatic nuclei and cytoplasmatic vacuoles resembling primitive vascular lumina. Mitotic index was high (46 mitoses/10 HPF).

By immunohistochemistry, tumor cells were positive for vimentin, CD31 and CD34 (Fig. 1B) and a strong immunoreactivity for anti-podoplanin was also observed (Fig. 1C). Anti-HHV-8 immunoreaction was negative. The proliferation rate, evaluated with anti-Ki-67 antibody, was about 70% (Fig. 1D).

The final histopathological diagnosis was cutaneous lymphangiosarcoma secondary to chronic lymphedema.

Cytogenetic results

Conventional cytogenetic study revealed a complex karyotype with the presence of a derivative from chromosome 3, gain of chromosome 21 and a ring chromosome; the neoplastic karyotype was defined as:

47–49,XX,der(3)t(3;?)(q27;?),+21,+r.

High-level of amplification was observed with MYC split signal probe.

SKY-FISH on metaphases from direct preparations allowed the definition of the final karyotype of lymphangiosarcoma as:

49,XX,der(3)t(1;3)(q12;q22),+der(8)(?),t(16;20)(q22;p12?),+20, +21.nuc ish(MYC amp) (Fig. 1E).

Discussion

We reported a rare case of cutaneous lymphangiosarcoma of the leg, including morphological and cytogenetic features. The tumor arose in a patient with a long dated history of chronic lymphedema, confirming that this condition is a risk factor for the development of lymphangiosarcoma [1,22,23,29]. The histopathological and immunohistochemical profile of the neoplastic cells were fully consistent with those of an angiosarcoma. The lack of HHV-8 ruled out a Kaposi's sarcoma. The strongly positive immunostaining for podoplanin, was a further support to the lymphatic origin of this neoplasm. According to Mankey et al. hobnail appearance of the tumor cells is more often observed in lymphangiosarcoma compared to angiosarcoma. So this feature, pointed out also in our case, is potentially useful in the distinction between these two sarcomas [13]. In conclusion pathological data can support the diagnosis of lymphangiosarcoma, formerly based only upon clinical findings [11,13].

Cytogenetic data on chromosomal aberrations in angiosarcomas of soft tissues are poor. A literature review shows that only seven cases have been analyzed by conventional cytogenetic [12,20,28]. Table 1 summarizes all described karyotypes: complex chromosome assessments with several structural and numerical alterations were observed in the majority of cases. Aneuploidy as the sole karyotypic aberration has been described in only one case (Case1 in Table 1). The most frequently involved region in structural rearrangements is 1q. This region is commonly involved also in multiple myeloma [6], in T cell lymphoma [17] and in numerous solid tumors as well as in different types of sarcomas [16,18,27]. Abnormalities of the heterochromatin region 1q12–q22 have been linked to disease progression an poor prognosis in all type of neoplasia [5] through modification of gene expression and induction of gene silencing via long range epigenetic mechanisms.

The other frequently involved chromosomes are 8 and 20. Interestingly, high level of amplification of MYC was observed with fluorescence in situ hybridization (FISH) analysis. Amplification of MYC has been previously reported as a recurrent genetic alteration found in angiosarcomas secondary to irradiation or chronic lymphedema but not in primary angiosarcomas regardless of anatomic location suggesting a split in pathogenesis of the two sarcoma types [7,14,15]. It is noteworthy that in a recent study [10] MYC amplification was identified in three out of six primary angiosarcomas of different sites (breast and bone) pointing out the possible role of this gene even in a minority of primary angiosarcomas [10]. In this contest the case here reported is an angiosarcomas secondary to chronic lymphedema in non-irradiated patients characterized