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Research Article

Vascular Tumors Preceding Ewing Sarcoma: Clinico-Pathological Observations

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Abstract

Background: Ewing sarcoma (ES) is an exclusively human aggressive tumor affecting mostly Caucasian adolescents and young adults. ES is characterized by a specific translocation that fuses *EWSR1* (22q12) to *FLI1* (11q24) or to a member of the ETS transcription factor family. Hemangioma is the most common tumor of infancy, affecting mainly Caucasian infants. The lesions are benign tumors of endothelial cells. We report the clinic-pathological features of two ES cases arising from pathological affected vascular lesions.

Methods: Diagnosis of ES was based on histology and immunohistochemical evaluation and molecular confirmation of *EWSR1* rearrangement. Retrospective review of paraffin embedded material studying *EWSR1* rearrangements was performed by fluorescence in-situ hybridization.

Results: the retrospective review of case #1 with recurrent hemangioma tumors removed 3.5 years and 6 months before ES diagnosis demonstrated the presence of *EWSR1* rearrangements in <1% of vascular tumor cells. Case #2 had a clinic and radiological diagnosis of vascular anomaly at age 2 years and developed a mixed mesenchymal-endothelial undifferentiated tumor that progressed over time to ES.

Conclusion: These observations support the hypothesis of a potential common precursor stem cell of origin of both the ES and the preceding vascular tumors.

Keywords: Ewing sarcoma; Vascular tumors; Hemangioma; Hemangioblast; Cancer stem cell

Introduction

Ewing sarcoma (ES) is an exclusively human aggressive tumor, most prevalent in Caucasians and rarely diagnosed in African or Asian-descent populations, affecting mostly adolescents and young adults [1]. ES is characterized by a specific translocation that fuses *EWSR1* (22q12) to *FLI1* (11q24) in approximately 85% of cases, or, in the majority of remaining cases, to a member of the *ETS* transcription factor family other than FLI1. *EWSR1-ETS* fusions encode aberrant transcription factors that promote cell transformation through abnormal regulation of specific target genes involved in the control of a variety of cellular processes as well as key signal transduction pathways [1].

ES is reported to be the second most common bone malignancy in children (after osteosarcoma) with an average annual incidence rate of 2.9 per million [1]. In Spain, however, the frequency of ES is almost twice higher than osteosarcoma, according to the national Spanish registry [2] and to our institutional experience being the most common bone malignancy in children and adolescents. The incidence of ES peaks in the second decade of life, and is extremely rare during the first five years of age [1].

The cause of ES remains largely unknown. ES has been reported in siblings or cousins and together with the peculiar epidemiological distribution it suggests the presence of genetic susceptibility factors, particularly in populations of European ancestry. In fact, a recent genome-wide association study reported 2 major risk haplotypes (near *TARDBP* on 1p36; and near *EGR2* on 10q21) associated with susceptibility to ES [3]. A number of studies have evaluated predisposing factors. Parental farming exposure, history of inguinal hernia, and family history of gastric cancer or melanoma have been reported as associated with increased risk of developing ES. These findings have been based on retrospective analysis and have not been reproducible in all studies [1,4]. Patients with ES appear to have an increased incidence of bone abnormalities, particularly rib and vertebral anomalies. Other congenital malformations have also been reported like cataracts and genitourinary anomalies. All these abnormalities however occur at very low incidence in ES patients and without a consistent pattern suggesting that their occurrence with ES may be coincidental [1,4]. Given the association between ES and adolescence, the pattern of physical growth and development has been studied. Differences in birth weight between patients and controls have not been observed and patients with ES are not taller than their peers. In summary, currently there is no convincing evidence that ES is associated with any disease, cancer predisposition syndrome, or environmental factors [4].

The cellular origin of ES remains controversial. Since the initial proposal of an endothelial origin by James Ewing in 1921 [5], a neural crest and mesenchymal stem cell origins of ES have been hypothesized [6-8]. Since some mesenchymal stem cells (MSC) are derived from the neural crest, this partially common ontogeny could

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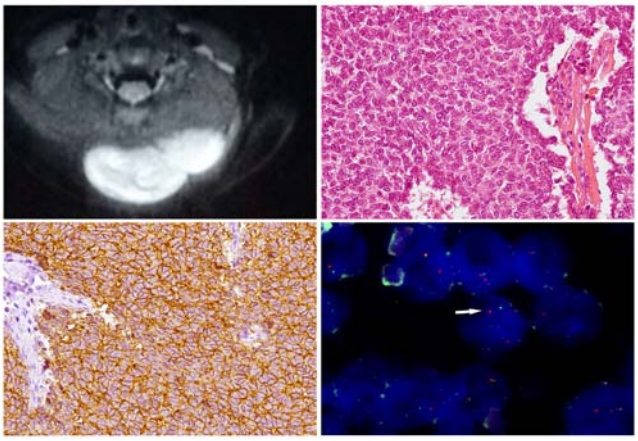

Figure 1: Initial presentation of patient #1. A: MRI image of the cervical region showing the intramuscular hemangioma. B: Microscopic view of the hemangioma showing the characteristic diffuse sheet of tumor cells, with no mitosis or atypical cells (original magnification 400x). C: Immunohistochemistry of the hemangioma showing positive membrane staining for the glucose transport protein GLUT-1. (Original magnification 400x). D: Fluorescence in-situ-hybridization using Vysis LSI EWSR1 (22q12) Dual Color, Break Apart Rearrangement Probe. Arrow shows the separation of the signals in single-cell nuclei indicating the rearrangement of *EWSR1*.

support a unifying hypothesis that ES might arise from malignant transformation of MSC of either mesodermal or neural crest origin. The hypothesis of a vascular differentiation potential of ES cells is supported by microarray data [9] and by the observation of vascular mimicry in ES associated with the ability of some Ewing cell lines to form vascular-like tubes *in vitro* [10]. In silico and experimental results suggest that some ES might derive from MSC that may have both mesenchymal and vascular potentials. ES may therefore constitute an appropriate model to further study the characteristics of a common mesenchymal vascular progenitor.

Hemangioma is the most common tumor of infancy, affecting approximately 4% of Caucasian infants. The lesions are benign tumors of endothelial cells (ECs) that exhibit a predictable evolution and duration [11,12]. In 1999 the erythrocyte-type glucose transporter protein, GLUT1 was first reported to be highly expressed by endothelial cells of infantile hemangiomas during both proliferation and involution providing what has become an invaluable tool for the diagnosis of infantile hemangioma [12]. The mystery of the origin of infantile hemangioma still remains. The persistent embryonic-like phenotype of the ECs of infantile hemangiomas has suggested a possible stem cell origin with delayed maturation [12]. A variable population of hemangioblast-like cells in infantile hemangiomas has been characterized by their cytometric phenotype [13]. These immature cell populations might represent the precursors of hemangioma ECs.

Here we report the clinic-pathological features of two cases displaying bona fide diagnosis of vascular tumors prior to the development of ES arising from pathological affected vascular lesions suggesting a common cell of origin.

Patients and Methods

From January 2002 to December 2014, 57 newly diagnosed, previously untreated, ES patients were managed at HSJD. The first 31 were previously reported [14]. Histologic and immunohistochemical evaluation, as previously reported [15], of primary tumors were consistent with the diagnosis of ES in all cases. Molecular diagnostic confirmation of *EWSR1* rearrangement was performed in all cases [14]. Retrospective review of paraffin embedded material studying *EWSR1* rearrangements was performed by fluorescence in-situ hybridization using the Vysis LSI *EWSR1* (22q12) Dual Color, Break Apart Rearrangement Probe (a mixture of two FISH DNA probes: a ~500 kb probe labeled in Spectrum Orange, flanking the 5' side of *EWSR1* and extending inward into intron 4; and a second probe of ~1100 kb labeled in Spectrum Green, flanking the 3' side of *EWSR1*).

Two (3.5%) of the 57 patients had previous surgical procedures because of preceding tumor lesions in the same pathological region of ES. Both prior diagnoses were vascular tumors in very young children that progressed over time to pathological diagnoses of ES.

Patient #1

AF was 11 months old when he presented with an indolent cervical mass. Imaging studies were performed including Doppler-ultrasound and MRI (Figure 1A) suggestive of vascular tumor. The patient was diagnosed of intramuscular hemangioma when resection of the tumor was undertaken from the posterior cervical area (Figure 1B). The tumor was described as a diffuse sheet of tumor cells, with no mitosis or atypical cells with the characteristic features of infantile hemangioma. Margins were affected. CD34 and Glut-1 was diffusely positive (Figure 1C). The patient did well until 3.5 years later when a recurrence of the tumor in the same posterior cervical region was again removed. The histology resembled the previous lesion with round to oval tumor cells with no cellular atypia and scant mitotic figures (Figure 2A). This time the tumor showed a lobular appearance. CD34 and GLUT1 remained intensely positive (Figure 2B). Six months later, when AF was 5 years of age, he started complaining of bone

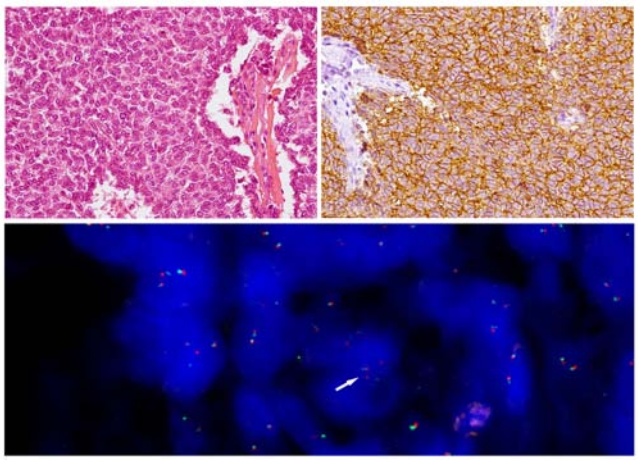

Figure 2: Three and half year's later local recurrence of patient #1. A: Microscopic view of the recurrent hemangioma showing the characteristic diffuse sheet of tumor cells (original magnification 400x). B: Immunohistochemistry of the hemangioma showing positive membrane staining for the glucose transport protein GLUT-1. (Original magnification 400x). C: Fluorescence in-situ-hybridization using the Vysis LSI EWSR1 (22q12) Dual Color, Break Apart Rearrangement Probe. Arrow shows the separation of the signals in single-cell nuclei indicating the rearrangement of *EWSR1*.

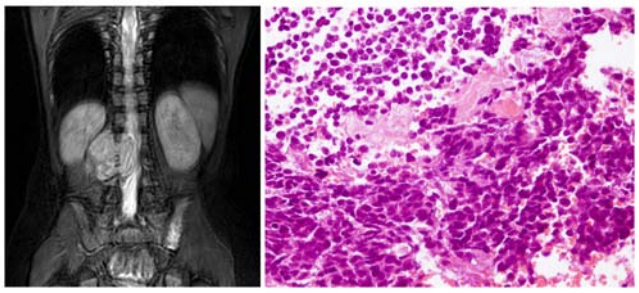

Figure 3: Disseminated presentation of ES of patient #1. A: MRI coronal image of lumbar region showing the right paravertebral tumor. B: Microscopic view of the tumor showing the characteristic features of Ewing tumor cells invading the muscle (original magnification 400x).

pain and limping. A paravertebral mass was found and the extent of disease work-up showed a T12 vertebral tumor with adjacent soft tissue mass (Figure 3A). The biopsy of the paravertebral mass showed characteristic features of ES (Figure 3B), and the *EWSR1-FLI1* fusion transcript was found expressed by qRT-PCR in the tumor cells (not shown). Bone marrow studies by qRT-PCR also showed expression of the fusion transcript. AF was enrolled in the mP6 institutional protocol as reported [14] and did well for 3 years when bone and soft tissue metastatic relapse was found. He underwent several rescue treatments and died of disease progression 4 years after ES diagnosis.

The retrospective review by FISH of the cervical tumors removed 3.5 years and 6 months before the ES diagnosis looking for *EWSR1* rearrangement showed mostly normal signals of the *EWSR1* break-a-part probe but <1% of rearranged cells in both vascular lesions (arrows in Figure 1D and 2C).

Patient #2

MT was referred to our institution when she was 2.5 years old because of a right thigh soft tissue tumor. Her family had seen the tumor grow for more than a year but since it was painless and the child was otherwise asymptomatic they did not seek for advice. An MRI and angiography was performed clearly suggestive of a vascular malformation affecting the muscle mass of the right thigh (Figure 4A). She was carefully followed in the vascular tumor clinic. When MT was 3 years old and because of an accelerated tumor growth, it was decided to perform a biopsy of the mass. Histology showed an undifferentiated tumor with vessels showing a hemangiopericytoma-like pattern (Figure 4B). Tumor cells expressed focal CD99 and S-100 and were negative for myogenin, MyoD1, CD68, CD31, CD34, actin, and desmin. Molecular studies were all negative for *EWSR1* and congenital fibrosarcoma translocations (not shown). Nine months later the tumor growth rate increased again significantly both clinically and by MRI and regional lymph nodes increased in number and size. Eventually, when MT was 4.5 years old, radical surgery was performed and the tumor was completely removed along with the locoregional lymph nodes. Histology showed undifferentiated, round blue cells infiltrating the muscle and invading the lymph nodes, diffusely positive for CD99 (Figure 5). Molecular studies confirmed the *EWSR1-FLI1* rearrangement and the extent of disease work-up was negative. MT was treated according to the institutional mP6 protocol and 1.5 years after the ES diagnosis remains in continued complete remission.

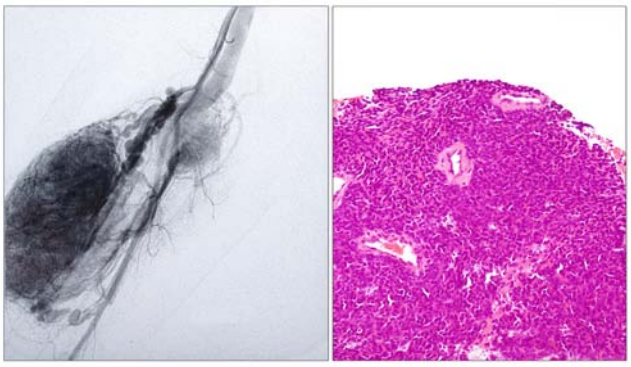

Figure 4: Initial presentation of patient #2. A: Angiogram of the right femoral artery showing the characteristic features of a vascular lesion with tortuous large vessels, increased and leaky perfusion of the mass. B: Microscopic view of the tumor showing an undifferentiated tumor with vessels showing a hemangiopericytoma-like pattern (original magnification 400x).

Discussion

The clinical observations here described are intriguing and have not been previously reported. The origin of ES from previous vascular lesions supports the hypothesis of a potential common precursor stem cell of origin. The origins of both ES and hemangioma remain unclear and involve a MSC with potential for both mesenchymal and vascular differentiation, an equivalent of the embryonic hemangioblast.

The first mesodermal cells to develop within the embryo contribute predominantly to the extra-embryonic tissues, giving rise to the hematopoietic and vascular cells of the yolk sac. From the extra-embryonic mesoderm arise the first hematopoietic and endothelial precursors which differentiate to form the blood islands in the yolk sac of the early embryo [16]. The close spatial and temporal development of these lineages provided the basis for the hypothesis of a common progenitor, a cell known as the hemangioblast [17]. Evidence in support of the concept of the hemangioblast has come from studies demonstrating that the hematopoietic and endothelial lineages express many genes in common and that some of these genes are essential for both blood cell and vascular development [18-21]. Very recently, Mazzarella L, et al. have shown that normal embryonic hemangioblasts remain epigenetically plastic with neural specifying genes like Nkx2-2, Nkx2-9, and Sox1 in bivalent chromatin state. Furthermore, conditional deletion of polycomb resulted in overt and inappropriate expression of neural genes in hemangioblasts [22]. The aberrant expression of neural genes in normal embryonic hemangioblasts secondary to epigenetic blockade is a suggestive

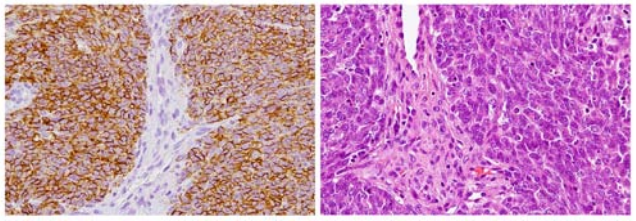

Figure 5: Progression of patient #2. A: Immunohistochemistry of the tumor showing diffuse, intense positive membrane staining for CD99 (original magnification 400x). B: Microscopic view of the tumor showing the characteristic features of Ewing tumor cells (original magnification 400x).

background for the pathogenesis of ES since most ES tumors characteristically express neural markers [1].

ES is characterized by a specific translocation that fuses *EWSR1* (22q12) to mainly *FLI1* (11q24). *FLI1* is a key regulator of early blood vessel formation [23] and during development FLI1 is preferentially expressed in hematopoietic and ECs and in the mesenchyme derived from neural crest cells [24]. In zebrafish embryos, FLI1 expression is detected in sites of vasculogenesis and the avian FLI1 is specifically expressed during embryogenesis in a subset of neural crest cells giving rise to developing mesenchyme. A study on Xenopus embryos described predominant FLI1 localization to territories invaded by neural crest cells. A comparison with ERG, the most closely FLI1-related gene and second most frequent *EWSR1* fusion partner in ES, revealed that both gene expressions partially overlap, suggesting that they may contribute to related or complementary functions in these tissues. *ERG* gene expression predominates in mesodermal tissues, including the endothelial, pre-cartilaginous and urogenital areas. Similar to *FLI1*, a specific *ERG* gene expression was also identified in migrating neural crest cells [25]. Overall, the embryonic patterns of expression of ETS genes and the prevalent role in early mesenchymal fate determination provide intriguing clues to many of the clinic-pathological aspects characteristic of ES.

While it was once believed that *EWSR1* rearrangements were specific to ES, it is now known that *EWSR1-ETS* fusions occur sporadically in other malignancies, including leukemia's [26-30] and biphenotypic tumors, which have features of both myogenic and neuroectodermal differentiation [31]. Most recently a novel EWRS1 translocation has been described in hemangioma of the bone, a benign bone tumor [32]. The finding of a t(18;22)(q23;q12) translocation resulting in *EWSR1-NFATC1* fusion in bone hemangioma further supports the common traits of vascular tumors and some *EWSR1* rearranged tumors.

In summary, we describe 2 cases where ES developed from tumors of mixed mesenchymal-endothelial origin supporting the hypothesis of hemangioblast-like cells as one of the potential cells of origin for ES.

Acknowledgment

JM, CdT and MS contributed to the writing and review of the manuscript. ER performed the IHC and FISH techniques. JM conceptualized and wrote the manuscript.

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