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Case Report Open Access

Extraskeletal Intracranial Chondroma: Ultrastructural and Immunohistochemical Features

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Abstract

The discrete microvasculature environment of the perivascular cell plays a dominant role in regulating stem cell activity and its proliferation behavior. Mesenchymal stem cells (MSC) reside in perivascular compartment, hence have the potential to contribute to the pathogenesis of extra-skeletal intracranial chondroma (ESICC). In this case report, for the first time, the immunohistochemical (IHC) markers and transmission electron microscpoe (TEM) features of perivascular cell are presented in ESICC. Microscopic examination revealed a well-differentiated chondroma with foci of ossification and islands of vacuolated-cells encircling thin-walled capillaries. TEM showed transitional morphological features suggestive of transformation to chondrocytes, with vacuolated cells. A subpopulation of spindle vacuolated-cells displayed reaction for S-100, ferritin, trypsin-anti-chymotrypsin and Vimentin. As there was no evidence of transitional forms to arachnoid cells, it can be concluded that the origin of the recognized ICC is a perivascular multipotential mesenchymal cell, MSC.

Key Words:

Neoplasm; Chondrosarcoma; Perivascular cells; Multipotential cells

Introduction

Intracranial chondromas (ICC) comprise 0.2-0.3% of intracranial tumors. However the present case of Extra-skeletal ICC (ESICC) is even rarer. The discrete microvasculature environment of the perivascular cell forms niches and that play a dominant role in regulating stem cell activity and its proliferation behavior. Recent studies suggest that committed stem cell progeny become indispensable components of the niche in a wide range of stem cell systems [1,2]. This provide heterogonous cell types that constitute the niche, hence they contribute to the dynamics of the microenvironment of the perivascular compartment. It has been suggested that mesenchymal stem cells (MSC) reside in the perivascular compartment and have characteristics identical to a subclass of pericytes [3-6]. Pericytes reside in the perivascular space and span the entire microvasculature. It had been established that the MSC are important regulators of angiogenesis and blood vessel function [7] they also contribute to the pathogenesis of diabetic microangiopathy, cancer, atherosclerosis and Alzheimer's disease [6]. Furthermore the MSC pericytes have been reported to be able to differentiate into osteoblasts [8,9] chondrocytes and adipocytes [10,11].

Clearly blood vessels and adjacent cells form the microvasculature environment of the perivascular MSC. Less is known about the immunohistochemical (IHC) of the extra-skeletal intra cranial chondroma (ESICC) and related ultrastructure alterations (USA). The purpose of this report was to present the IHC and related USA of Extra-skeletal ICC tumor with, no bone attachment, of a falx chondroma in a 15 year old child.

Clinical History

A 15-year-old female was admitted to pediatrics neurosurgery clinic with frequent headaches for the past 6 months. Physical examination including nervous system examination, chest X ray, ECG, and lungs functions were within normal range. The diagnosis was consistent with meningioma. The meningioma was removed by a left frontal craniotomy for a "Simpson grade 1" excision of an extra-axial tumor with duraplasty using periosteum. On gross the mass was 3 cm, well defined and of elastic consistency, with convexity dural attachment. The results of the frozen section analysis revealed a benign lesion, chondroma. The postoperative period was uneventful and the incision healed. Patient was in good condition for the next 6 months period of follow up.

Materials and Methods

Tissue preparation: Tissue from patients with exatraskeletal chondroma was operated at the Hamilton General Hospital, Hamilton, Canada. First the tissue was rinsed of blood, prior to preservation to prevent the detection of hematologic antigens which could interfere with the detection of target antigens, hence was immediately fixed with formaldehyde.

Immunohistochemistry (IHC)

Immunohistochemical staining was performed using 4-mm sections of formalin-fixed, paraffin-embedded tissue, which were mounted on capillary gap microscope slides (DAKO ChemMate, A/S BioTek Solutions) and dried at room temperature overnight followed by 1-2 h at 60°C. The tissue sections were deparaffinized and rehydrated. Antigen retrieval was achieved by microwave treatment in 1 mM EDTA, pH 9.0, at 900 W for 8 minutes followed by 15 min at 350 W. The slides were then allowed to cool for at least 20 min in the EDTA solution.

Primary antibodies were mouse monoclonal IgG antibodies to hMSH2 (clone 25D12, dilution 1:50, Novocastra/Leica) and hMSH6 (clone 44, dilution 1:10, ABCAM). Staining was performed in an automated immunostainer (Bondmax, Leica/Novocastra), according to the manufacturer's instructions. DAKO ChemMate kit peroxidase/3,30-diaminobenzidine was used for hMSH2 and DAKO Envision TM/HRP rabbit/mouse for hMSH6, with rabbit anti-mouse IgG, dilution 1:400, as a link to amplify between the primary antibody and the Envision step. Diamino benzidine was used as a chromogen. The sections were counterstained with haematoxylin, dehydrated in ascending concentrations of alcohol to xylene and mounted. The Bond polymer refine detection kit was used (catalogue no. DS9800). Loss of expression of the respective mismatch repair genes protein was defined as absence of nuclear staining in the tumour cells, and normal nuclear staining in lymphocytes and normal epithelial or stromal cells was required serving as internal control. The expression was classified by 2 pathologists as present, absent or non-valuable without grading of the staining intensity.

Ultra-structure procedures

Tissue samples were fixed by immersion in 3% buffered cold (4°C) of glutaraldhyde at 4°C then post fixed in 1% osmium tetroxide. Fixed tissue were dehydrated in graded concentrations of ethyl alcohol, (30%, 50%, 70%, 90%) for 30 min and finally in absolute ethanol (100%) for 40 min. Dehydrated tissue samples were then placed in propylene oxide to get rid off ethanol and render the tissues to become penetrate-able for the embedding media. This step was done at room temperature for 60 min. Tissue samples were infiltrated by transferring them from propylene oxide to a mixture of epoxy resins. First, samples were placed in a mixture of propylene oxide and resins at the ratio of 1:1 for 2 hr and lastly placed in pure epoxy mixture overnight. Tissue samples were embedded in the epoxy mixture using polyethylene been capsules. Polymerization of the resin was done at 60°C for 48 hr. The adequacy of each sample was checked on the semi thin sections. Ultra sections (70nm) were made and double stained with uranyl acetate and lead citrate. Ultra sections were mounted on carbon-coated grids, then examined and photographed by transmission electron microscope (JEOL-100 CX) at 80KV.

Magnetic resonance findings (MRI)

MRI (Figure 1) revealed no signal abnormality seen in the underlying cerebrum. The mass showed irregular post contrast enhancement, with heterogonous characteristics and an ovoid shaped 3.9 × 2.4 × 1.5, extra-axial, parasaggital, left anterior frontal mass. The mass showed falcine, consistent with meningioma. No definite dural tail of enhancement was noted. There was compression of the frontal lobe with minimal displacement of the midline. No evidence of boney erosion was noted. The remainder of the MRI examination was normal.

Light microscopic (LM) findings

Microscopic examination of the present study revealed a well-differentiated chondroma with multiple foci of ossification and islands of spindle and polygonal, vacuolated-cells encircling thin-walled capillaries of various dimensions. Furthermore gross examination revealed a 3.5 × 3.0 × 3.0 firm to hard mass (Figure 2a). The external surface was bosselated, glistening and pearly white. The cut surface was rough and grey (Figure 2b).

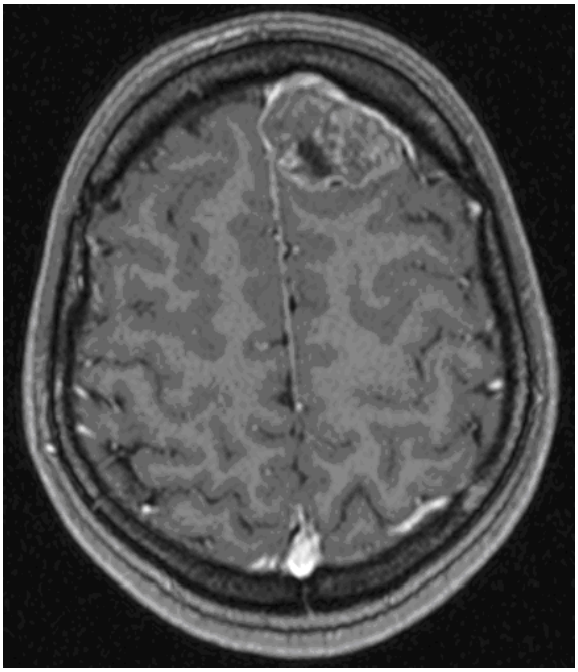

Figure1: MRI; Post-Gadolinium transverse image revealed an ovoid shaped 3.9 × 2.4 × 1.5, extra-axial, parasaggital, left anterior frontal mass.

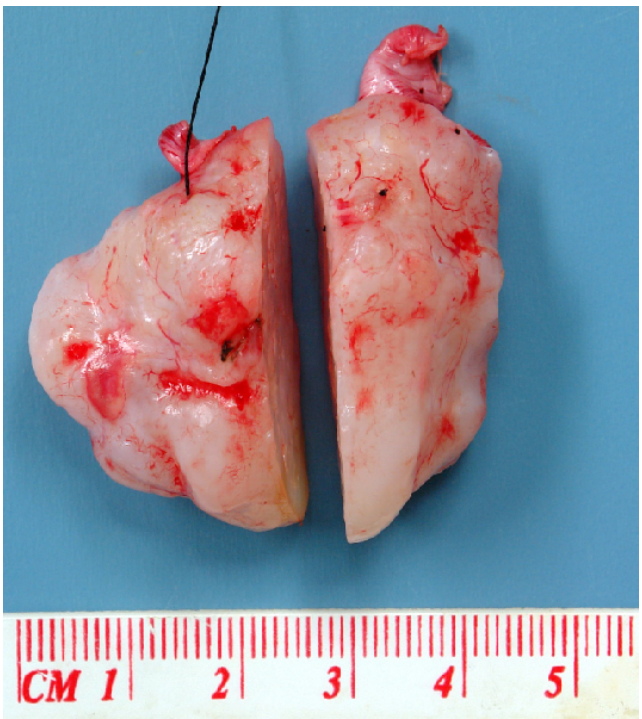

Figure 2a: Gross examination revealed a 3.5 × 3.0 × 3.0 firm to hard mass with a bosselated, glistening and pearly white external surface.

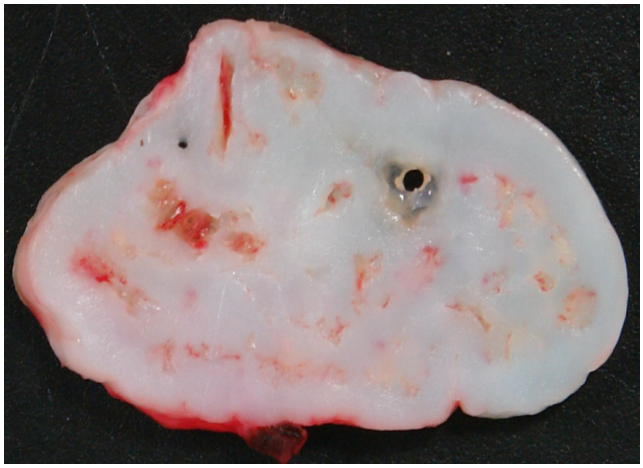

Figure 2b: Cut surface: rough, grey with numerous gelatinous islands.

There were numerous gelatinous islands and small foci of ossification. Hematoxylin and eosin stain (H&E) sections revealed a well-differentiated cartilage (Figure 3a) with striking islands polygonal and spindle foamy cells surrounding thin walled vessels. At the interface of these islands with the cartilage, there were multiple transitional forms between the perivascular vacuolated-spindle-cells and chondrocytes. As if the perivascular-vacuolated-spindle-cells are gradually merging and/or transforming into chondrocytes (Figure 3b). There were islands of ossification and a peripheral single small focus of adipose tissue.

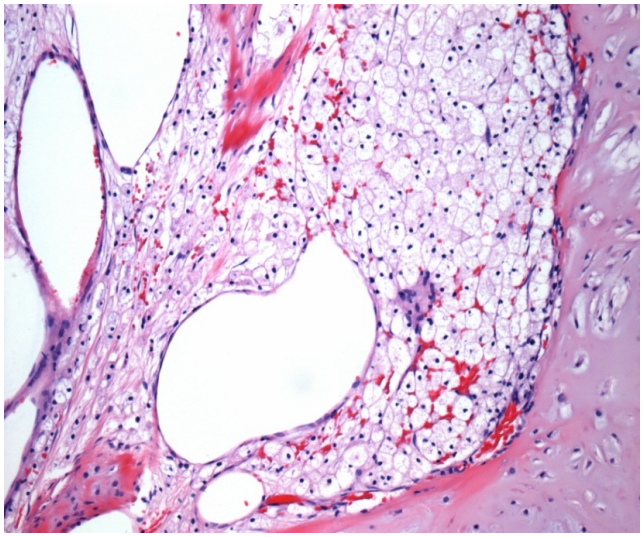

Figure 3a: H&E sections revealed a well-developed proteoglycan-like matrix with multiple islands of vasculature 20X

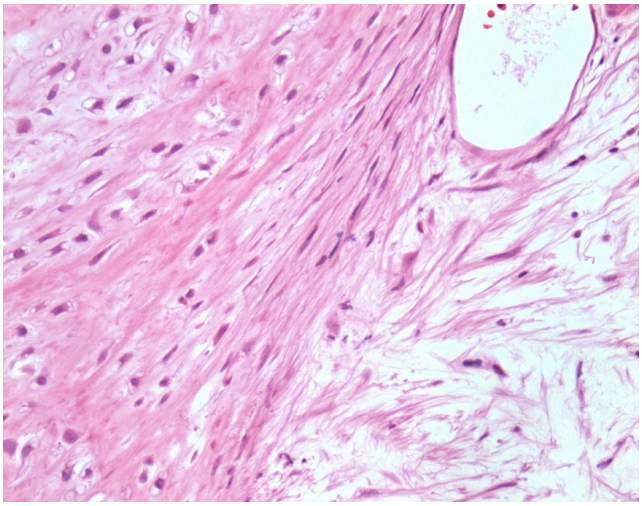

Figure 3b: H&E showing transitional cells including vacuolated spindle cells (arrow-head) and chondrocytes (arrow) 40X

Immunohistochemistry (IHC) findings

Immunohistochemistry findings are presented in Table 1.

Marker/Cell Type	Chodrocytes	PVPVC	PVSVC	Dura (arachnoid cells)
VIMENTIN	+	+	+	
S100	+	+	+	-
CD1a	+	+	+	-
FERRITIN	+	+	+	-
A1AT/AACT	+	+	+	-
Merosin	+	+	+	-
NSE	+	-	-	-
CD68	-	+	+	-
CD31	TD	+	+	-
EMA	-	-	-	+
CAM 5.2, AE1/AE3, GFAP, ACTIN, SMA, SMMHC, Caldesmon, CD3, CD10, CD20, CD21, CD34, CD45, Mac387, HMB45, MELANOMA CT were negative in all cell lines.				
KI67 and P53 showed technical difficulties (TD) in all cell lines and therefore immunoreactivity could not be examined. Progesterone and CD15 showed TD with chondrocytes only but, were negative for other cell lines.				

Table 1: Immunohistochemical properties of: chondrocytes, perivascular-vacuolated-polygonal-cells (PVVPC), perivascular-vacuolated-spindle-cells (PVVSC) and dura (arachnoid cells).

CD68 and Ferritin showed reactivity within the lacunated cells and the perivascular (and dural) spindle and polygonal cells (Figure 4a). Ferritin revealed reactivity within the chondrocytes and perivascular-vacuolated-spindle-cells (Figure 4b). The majority of vacuolated-cells

were represented by PAS, Oil-red-O, Vimentin, CD68, CD31, trypsin-anti-chymotrypsin and ferritin-positive macrophages. In addition a subpopulation of spindle vacuolated-cells displayed reaction for S-100, ferritin, trypsin-anti-chymotrypsin and Vimentin.

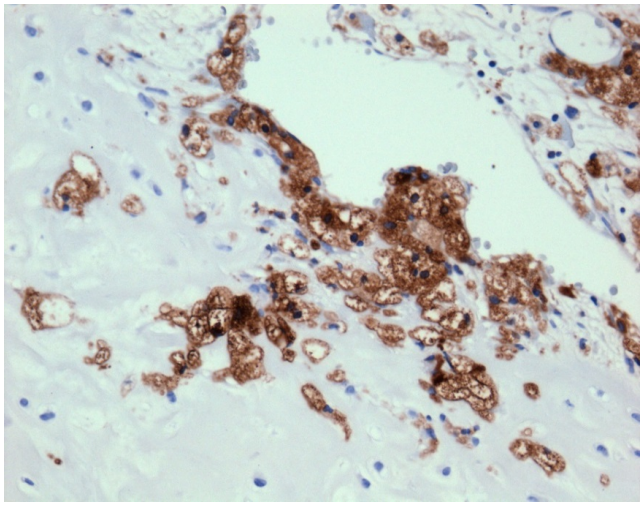

Figure 4a: CD68 reactivity within the lacunated cells and the perivascular spindle and polygonal cells 40X.

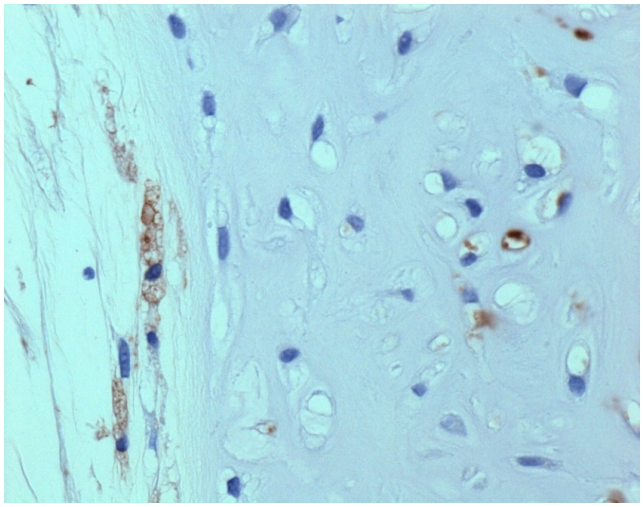

Figure 4b: Ferritin reactivity within the lacunated cells and the perivascular spindle and polygonal cells 40X

Transmission electron microscopy (TEM) findings

Electron microscopy revealed well differentiated chondrocytes, surrounded by a proteoglycan matrix. These cells contained free glycogen and intermediate filaments. Perivascular cells (PVC) displayed two patterns of differentiation:

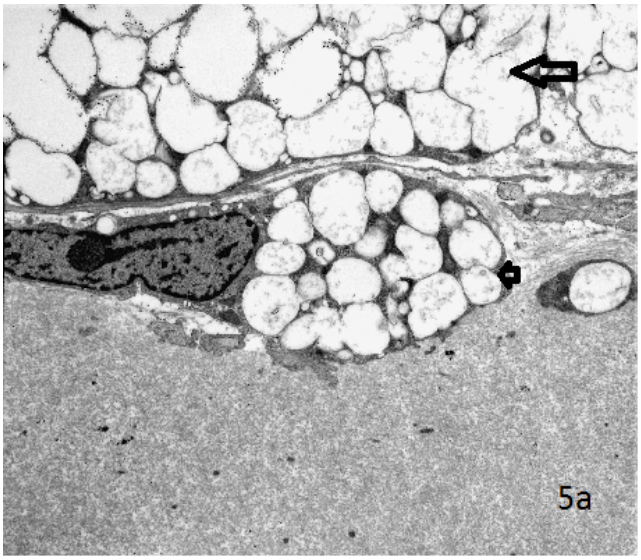

Figure 5a: Electron-microscopy shows transitional perivascular-vacuolated-spindle-cells 10,000X

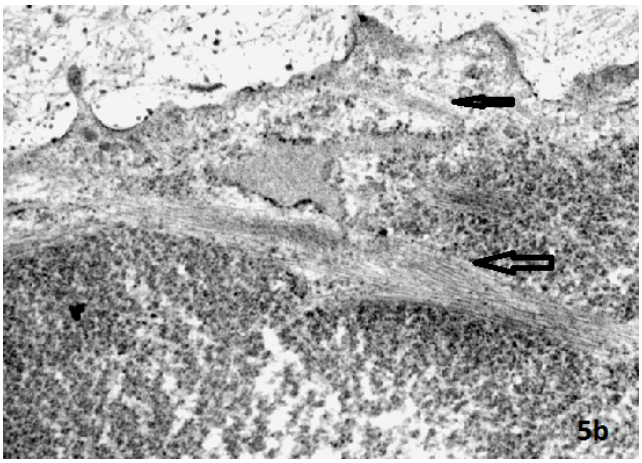

Figure 5b: Electron-microscopy shows subplasmalemmal tracts of filaments (arrow) 10,000X

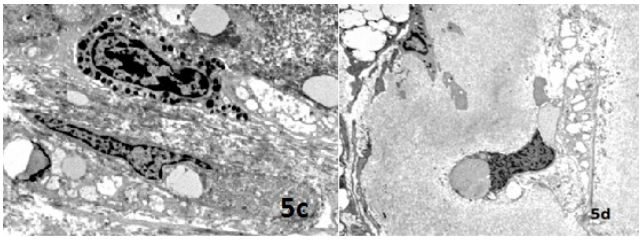

Figure 5c&d: Electron-microscopy shows transitional cells between myofibroblasts and chondrocytes 10,000X

(1) Typical macrophages; with vacuolated cytoplasm contains lipid droplets (Figure 5a) and (2) spindle cells with well-developed RER, subplasmalemmal tracts of filaments with focal densities (stress-fibres) (Figure 5b) and discontinuous external lamina, consistent with myofibroblastic differentiation. There were cells with transitional morphological features suggestive of transformation from perivascular-vacuolated-spindle-cells/myofibroblasts to chondrocytes (Figures 5c and 5d). There was no evidence of transitional forms from arachnoid cells.

Discussion

The major findings of the present case report is the IHC and TEM features of extraskeletal "falcine and convexity" chondroma originated from a perivascular multipotential mesenchymal cell (MSC).

Perivascular cells and MSC have previously been linked [3-6,10,12]. The primary trophic property of MSCs is the secretion of growth factors and other chemokines to induce cell proliferation and angiogenesis. MSCs express mitogenic proteins such as transforming growth factor-alpha (TGF-α), TGF-β, hepatocyte growth factor (HGF), epithelial growth factor (EGF), basic fibroblast growth factor (FGF-2) and insulin-like growth factor-1 (IGF-1) to increase fibroblast, epithelial and endothelial cell division. [13-16]. Vascular endothelial growth factor (VEGF), IGF-1, EGF and angiopoietin-1 are released to recruit endothelial lineage cells and initiate vascularization [17].

Pericytes reside on the abluminal surface of endothelial cells in the perivascular space and span the entire microvasculature [1,8,17], hence they are important regulators of angiogenesis and blood vessel function. It had been reported that pericytes differentiates into osteoblasts [4,14], chondrocytes and adipocytes [3,18,24]. From pathological standpoint, it was reported that pericytes contribute to the pathogenesis of diabetic microangiopathy, cancer, atherosclerosis, Alzheimer's disease [19] and muscular dystrophy [13].

Mesenchymal stem cells (MSC) are the conceptual postnatal progenitors of most derivatives of mesoderm [2,5]. They were initially isolated from the bone marrow [23], but subsequently also from several other tissues e.g. the umbilical cord, bone trabeculae, muscle, synovial, dental pulp, periodontal ligament and adipose tissue [11,23]. Mesenchymal stem cells were isolated by adherence to plastic and characterized by the expression of a panel of surface markers [15] and their capacity to differentiate along mesodermal lineages into adipocytes, chondroblasts and osteoblasts [23]. Until recently, the exact in vivo identity of MSC was elusive. However, now it has been suggested that MSC resides in the perivascular compartment and have characteristics identical to a subclass of pericytes [2,5,6,9,11,12].

Conclusion

Immunihistochemical (IHC) and transmission electron microscope (TEM) revealed evidence that portrays the origin of extra skeletal intracranial chondroma (ESICC) is perivascular multipotential, mesechymal stem cells.

Acknowledgement

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