Pathological Study of Pigmented Dermatofibrosarcoma Protuberans

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Abstract : **In this paper we performed morphological studies of Bednar tumor, which is a rare pigmented variant of dermatofibrosarcoma protuberans (DFSP) for evidence of histogenetic origin. Presence of melanosome-containing cells is the only histologic criterion of Bednar tumor, which is different from the ordinary DFSP. Immunohistochemical and ultrastructural examinations revealed that the melanosome-containing cells gave positive reactivity to antibodies to vimentin and S-100 protein, but negative to HMB-45, CD38, Factor VIII, NSE, and KP-1. The melanosomes are in Stage II, III, and IV, suggesting that the pigment was produced within the cell rather than phagocytosed from other cells. The rest of the tumor had similar pattern of immunoreactivity to DFSP such as positivity for CD34 and vimentin, indicating that Bednar tumor and DFSP originated from the same cell line. The ultrastructural feature of non-pigmented cells was similar to perineural cells in the first group and similar to fibroblasts in the second group. DNA flow cytometry in our cases revealed diploid cells with low to intermediate S-phase fraction and no aneuploid cells which were reported in a previous study.**

Keywords: Pigmented dermatofibrosarcoma protuberans; Bednar; CD 34; Ultrastructure; DNA flow cytometry

เรื่องย่อ : **การศึกษาลักษณะทางพยาธิสภาพของเนื้องอกชนิด Pigmented Dermatofibrosarcoma Protuberans (Bednar tumor)**

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รายงานฉบับนี้เป็นการศึกษาลักษณะทางพยาธิวิทยาเพื่อบอกพยาธิกำเนิดของเนื้องอก Bednar ซึ่งจัดเป็นประเภทหนึ่งของเนื้องอก Dermatofibrosarcoma protuberans (DFSP) พบว่าเนื้องอก Bednar มีเซลล์ที่บรรจุสารรงควัตถุคือ melanosome ไว้ภายใน ซึ่งเซลล์เหล่านี้ให้ผลบวกต่อการย้อมอิมมูโนเคมีชนิด S100 และ vimentin และให้ผลลบต่อการย้อมชนิด HMB 45, CD 34, Factor VIII, NSE และ KP-1 ในขณะที่เซลล์ของเนื้องอกประเภทอื่นของเซลล์เนื้องอก DFSP ให้ผลบวกต่อการย้อมอิมมูโนเคมีชนิด CD 34 และ vimentin แสดงว่า Bednar tumor และ DFSP มีเซลล์ต้นกำเนิดจากเซลล์กลุ่มเดียวกัน

ลักษณะทางจุลทรรศน์อิเลคตรอนของสารรงควัตถุพบว่าเป็น melanosome ระยะที่ 2, 3 และ 4 แสดงว่าเซลล์ของเนื้องอก Bednar ผลิตรงควัตถุขึ้นได้เองไม่ได้เกิดจากขบวนการ phagocytosis เข้ามา ส่วนลักษณะของเซลล์รูปกระสวยที่ไม่มีรงควัตถุ มีทั้งลักษณะที่คล้ายกับ fibroblast และ perineural cell นอกจากนี้ยังได้ศึกษาคุณสมบัติทางชีววิทยาของเนื้องอก Bednar ด้วยวิธี DNA flow cytometry และพบว่าเนื้องอกทั้งสองรายเป็นชนิด Diploid ที่มี S-phase fraction ต่ำถึงปานกลาง ซึ่งแตกต่างจากที่เคยมีรายงานใน DFSP ว่ามีทั้ง diploid และ aneuploid

INTRODUCTION

Bednar tumor or pigmented dermatofibrosarcoma protuberans was described by Bednar in 1957 as a variant of neurofibroma.[1] It is a rare neoplasm accounting for approximately 1-5% of all cases of dermatofibrosarcoma protuberans (DFSP), a rare tumor that constitutes less than 0.1% of all malignancy.[1] The histogenic origin of this tumor is still a matter of controversy. Stout initially regarded the lesion to be a variant of fibrosarcoma afterward reported a similar lesion as a neoplasm of uncertain histogenesis and finally melanosarcoma.[2,3] Dupree and Weiss reported 20 cases in 1985 emphasizing clinical, morphological, electron microscopic and immunohistochemical evidence supporting a close relationship between this tumor and nonpigmented DFSP.[4] Bednar tumor commonly presents as an exophytic multinodular neoplasm in the dermis or subcutaneous tissue. The trunk is the most common anatomic site, with the remaining sites almost equally distributed in the upper and lower extremities, the head and neck. At the light microscopic level, spindled cells are focally arranged in a tight storiform pattern. Melanin-containing dendritic cells admixed with the spindle cell elements is the distinguishing feature between this neoplasm and nonpigmented DFSP.

In a six years database (1995-2000), we found two new cases of Bednar tumor out of twenty new cases of DFSP tumor, the same range of incidence mentioned above (1%). We studied the immunoreactivity, ultrastructural features of the spindle cells and pigmented cells of Bednar tumor for evidence supporting the histogenesis and DNA flow cytometry was also performed to elucidate biologic features.

MATERIALS AND METHODS

Formalin-fixed, paraffin embedded tissue specimens from two Bednar tumors proven by histology were used for immunohistochemical, ultrastructural, and DNA flow cytometry studies.

Immunohistochemical studies was done by using enzyme-labeled conjugated, three-step indirect method (Thomas Boenisch, DAKO Corporation.). The studied antigens included Vimentin (monoclonal antibody, clone v9, DAKO, dilution 1:3,000), CD-34 (monoclonal antibody, clone 4Bend10, DAKO, dilution 1:250), S-100 (polyclonal Rabbit anti-cow S-100 protein, DAKO, dilution 1:3,000), KP-1 (monoclonal antibody, clone KP-1, DAKO, dilution 1:2,500), Factor VIII related antigen (polyclonal Rabbit anti-human factor VIII, DAKO, dilution 1:500), Neuron specific enolase, NSE (Monoclonal mouse

anti-human neuron specific enolase, DAKO, dilution 1:100), and HMB-45 (monoclonal antibody, clone HMB-45, DAKO, dilution 1:500).

The specific areas of pigment-containing cells and spindle cells were selected for ultrastructural studies by Transmission electron microscope (TEM) model JEM-100SX, JEOL Co.

Sixty-micrometer thick paraffin embedded tissue was deparaffinized, and digested with 1% collagenase and 1% pepsin solution, then labelled with propidium iodine[5] before being analyzed by Coulter EPICS flow cytometer. The data was then analyzed with Multicycle for windows computer program (PHOENIX FLOW SYSTEMS, Inc, San Diego, California, USA) for DNA ploidy and S-phase fraction measuring.

RESULTS

The Bednar tumor was obtained from 2 patients. The first case was a 34-year-old man with 6 cm. diameter tumor on his right shoulder; the second case was a 43-year-old woman with a 2.5 cm.diameter mass on her left buttock. Grossly, both tumors were well-demarcated, grayish black, subcutaneous nodules, without ulceration. Routine histologic studies revealed that the tumors were composed of cellular uniform, pale eosinophilic cytoplasm with mild nuclear atypia and mitotic figure spindle cell tumor arranged in storiform pattern; and scattered melanin containing multipolar dendritic cells. (Figure 1.1)

Immunohistochemical studies

Both cases demonstrated the same immunoreactivity pattern (Table 1). The spindle cells showed positive immunoreactivity to vimentin and CD 34. (Some spindle cells also showed positive immunoreactivity to Factor V III.), and showed negative reactivity to S-100 protein, HMB-45, KP-1, and NSE. The pigmented cells showed positive immunoreactivity to S-100 protein and vimentin. Only some inflammatory cells in the lesion, not tumor cells, showed positive immunoreactivity to KP-1. (Figure 1.2)

Table 1. Demonstration of immunohistochemical staining pattern of Bednar tumor.

Cell types	Immunohistochemical stains						
	Vimentin	CD 34	Factor VIII	S-100	HMB-45	KP-1	NSE
Pigmented cells	+	-	-	+	-	-	-
Spindle cells	+	+	+/-	-	-	-	-

Ultrastructural studies

Ultrastructurally, three cell populations were identified. Non-melanin containing cells showed fibroblastic and perineural-like features. Thin basal lamina were seen in non-pigmented perineural-like cells and pigmented cells and were absent in non-pigment fibroblast-like cells. The melanin-containing cells revealed stage two, three, and four melanosomes, in their cytoplasm. (Figure 2.1 and 2.2)

DNA flow cytometry analysis

Both lesions revealed diploid tumor, with the percentage of population in G_1, S, and G_2 being 92.2%, 4.8% in the first case, and 3.0%, and 90.6%, 7.3%, and 2.1%, in the second case. (Figure 3)

Table 2. Ultrastructural findings in different cells found in Bednar tumor.

Cell types	Ultrastructural findings			
	Shape	Cytoplasmic organelles	Nucleus	Others
Fibroblast-like cells	Spindle	Rough-endoplasmic reticulum, few mitochondria	Oval shape	Absence of basal lamina
Perineural-like cells	Spindle with slimmer shape	Wavy filament and picnocytic vacuoles in cytoplasm	Oval shape	Thin basal lamina
Pigmented cells	Large pigmented	Presence of type three and four melanosomes, picnocytic vacuoles	Plump	Thin basal lamina

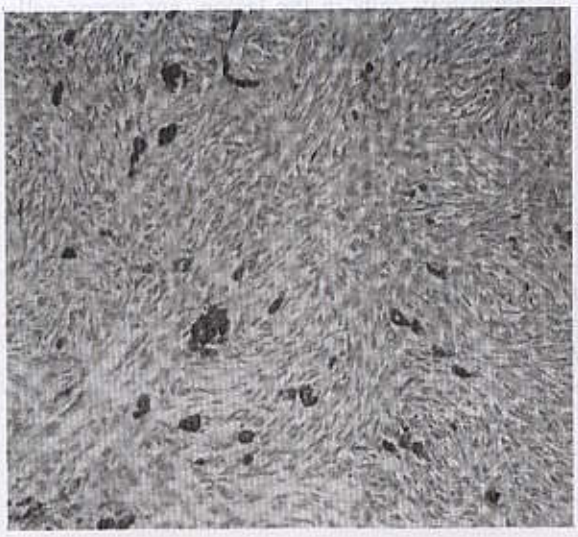

Figure 1.1 Histological feature of Bednar tumor reveals the classic spindle cells of DFSP and pigmented cells (H&Ex200).

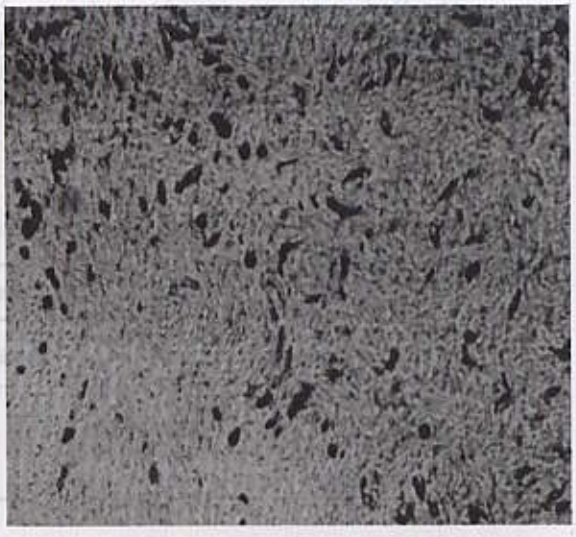

Figure 1.2 Histological feature of Bednar tumor reveals the classic spindle cells of DFSP and pigmented cells (Vimentinx100).

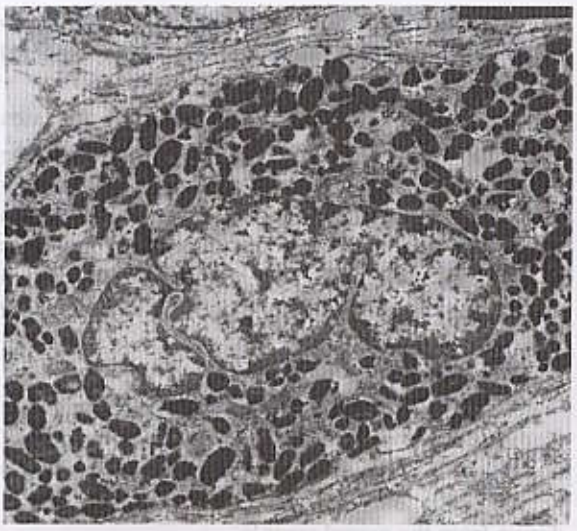

Figure 2.1 Electron microscopic features of a pigmented cell shows several mature melanosomes. (Magnification X12,320)

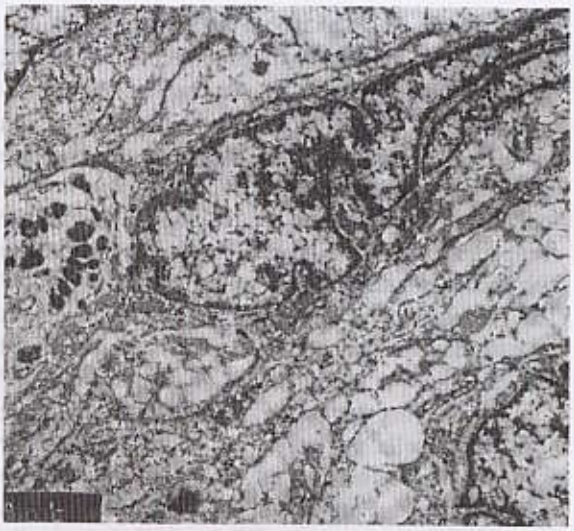

Figure 2.2 Electron microscopic feature of a non-pigmented cell shows the basal lamina. (Magnification X12,320)

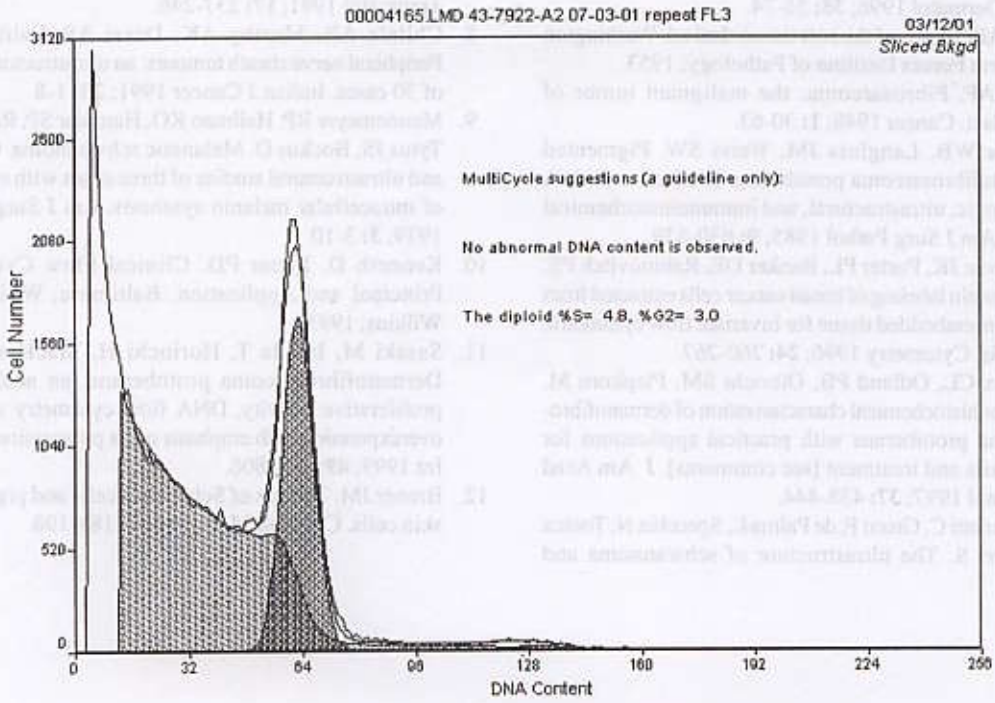

Figure 3. DNA histogram of Bednar tumor shows diploid tumor.

DISCUSSION

The non-pigment spindle cells in these 2 cases of Bednar tumors shared the same immunoreactivity as DFSP by positive staining of vimentin and CD 34.[6] Vimentin which indicates connective tissue origin has not proved to be a reliable marker for DFSP.[1] CD34 seems to be highly specific for DFSP compared with other fibrohistiocytic tumors and was also demonstrated in a variety of neurogenic tumors.[1] The ultrastructural studies in non-pigmented cells gave similar results in both tumors and also resembled the cells found in peripheral nerve sheath tumor.[7,8,9]

Varying stages of melanosomes found in the pigmented cells suggests that these cells rather produce melanin themselves rather than phagocytose from other cells. Furthermore, these pigmented cells showed negative result for KP-1, which is basically reacting positively in macrophage. These pigment-loaded cells have ultrastructural features similar to the pigmented cells found in a rare variant peripheral nerve sheath tumor, melanotic schwannoma.[9]

In DNA flow cytometry studies, both cases gave diploid patterns with low to intermediate S-phase fraction. Because no previous documentation of DNA cytometry of Bednar tumor has been published, we compared our results with some studies in conventional DFSP. The previous studies stated that both diploid and aneuploid DNA pattern could be found in DFSP; and approximately 26-28% of DFSP were aneuploid.[10,11]

In conclusion, Bednar tumor could originate from the same cell line as the conventional DFSP, and might have a close relationship to the peripheral nerve sheath tumor and melanocytic tumor which derive from the neural crest.[12]

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