PATHOLOGY ONCOLOGY RESEARCH Vol 7, No 1, 2001

10.1053.paor.2001.0282 available online at http://www.idealibrary.com on IDEAL

CASE REPORT

Poorly Differentiated Synovial Sarcoma: A Case Report

Francesco CAPPELLO,[1,2] M BELLAFIORE,[2] F BUCCHIERI,[2,3] G BALSANO,[4] A PALMA,[2] G ZUMMO,[2]

[1]Institute of Pathological Anatomy, [2]Institute of Human Anatomy, University of Palermo,
[3]Institute of Internal Medicine and Pneumology, Ospedale v. Cervello,
[4]Institute of General and Endoscopic Surgery, University of Palermo, Palermo, Italy

Poorly differentiated synovial sarcoma is a rare soft tissue tumor. We studied a case arising in the pleural cavity of a young subject, characterised by the presence of spindle cell, small cell, and large epithelioid cell areas. We performed stains for mucosubstances and analysed the expression of cytokeratins 5/6, 7, 8, 18, 19, CEA, CD34, Ber-Ep4 and calretinin to characterize the phenotype of this neoplasm. We furthermore assessed immunohistochemically the presence of p53, Bcl-2, Bax and caspase 3, four apoptotic markers, to evaluate a relationship between apoptotic activity and the behaviour of this tumor. Our findings showed a strong presence of calretinin, p53 and Bcl-2 in all three areas. The possibility that poorly differentiated synovial sarcoma could be calretinin-positive was a new data, to our knowledge, and it could be of some importance in diagnostic pathology. Moreover, the negligible positivity for Bax and caspase 3 suggested that the minor role of programmed cell death could be one of the causes of the aggressive behaviour of this tumor. These data also suggest that the reduction of apoptotic phenomena in poorly differentiated synovial sarcoma could be considered one of the major mechanisms of tumoral growth. (Pathology Oncology Research Vol 7, No 1, 63–66, 2001)

Keywords: synovial sarcoma, poorly differentiated synovial sarcoma, soft tissue tumors, calretinin, apoptosis

Introduction

Synovial sarcoma is a relatively frequent malignant soft tissue tumor. Commonly, this neoplasm arises in the extremities and, less frequently, in parapharyngeal region, abdominal wall or other extraarticular sites. While the name implies an origin from synovia, generally it arises in sites devoid of normal synovium. In fact, it is thought to originate from primitive pluripotential mesenchimal cell.

Generally, the microscopic pattern is the classic biphasic type. Nevertheless, synovial sarcoma can be characterised by a predominant monophasic spindle or, more rarely, epithelial pattern, or by a poorly differentiated aspect. The latter is the variant with the poorest prognosis. Therefore, three patterns of poorly differentiated synovial sarcoma (PDSS) have been recognised: spindle cells (herringbone pattern), large epithelioid cells and small cells.[16]

The differential diagnosis includes a variety of soft tissue sarcomas, as malignant peripheral nerve sheath tumors, fibrosarcomas and leiomyosarcomas. The histologic criteria have been well characterised[7], but sometimes histologic and immunohistochemical aspects can make the diagnosis harder.[9]

We therefore aimed to study histochemically and immunohistochemically a case of PDSS. We selected different histochemical stains and immunohistochemical markers, present in literature, to address the diagnosis. We also assessed the presence of some apoptotic markers to evaluate the role of apoptosis in the pathogenesis and in the behaviour of this neoplasm.

Materials and Methods

We studied a case of PDSS arising in the lower right pleural cavity of a 33-years old non-smoking man. Diagnosis was made using traditional techniques (H&E), histo-

Received: July, 12, 2000; *revised:* Jan 10, 2001; *accepted:* Jan 21, 2001

Correspondance: Franceso CAPPELLO, M.D., Via Alla Falconara 120, 90136 Palermo, Italy; Tel: +39-0339-5675268, fax: +39-091-6553508; email: FRANCAPP@HOTMAIL.COM

 1219-4956/01/010063+04 $ 12.00/0

chemistry and immunohistochemistry. We used 7-μm paraffin sections. Histochemical stains for reticulin, PAS (with and without diastase digestion), mucicarmine and alcian blue (with and without hyaluronidase digestion) were performed. Immunostaining by avidin-biotin complex method was performed (DAKO, LSAB2), using primary antibodies against cytokeratin (CK) 5/6 (Boehringer-Mannheim, monoclonal, 1:25), CK7 (DAKO, monoclonal, 1:100), CK8 (Sigma, monoclonal, 1:100), CK18 (Sigma, monoclonal, 1:500), CK19 (DAKO, monoclonal, 1:50), CEA (DAKO, monoclonal, 1:50), CD34 (DAKO, monoclonal, 1:40), Ber-Ep4 (DAKO, monoclonal, 1:20), calretinin (Zymed, polyclonal, 1:20), p53 (Biogenex, monoclonal, 1:100), Bcl-2 (DAKO, policlonal, 1:10), Bax (Biogenex, monoclonal, 1:30), caspase 3 (Pharmingen, polyclonal, 1:500) and isotype-matched control. Diaminobenzidine (DAB chromogen, DAKO) was used as develop chromogen.

Results

Histologic findings

Fifty percent of the tumor was composed of spindle cell areas, with a herringbone pattern (***Figure 1a***). Furthermore, these areas showed a high mitotic rate and, focally, necrosis. Moreover, the neoplasm consisted of small cell (***Figure 1b***) and large cell (***Figure 1c***) areas. Small cell areas showed cells with less cytoplasm and rounded nuclei. By contrast, in the large cell areas, the nuclei had an irregular contour and cytoplasm was increased, giving an epithelioid appearance to the cells.

Histochemical findings

This sarcoma was positive for reticulin. In particular, the small cell component of tumor was abundantly positive. Moreover, the neoplasm showed PAS-positive, alcian blue positive and mucicarmine negative mucosubstances, inside and among the tumoral cells. PAS and alcian blue positivity was respectively diastase and hyaluronidase resistant (data not shown).

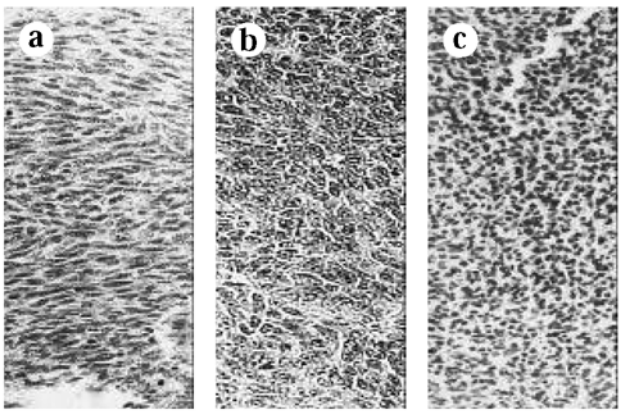

Figure 1. a) *herringbone area, H&E (25x);* ***b)*** *small cell area, H&E (25x);* ***c)*** *large cell area, H&E (25x).*

Immunohistochemical findings

Interestingly, calretinin was strongly present, particularly in the spindle component (***Figure 2a***). By contrast, CKs, CEA, CD34, and Ber-Ep4 were negative in all components of the tumor. Furthermore, this tumor was characterised by a strong presence of p53 ***(Figure 2b)*** and Bcl-2 (***Figure 2c***) in all components. By contrast, there was weak positivity for Bax (***Figure 2d***) and a negligible presence of caspase 3 (***Figure 2e***), in both small and large cells more than in herringbone areas.

Discussion

PDSS is the synovial sarcoma with the poorest prognosis. Differential diagnosis is often very difficult and speculative hypotheses about the pathogenesis of these neoplasms rarely support definite conclusions. In particular, the difficulty to study the synovial sarcoma lies not only in the morphologic aspect, but also in the variability of the site. Nevertheless, the simultaneous presence of all three patterns (herringbone, large epithelioid cells and small cells) wakes the diagnosis.

Histochemical and immunohistochemical studies often can not produce a reliable diagnosis without risks and, sometimes cytogenetic studies are indispensable. Histochemical positivity is a characteristic of these tumors. In particular, synovial sarcoma is generally PAS and/or mucicarmine and/or alcian blue positive, diastase/hyaluronidase resistant. This resistance to digestion can be discriminative in differential diagnosis between these and other sarcomatoid tumors, as malignant mesothelioma.[10]

We performed the diagnosis using the histological criteria and the histochemical results more than the immunohistochemical data. In particular, this case was PAS/alcian blue positive, digestion resistant, but mucicarmine negative. This fact confirmed the importance of performing all three mucosubstances staining to increase the diagnostic certainty.[10]

Concerning of immunohistochemical studies, there are many publications about the use of antibodies to diagnose PDSS. Generally, CKs and CEA are positive in 50-100% of cases.[1,5,8,9] This may be of some utility in a differential diagnosis between this entity and others round cell sarcomas, such as primitive neuroectodermal tumors and malignant nerve sheath tumors. Conversely, positivity to CD34 and Ber-Ep4 is less frequent.[1,20,22,24]

Calretinin is a recent immunohistochemical marker for mesothelioma, but it was also found to be present in other epithelial and spindle tumors.[21] In particular, biphasic syn-

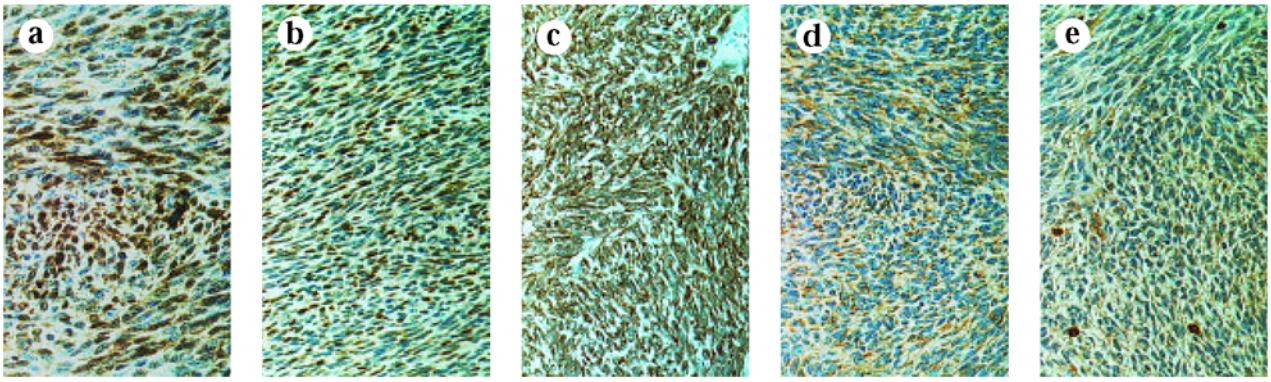

Figure 2. ***a)*** *anti-calretinin (25x);* ***b)*** *anti-p53 (25x);* ***c)*** *anti-Bcl-2 (25x);* ***d)*** *anti-Bax (25x);* ***e)*** *anti-caspase 3 (25x).*

ovial sarcoma showed little positivity to this antibody.[6] The importance of this datum could lie in the possibility to differentiate the biphasic synovial sarcoma from other sarcomas arising in the pleural cavity, to be as, biphasic malignant mesothelioma, in which, by contrast, there is a strong presence of calretinin. This tumor was strongly positive to calretinin; and in particular, this marker was expressed mainly in the herringbone component. CKs, CEA, CD34 and Ber-Ep4 were absolutely negative in all areas. These results suggested that in the presence of a strong calretinin-positive pleural malignant tumor, the possibility of PDSS should not be excluded in differential diagnosis.

In this PDSS, p53 was strongly positive in all components. P53, the „guardian of genome", is a tumor suppressor gene. Its product is a nuclear phosphoprotein, which half-life is normally very short. If a mutagenic agent stresses the cell, p53 blocks the cell cycle to allow DNA repair. However, if the repair process fails, p53 induces the expression of pro-apoptotic genes, among which is Bax, to stimulate the apoptotic process. By contrast, when p53 is mutated, its half-life becomes longer, it accumulates at nuclear level and DNA-damaged cells can continue to proliferate, resulting in tumor. Recently, Nakanishi[17] found that 27% of synovial sarcoma were p53 positive and he consiclered this data indicative of abnormal accumulation of p53. P53 presence in this tumor could be expression of nuclear damage, but this fact alone is not helpful in pathogenetic speculation.

Bcl-2 was also strongly positive in PDSS. Bcl-2 is a protein normally expressed in mitochondria, endoplasmic reticulum and the nuclear envelope. Generally, Bcl-2 is an homodimer that binds a pro-apoptotic protease-activating factor (APAF-1), which is bound to a cysteine-aspartic protease, caspase 9. Bcl-2 suppresses apoptosis both preventing an increase of mitochondrial membrane permeability (direct way) and interacting with other pro-apoptotic proteins (mediated way). If DNA is irreversibly damaged, p53 induces the synthesis of Bax, which binds Bcl-2, forming an heterodimer Bax-Bcl-2. The formation of this heterodimer causes increased of permeability of mitochondrial membranes and the release of an apoptotic trigger, cytochrome C. The later disrupts the binding between Bcl-2 and APAF-1 and primes the caspase cascade, inducing apoptosis.

Over-expression of Bcl-2 can inhibit apoptotic phenomena in vitro[2]. Moreover, an inverse correlation between Bcl-2 expression and apoptotic events has been confirmed in vivo.[13,26] Chilosi[4] found that synovial sarcoma was Bcl-2 positive. Analogously, Nicholson[18] and Suster[25] found Bcl-2 positivity in 100% of synovial sarcomas. Segers[23] and Nakanishi[17] found that synovial sarcoma could be also Bcl-2 negative. In addition, Nakanishi[17] underlined the possibility that patients with Bcl-2 positive soft tissue tumors showed more favourable prognosis than those Bcl-2 negative. He suggested that the immunohistochemical detection of Bcl-2 could be useful to predict the prognosis of patients with soft tissue tumors, as confirmed by other studies.[12,14] Nevertheless, this datum was in contrast with the study of Bubendorf[3], that studied the clinical course of prostatic cancer and found a better prognosis in Bcl-2 negative tumors.

The strong positivity to Bcl-2 that we found in PDSS could indicate a reduction of apoptotic phenomena and could suggest that p53 was mutated because it could not induce the synthesis of Bcl-2 suppressor proteins, such as Bax. The consequent reduction of apoptosis could be a mechanism of tumoral growth.

In addition, we found scarce positivity of Bax in PDSS. Bax is a 21-kD molecule of a growing family of proteins that may both promote and inhibit cell death. In particular, Bax may overcome the death-suppressive functions of Bcl-2.[19] Recently, McPake[15] demonstrated that Bax may be an important determinant of chemosensitivity, proposing the measurement of Bax expression as a clinical prognostic indicator for tumor response to therapy. The weak presence of Bax in PDSS could confirm the hypothesis that this neoplasm presented a reduction of apoptotic events as mechanism of tumoral growth. Moreover, the contrast between the strongly positivity of p53 and the scarce positivity of Bax suggests the hypothesis that p53 is present but mutated.

Finally, PDSS showed also scarce caspase 3 positivity. Caspase 3 is a member of a family of proteases that get their name because they cleave proteins at the aspartic acid residues. Caspases are subdivided in initiators and executors. In particular, caspase 3 belongs to the second group, determining fragmentation of the cytoskeleton. In fact, the sequential activation of caspases creates an expanding cascade of proteolytic factors, which leads to the digestion of structural protein of the cytoskeleton and to the degradation of chromosomal DNA in the nucleus, with the formation of apoptotic bodies and cell death.

We found negligible presence of caspase 3 in all tumoral areas. This could be a further confirmation of the reduction of apoptosis in this tumor.

In conclusion, our findings indicate that Bcl-2 can also be present in the poorly differentiated variant of synovial sarcoma. The reduction of apoptosis in this tumor could be the mechanism of tumoral growth. Although p53 was found strongly present, it seems not to be able to induce the activation of apoptotic proteins, as Bax.

The continuous assembly disassembly cycle of the complex network of morphologically distinct filaments and proteins into the neoplastic mesenchymal cell[11] can confuse histologic and immunohistochemical features in all poorly differentiated tumors. This can explain why calretinin, negligibly positive in byphasic synovial sarcoma, can be strongly present in PDSS.

References

1. *Abenoza P, Manivel JC, Swanson PE, et al:* Synovial sarcoma: ultrastructural study and immunohistochemical analysis by a combined peroxidase-antiperoxidase/avidin-biotin complex procedure. Hum Pathol 17:1107-1115, 1986.
2. *Bissonnette RP, Echeverri F:* Apoptotic cells induced by c-myc are inhibited by Bcl-2. Nature 359:552-554, 1992.
3. *Bubendorf L, Sauter G, Moch H, et al:* Prognostic significance of Bcl-2 in clinically localized prostate cancer. Am J Surg Pathol 148:1557-1565, 1996.
4. *Chilosi M, Facchetti F, Dei Tos AP, et al:* Bcl-2 expression in pleural and extrapleural solitary fibrous tumors. J Pathol 181:362-367, 1997.
5. *Dei Tos AP, Wadden C, Calonje E:* Immunohistochemical demonstration of glycoprotein p30/30 MIC2 (CD99) in synovial sarcoma. A potential cause of diagnostic confusion. Applied immunohistochemistry 3:168-173, 1995.
6. *Doglioni C, Dei Tos AP, Laurino L, et al:* Calretinin: a novel immunocytochemical marker for mesothelioma. Am J Surg Pathol 20:1037-1046, 1996.
7. *Enzinger FM, Weiss SW:* Soft tissue tumors. St. Louis, Mosby, 1995.
8. *Fisher C:* Ultrastructural and immunohistochemical features of epithelial differentiation in monophasic and biphasic tumors. Hum Pathol 17:996-1008, 1986.
9. *Folpe AL, Schmidt RA, Chapman D, et al:* Poorly differentiated synovial sarcoma. Immunohistochemical distinction from primitive neuroectodermal tumors and high grade malignant peripheral nerve sheath tumors. Am J Surg Pathol 22:673-682, 1998.
10. *Gaertner E, Zeren EH, Fleming MV, et al:* Biphasic synovial sarcoma arising in the pleural cavity. A clinicopathologic study of five cases. Am J Surg Pathol 138:505-513, 1991.
11. *Hall PA, Levison DA:* Biphasic tumor: clues to possible histogenesis in developmental processes. J Pathol 159:1-2, 1989.
12. *Joensuu H, Pylkkanen L, Toikkanen S:* Bcl-2 protein expression and long term survival in breast cancer. Am J Pathol 145:1191-1198, 1994.
13. *Kulig E, Jin L, Qian X, et al:* Apoptosis in non-tumorous and neoplastic human pituitaries. Expression of the Bcl-2 family of proteis. Am J Pathol 154:767-774, 1999.
14. *Lu QL, Abel P, Foster CS, et al:* Bcl-2: role in epithelial differentiation and oncogenesis. Hum Pathol 27:102-110, 1996.
15. *McPake CR, Tillman DM, Poquette CA, et al:* Bax is an important determinant of chemosensitivity in pediatric tumor cell lines independent of Bcl-2 expression and p53 status. Oncol Res 10:235-244, 1998.
16. *Meis-Kindblom JM, Stenman G, Kindblom LG:* Differential diagnosis of small round cell tumors. Semin Diagn Pathol 13:213-241, 1996.
17. *Nakanishi H, Ohsawa M, Naka N, et al:* Immunohistochemical detection of Bcl-2 and p53 proteins and apoptosis in soft tissue sarcoma: their correlation with prognosis. Oncology 54:238-244, 1997.
18. *Nicholson AG, Goldstraw P, Fisher C:* Synovial sarcoma of the pleura and its differentiation from other primary pleural tumors: a clinicopathological and immunohistochemical review of three cases. Histopathology 33:508-513, 1998.
19. *Oltavi ZN, Milliman CL, Korsmeyer SJ:* Bcl-2 hemerodimer in vivo with a conserved homologue, Bax, that accelerates programmed cell death. Cell 74:609-619, 1993.
20. *Ordonez NG, Mahfouz SM, MacKay B:* Synovial sarcoma: an immunohistochemical and ultrastructural study. Hum Pathol 21:733-749, 1990.
21. *Ordonez NG:* The immunohistochemical diagnosis of epithelial mesothelioma. Hum Pathol 30:313-323, 1999.
22. *Salisbury JR, Isaacson PG:* Synovial sarcoma: an immunohistochemical study. J Pathol 147:49-57, 1985.
23. *Segers K, Ramael M, Singh Sk, et al:* Immunoreactivity for Bcl-2 protein in malignant mesothelioma and non-neoplastic mesothelium. Virchows Arch 424:631-634, 1994.
24. *Singer S, Baldini EH, Demetri GD, et al:* Synovial sarcoma: prognostic significance of tumor size, margin sesection and mitotic activity for survival. J Clin Oncol 14:1201-1208, 1996.
25. *Suster S, Fisher C, Moran CA:* Expression of Bcl-2 oncoprotein in benign and malign spindle cell tumors of soft tissue, skin, serosal surfaces and gastrointestinal tract. Am J Surg Pathol 22:863-872, 1998.
26. *Tormanen U, Eerole AK, Rainio P, et al:* Enhanced apoptosis predicts shortened survival in non-small cell lung carcinoma. Cancer Res 55:595-602, 1995.