

Original Article

β -catenin expression in benign and malignant pleural disorders

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Abstract: Benign and malignant pleural processes display a large and overlapping spectrum of morphological appearances, and can be difficult to distinguish, histologically, from each other. β -catenin, a participant in the wingless-type (Wnt) transduction pathway, is involved in the pathogenesis of malignant mesothelioma and has received limited evaluation for its ability to serve as a diagnostic aid for distinguishing between individual pleural disorders. We performed immunohistochemistry for β -catenin on 10 pleural malignant mesotheliomas, 10 examples of mesothelial hyperplasia and 18 cases of organizing pleuritis. Although differences were noted in staining intensity between the mesothelioma and mesothelial hyperplasia groups, extensiveness and cellular location were similar. Staining intensity (mean $\pm$ s.d.) in mesotheliomas (2.00 $\pm$ 0.67) was significantly less intense than in mesothelial hyperplasia cases (3.00 $\pm$ 0.00) ($p=0.0005$). Stromal cell staining was cytoplasmic in all cases, and endothelial cell staining was membranous, submembranous and cytoplasmic. Nuclear expression of β -catenin was not observed in any of the cases studied. This lack of nuclear staining in the stromal cells of organizing pleuritis differs markedly from the previously reported high frequencies of nuclear β -catenin expression in other pleural spindle cell proliferations (desmoid tumors and solitary fibrous tumors). In summary, the current study adds to previous work indicating a role for β -catenin in the genesis of pleural conditions including organizing pleuritis, mesothelial hyperplasia and malignant mesothelioma. Although IHC for β -catenin does not appear to be conclusive for separating benign from malignant mesothelial proliferations, it may be valuable for assisting in the differential diagnosis of mesothelial and spindle cell proliferations in the pleura.

Keywords: β -catenin, mesothelioma, pleura, mesothelial hyperplasia, Wnt

Introduction

Pleural diseases can present with a wide range of morphologic appearances, leading to challenges in the histologic diagnosis of pleural neoplasms and benign pleural disorders. Differentiating malignant mesothelioma and metastatic carcinomas from other causes of pleural thickening and effusion, however, remains critically important for determination of therapy. Although these processes can resemble each other grossly and microscopically, immunohistochemistry (IHC) can often help to resolve the differential diagnosis. It has been a particularly valuable tool for diagnosis of metastatic adenocarcinomas involving the pleural space, and for confirmation of mesothelial differentiation. IHC has also been useful for distinguishing between solitary fibrous tumor and desmoid tumor. Un-

fortunately, however, despite numerous investigations utilizing a variety of antibodies, no antibody has emerged as a reliable discriminator between benign and malignant mesothelial proliferations, although some studies suggest a helpful role for epithelial membrane antibody (EMA), desmin, glucose transporter-1 (GLUT-1), tenascin-X, CD146, p53, Ki67, hTERT, insulin-like growth factor-II mRNA-binding protein 3 (IMP3), and K homolog domain containing protein overexpressed in cancer (KOC) [1-15]. The specific EMA antibody clone has also been reported to be important, regarding its utility for differentiating between reactive mesothelial cells and malignant mesothelioma [16].

The wingless-type (Wnt) transduction pathway plays an important role in the genesis of many neoplasms [17, 18], and appears to be impor-

tant in the pathogenesis of mesothelioma [19, 20]. β -catenin, a participant in the Wnt pathway, has undergone limited study for its value in assisting in the morphologic differentiation between mesotheliomas and reactive mesothelial proliferations [21, 22]. β -catenin's roles in the cell include functioning in cell-cell adhesion and participating in Wnt signaling [17, 23]. Cytoplasmic accumulation of β -catenin occurs with activation of the Wnt pathway through Wnt ligand binding to the frizzled transmembrane receptors, leading to the stabilization and accumulation of β -catenin [20]. Interactions of β -catenin with transmembrane cadherins promote cell-cell adhesion. Nuclear translocation of β -catenin leads to interactions with TCF/LEF transcription factors to promote expression of the oncogenes c-myc and cyclin D1. Nuclear localization of β -catenin was reported in 19% of mesotheliomas and no examples of mesothelial hyperplasia, which demonstrated immunoreactivity localized to the cell membrane [21]. Another study of β -catenin expression, in a spectrum of benign and malignant mesothelial proliferations, revealed increased cytoplasmic and nuclear staining in mesotheliomas as compared with normal and reactive mesothelial proliferations which showed membranous and/or submembranous staining [22]. Given these observations, we decided to evaluate the utility of β -catenin IHC for distinguishing between malignant mesothelioma and reactive pleural processes, as a potential approach for assisting in the interpretation of difficult cases.

Materials and methods

Institutional review board approval

This study was reviewed and approved by the Penn State College of Medicine Institutional Review Board prior to its initiation, and was given exemption from the requirement for informed consent.

Cases

Cases were identified from a text search of surgical pathology reports at Penn State Milton S. Hershey Medical Center. They included 18 examples of organizing pleuritis (5 with a component of acute pleuritis), 10 cases of mesothelial hyperplasia, and 10 malignant mesotheliomas (7 epithelioid, 3 biphasic). All cases were reviewed by two of the authors (WA, DSZ) to con-

firm the diagnoses.

Immunohistochemical staining and assessment

Immunohistochemistry was performed on 5-mm recut sections of the formalin-fixed paraffin-embedded tissues, using a monoclonal mouse antibody to β -catenin-1 (M3539, Dako, Carpinteria, CA). Heat-induced antigen retrieval with 1 mM ethylenediaminetetraacetic acid, pH 8.0, was followed by incubation in primary antibody (diluted to 1:400) at room temperature for 30 min. Detection of the primary antibody was carried out with Envision+ antimouse/horseradish peroxidase-labeled polymer detection system (Dako, Carpinteria, CA) followed by visualization with 3,3'-diaminobenzidine (DAB) (Dako) and counterstaining with Mayer's modified hematoxylin. Formalin-fixed paraffin-embedded colonic tissue sections were used as positive controls and were stained with the same protocol as the experimental cases. Negative control slides were subjected to the same staining protocol modified by the substitution of diluent for the primary antibody.

The types of cells staining, the cellular localization of the staining, and extensiveness of the staining (represented by the percentage of cells staining in each cell population) were evaluated simultaneously by two of the authors (WA and DSZ). Between 25 and 200 cells were counted in each population. Staining extensiveness was scored as positive ($\geq 10\%$ of cells staining) or negative ($<10\%$ of cells staining). The staining intensity was also evaluated by the same two authors and assigned a value of 0 (no staining), 1+ (weak staining), 2+ (moderate staining) or 3+ (strong staining), based upon the predominant staining intensity.

Statistical analysis

Data are expressed as mean $\pm$ standard derivation. Statistical significance was determined using the U-test and statistical significance was achieved when the p value was <0.05 .

Results

Important differences were noted in staining intensity between the mesothelioma and mesothelial hyperplasia groups, while extensiveness and location were similar (**Figures 1, 2 and 3**). Staining intensity (mean $\pm$ s.d.) in mesothelio-

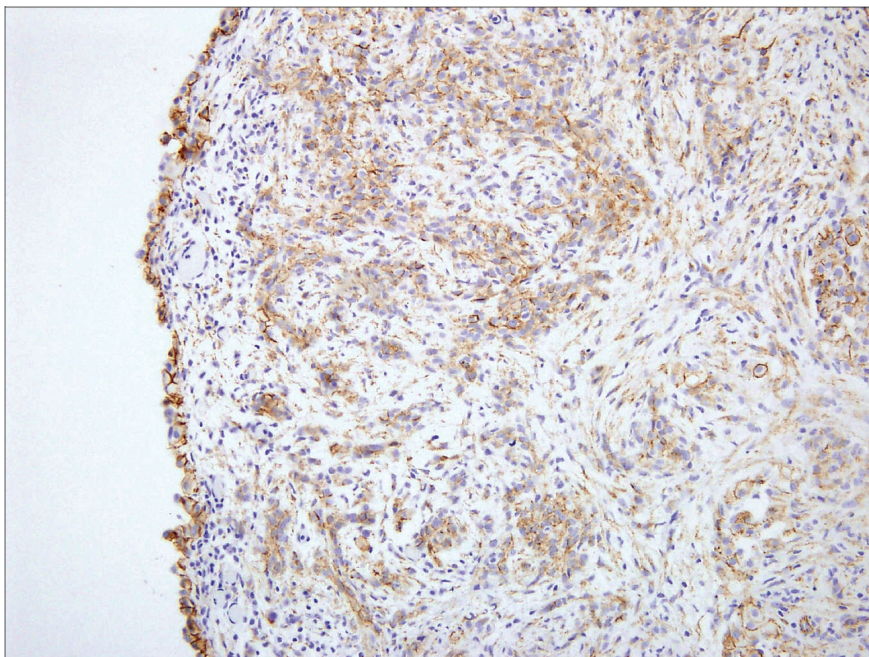


Figure 1. Malignant mesothelioma, invasive and involving the pleural surface, β -catenin IHC. There is diffuse staining of the tumor cells for β -catenin with more intense staining of the neoplastic cells on the pleural surface than the invasive component. 200x magnification.

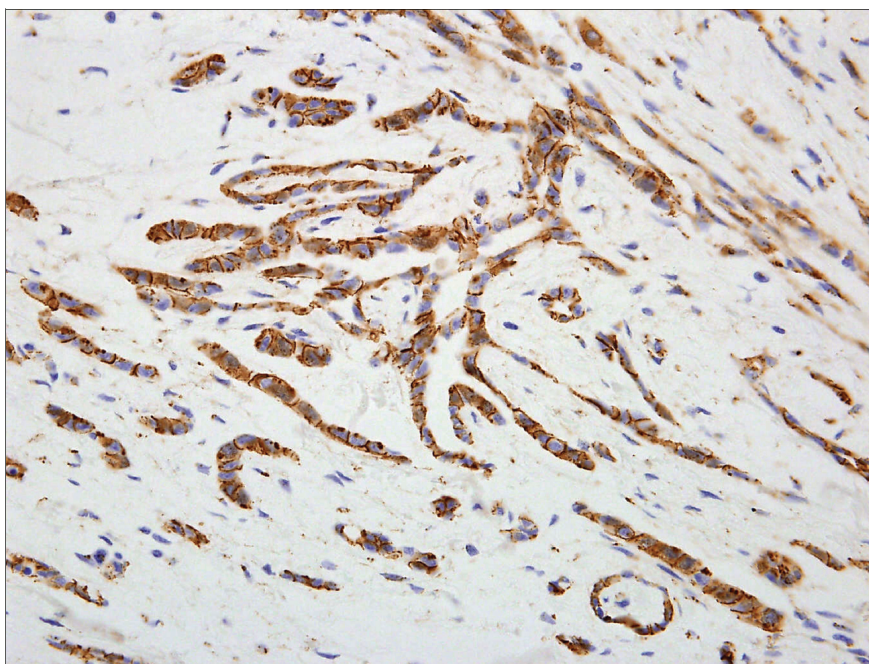


Figure 2. Malignant mesothelioma, β -catenin IHC. Tumor cells demonstrate strong staining for β -catenin in a membranous, submembranous, and cytoplasmic distribution. 400x magnification.

mas (2.00 ± 0.67) was significantly less intense than in mesothelial hyperplasia cases (3.00 ± 0.00) ($p=0.0005$). Staining intensity tended to decrease in the more deeply invasive areas of the mesotheliomas as compared to the areas closer to the pleural surface. Diffuse staining for β -catenin was observed in all cases of malignant mesothelioma, mesothelial hyper-

plasia, and pleuritis, however. In the cases of malignant mesothelioma, 90% or more of the tumor cells stained in all cases, and the localization was membranous, submembranous and cytoplasmic in 8/10 cases and membranous-only in 2/10 cases. Similarly, in the mesothelial hyperplasia cases, at least 90% of the hyperplastic mesothelial cells expressed β -catenin,

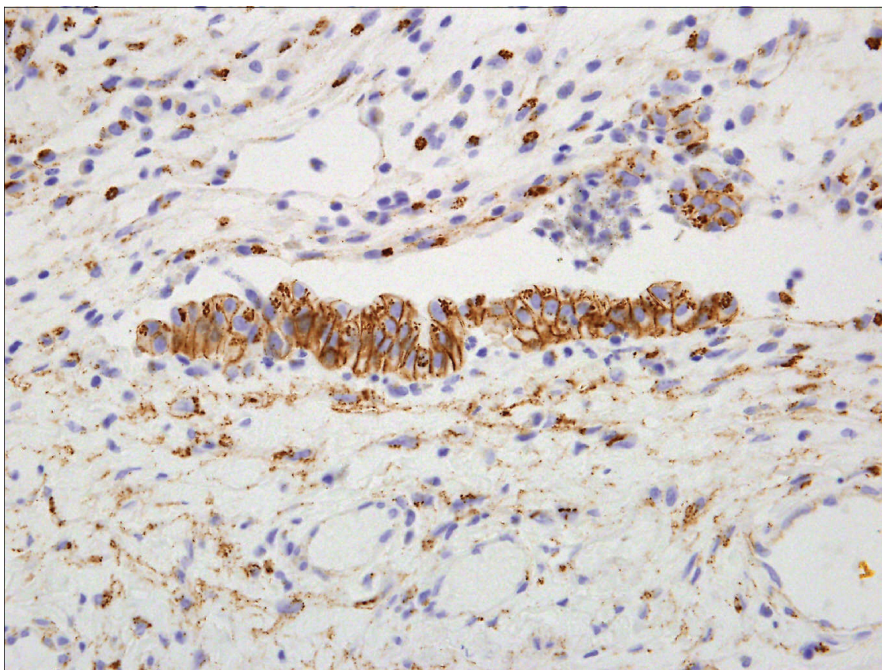


Figure 3. Hyperplastic mesothelial cells, β -catenin IHC. This small focus of mesothelial hyperplasia shows strong staining for β -catenin. Scattered stromal cells and inflammatory cells also stain. 400x magnification.

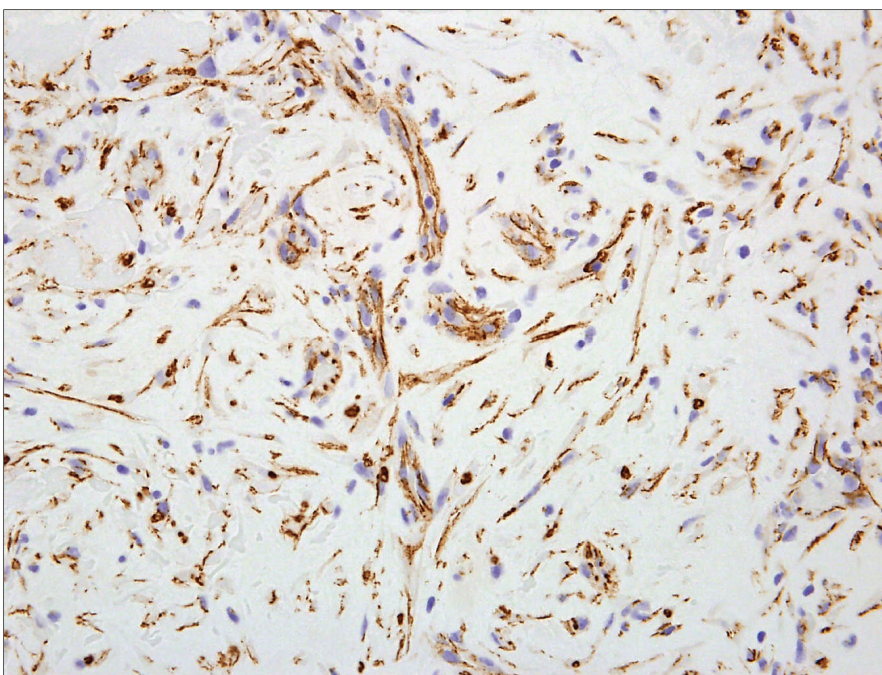


Figure 4. Organizing pleuritis, β -catenin IHC. Stromal cells and endothelial cells demonstrate extensive β -catenin expression. 400x magnification.

with a membranous, submembranous and cytoplasmic distribution in 9/10 cases, and a solely membranous location in 1/10 cases. At least 90% of the stromal and endothelial cells were immunoreactive for β -catenin in all of the cases of organizing pleuritis (**Figure 4**). Stromal cell staining was cytoplasmic in all cases, and endothelial cell staining was membranous, submembranous and cytoplasmic. No nuclear expres-

sion of β -catenin was observed in any of the samples.

Discussion

Our results suggest that staining intensity for β -catenin differs between mesothelioma and mesothelial hyperplasia, while staining extensiveness and cellular localization are similar.

These results have similarities and differences compared to previous studies, which utilized a different primary antibody for β-catenin. Previous studies similarly found high frequencies of membranous, submembranous and cytoplasmic staining for β-catenin in mesotheliomas, but report nuclear staining in some cases, which was not observed in the current investigation. Dai et al. found that invasive mesotheliomas demonstrated more frequent cytoplasmic (72%) and nuclear staining (37%) and reduced membranous and/or submembranous staining (21%) with β-catenin than normal or reactive mesothelium, which showed distinct membranous and/or submembranous β-catenin in 20/23 (87%) cases [22]. Faint cytoplasmic staining, but no nuclear expression, of β-catenin was observed in three cases of florid reactive mesothelium, but nuclear expression was not found. Similarly, Abutailly and colleagues reported purely membranous staining in nine cases of mesothelial hyperplasia, while membranous and cytoplasmic staining for β-catenin was found in all 63 mesotheliomas and nuclear staining occurred in 19% [21]. Comparison with our results shows a higher frequency of cytoplasmic staining in our reactive cases than in these other studies, while our observation of membranous staining as a feature of mesotheliomas is in agreement with the report of Abutailly et al [21]. It is likely that the differences in staining pattern and intensity stems from the use of different antibodies, and argues for the importance of correlating staining results observed in practice with the results reported for the specific antibody clone chosen for staining.

Our results expand on the work done previously by a member of our group and colleagues [24], and support a role for β-catenin IHC in separating organizing pleuritis from solitary fibrous tumors (SFTs) and desmoid tumors (DTs) involving the pleura. In this earlier study, extensive (≥70% of the tumor cells) nuclear staining for β-catenin was observed in all (4/4) of the DTs, 4/5 of the histologically benign SFTs, and 2/6 of the histologically malignant SFTs, with intermediate or high intensity, as compared to the absence of nuclear staining in all of the examples of organizing pleuritis assessed in the current study. The antibody used by Andino et al was the same clone as the antibody employed in the current study. Given the diagnostic challenges presented by some spindle cell proliferations involving the pleura, we believe that β-catenin IHC

can offer help for differentiating between organizing pleuritis, SFT, and DT.

In conclusion, the current study supports a role for β-catenin in the genesis of a broad spectrum of pleural conditions including organizing pleuritis, mesothelial hyperplasia and malignant mesothelioma. Although IHC for β-catenin does not appear to be valuable for separating benign from malignant mesothelial proliferations, it may be valuable for the differential diagnosis of spindle cell proliferations in the pleura.

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References

- [1] Attanoos RL, Webb R and Gibbs AR. CD44H expression in reactive mesothelium, pleural mesothelioma and pulmonary adenocarcinoma. *Histopathology* 1997; 30: 260-263.
- [2] Attanoos RL, Griffin A and Gibbs AR. The use of immunohistochemistry in distinguishing reactive from neoplastic mesothelium. A novel use for desmin and comparative evaluation with epithelial membrane antigen, p53, platelet-derived growth factor-receptor, P-glycoprotein and Bcl-2. *Histopathology* 2003; 43: 231-238.
- [3] Cai YC, Roggli V, Mark E, Cagle PT and Fraire AE. Transforming growth factor alpha and epidermal growth factor receptor in reactive and malignant mesothelial proliferations. *Arch Pathol Lab Med* 2004; 128: 68-70.
- [4] Cakir C, Gulluoglu MG and Yilmazbayhan D. Cell proliferation rate and telomerase activity in the differential diagnosis between benign and malignant mesothelial proliferations. *Pathology* 2006; 38: 10-15.
- [5] Dejmek A, Hjerpe A. Reactivity of six antibodies in effusions of mesothelioma, adenocarcinoma and mesotheliosis: stepwise logistic regression analysis. *Cytopathology* 2000; 11: 8-17.
- [6] Hasteh F, Lin GY, Weidner N and Michael CW. The use of immunohistochemistry to distinguish reactive mesothelial cells from malignant mesothelioma in cytologic effusions. *Cancer Cytopathol* 2010; 118: 90-96.

- [7] Hanley KZ, Facik MS, Bourne PA, Yang Q, Spaulding BO, Bonfiglio TA and Xu H. Utility of anti-L523S antibody in the diagnosis of benign and malignant serous effusions. *Cancer* 2008; 114: 49-56.
- [8] Ikeda K, Tate G, Suzuki T, Kitamura T and Mitsuya T. Diagnostic usefulness of EMA, IMP3, and GLUT-1 for the immunocytochemical distinction of malignant cells from reactive mesothelial cells in effusion cytology using cytopspin preparations. *Diagn Cytopathol* 2011; 39: 395-401.
- [9] Ikeda K, Tate G, Suzuki T, Kitamura T and Mitsuya T. IMP3/L523S, a novel immunocytochemical marker that distinguishes benign and malignant cells: the expression profiles of IMP3/L523S in effusion cytology. *Hum Pathol* 2010; 41: 745-750.
- [10] Kato Y, Tsuta K, Seki K, Maeshima AM, Watanabe S, Suzuki K, Asamura H, Tsuchiya R and Matsuno Y. Immunohistochemical detection of GLUT-1 can discriminate between reactive mesothelium and malignant mesothelioma. *Mod Pathol* 2007; 20: 215-220.
- [11] Sato A, Torii I, Okamura Y, Yamamoto T, Nishigami T, Kataoka TR, Song M, Hasegawa S, Nakano T, Kamei T and Tsujimura T. Immunocytochemistry of CD146 is useful to discriminate between malignant pleural mesothelioma and reactive mesothelium. *Mod Pathol* 2010; 23: 1458-1466.
- [12] Schonherr A, Bayer M and Bocking A. Diagnostic and prognostic value of Ki67 proliferation fraction in serous effusions. *Cell Oncol* 2004; 26: 57-62.
- [13] Shi M, Fraire AE, Chu P, Cornejo K, Woda BA, Dresser K, Rock KL and Jiang Z. Oncofetal protein IMP3, a new diagnostic biomarker to distinguish malignant mesothelioma from reactive mesothelial proliferation. *Am J Surg Pathol* 2011; 35: 878-882.
- [14] Wolanski KD, Whitaker D, Shilkin KB and Henderson DW. The use of epithelial membrane antigen and silver-stained nucleolar organizer regions testing in the differential diagnosis of mesothelioma from benign reactive mesothelioses. *Cancer* 1998; 82: 583-590.
- [15] Yuan Y, Nymoen DA, Stavnes HT, Rosnes AK, Bjorang O, Wu C, Nesland JM and Davidson B. Tenascin-X is a novel diagnostic marker of malignant mesothelioma. *Am J Surg Pathol* 2009; 33: 1673-1682.
- [16] Saad RS, Cho P, Liu YL and Silverman JF. The value of epithelial membrane antigen expression in separating benign mesothelial proliferation from malignant mesothelioma: a comparative study. *Diagn Cytopathol* 2005; 32: 156-159.
- [17] Morin PJ. beta-catenin signaling and cancer. *Bioessays* 1999; 21: 1021-1030.
- [18] Polakis P. The many ways of Wnt in cancer. *Curr Opin Genet Dev* 2007; 17: 45-51.
- [19] Uematsu K, Kanazawa S, You L, He B, Xu Z, Li K, Peterlin BM, McCormick F and Jablons DM. Wnt pathway activation in mesothelioma: evidence of Dishevelled overexpression and transcriptional activity of beta-catenin. *Cancer Res* 2003; 63: 4547-4551.
- [20] Lee AY, Raz DJ, He B and Jablons DM. Update on the molecular biology of malignant mesothelioma. *Cancer* 2007; 109: 1454-1461.
- [21] Abutailly AS, Collins JE and Roche WR. Cadherins, catenins and APC in pleural malignant mesothelioma. *J Pathol* 2003; 201: 355-362.
- [22] Dai Y, Bedrossian CW and Michael CW. The expression pattern of beta-catenin in mesothelial proliferative lesions and its diagnostic utilities. *Diagn Cytopathol* 2005; 33: 320-324.
- [23] Ben Ze'ev A, Shtutman M and Zhurinsky J. The integration of cell adhesion with gene expression: the role of beta-catenin. *Exp Cell Res* 2000; 261: 75-82.
- [24] Andino L, Cagle PT, Murer B, Lu L, Popper HH, Galateau-Salle F, Sienko AE, Barrios R and Zander DS. Pleuropulmonary desmoid tumors: immunohistochemical comparison with solitary fibrous tumors and assessment of beta-catenin and cyclin D1 expression. *Arch Pathol Lab Med* 2006; 130: 1503-1509.