Title*:* Is immunohistochemical staining for β-catenin the definitive pathological diagnostic tool for desmoid-type fibromatosis? : A multi-institutional study

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Running head: Significance of β-catenin staining for desmoid

Declarations of interest: none

Funding disclosures

This work was supported in part by the Ministry of Health, Labor and Welfare of Japan, and the Ministry of Education, Culture, Sports, Science and Technology of Japan

[Grant-in-Aid 17H01585 for Scientific Research (A)], and the National Cancer Center Research and Development Fund (29-A-3).

Keywords: Desmoid-type fibromatosis; β-catenin; immunohistochemical staining; *CTNNB1* mutation

Abstract

Immunohistochemical staining with anti-β-catenin antibody has been applied as a diagnostic tool for desmoid-type fibromatoses (DF). In recent years, specific gene mutation (*CTNNB1*) analysis has also been reported to be useful for diagnosis of DF, however the association between *CTNNB1* mutation status and immunohistochemical staining pattern of β-catenin is rarely reported. The purpose of this study is to clarify the relationship of the staining pattern of β-catenin with the *CTNNB1* mutation status and various clinical variables, and investigate the significance of immunohistochemical staining of β-catenin in cases diagnosed with DF. Between 1997 and 2017, 104 cases diagnosed with DF from 6 institutions in Japan were enrolled in this study: Nagoya University; National Cancer Center Hospital; Niigata University; Okayama University; Kyushu University, and Cancer Institute Hospital. For all cases, immunohistochemical staining of β-catenin and gene mutation analysis of *CTNNB1* were performed. Of 104 cases, 87 (84%) showed nuclear staining of β-catenin, and 95 (91%) positive staining in cytoplasm. The proportion of cases showing strong nuclear staining of β-catenin was significantly higher in the cases with S45F than in those with T41A or wild type (WT). The proportion of cases stained strongly in the cytoplasm rather than in the nucleus was significantly higher in the group of T41A than that of S45F or WT. Among 17 cases in which nuclear immunostaining were absent, *CTNNB1* mutation was observed in 5 cases (29.4%). There were unignorable cases of DF with negative β-catenin immunostaining despite a definitive clinical and pathological diagnosis of DF and/or positive *CTNNB1* mutation.

Keywords: Desmoid-type fibromatosis; β-catenin; immunohistochemical staining;

CTNNB1 mutation

1. Introduction

Desmoid-type fibromatoses (DF) are soft tissue tumors with clonal origin and mesenchymal fibroblastic/myofibroblastic proliferation, with an incidence of two to four cases per million persons per year [1]. Most cases develop sporadically in adults, whereas a few occur against a family background of familial adenomatous polyposis (FAP) [2]. DF are generally highly invasive and locally aggressive, but do not metastasize [3,4], and are categorized as intermediate tumors in the World health organization (WHO) classification [5].

Somatic mutations at exon 3 of *CTNNB1* gene have been reported as a molecular pathogenesis of sporadic DF. The mutation generally occurs at codon 41 or 45. These mutations inhibit phosphorylation of β-catenin, which protect β-catenin from degradation by APC (adenomatous polyposis coli) complex, resulting in nuclear accumulation of β-catenin, and activation of the T cell factor/lymphoid enhancer factor (TCF/LEF) pathway [6].

A recent study showed that *CTNNB1* mutations were highly prevalent and detected in 88% of sporadic DF, in contrast to no hot spot *CTNNB1* mutations in any of the spindle cell non-desmoid lesions [7]. The hot spot mutation of *CTNNB1* is thought to be very specific for DF. Recent studies exhibited that tumors with *CTNNB1* S45F mutation were reported to exhibit resistance to surgical and conservative treatment [8,9], suggesting *CTNNB1* mutation analysis might be important not only for the diagnosis of DF but also for determining the subsequent treatment options. However, *CTNNB1* mutation analysis

is performed only in limited facilities in Japan and has not yet been generally applied for clinical use as a diagnostic tool. Whereas immunohistochemical staining of β-catenin has been considered useful for the diagnosis of DF [6,10–16]. These studies investigated the significance of immunohistochemical staining of β-catenin or *CTNNB1* mutation status independently, few reports demonstrated the correlation between immunohistochemical staining pattern of β-catenin and *CTNNB1* mutation status.

In the present study, we aimed to clarify the association between staining pattern of β-catenin, patient characteristics, and *CTNNB1* mutation status and assess the significance of immunohistochemical staining for nuclear or cytoplasmic β-catenin in 104 sporadic DF cases.

2. Materials and Methods

2.1. Tissue samples

This study has been performed according to the Declaration of Helsinki, and ethical approval from the appropriate committees of Nagoya University was obtained for this study. Formalin-fixed, paraffin-embedded specimens accumulated between 1994 and 2017 were retrieved from 6 institutions: Nagoya University; National Cancer Center Hospital; Niigata University; Okayama University; Kyushu University, and Cancer Institute Hospital. There were 113 cases diagnosed as sporadic DF. To confirm the histological diagnosis of DF, sections of all cases were transmitted to Pathology lab at Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital for central pathology review (T.M.). Based on the evaluation of central review and careful discussion between the pathologist and physicians, nine cases were excluded. No cases with an obvious history of FAP were included. Finally, this study is composed of

one-hundred four cases of DF. Specimens obtained by biopsy or surgical excision were formalin-fixed and paraffin-embedded, and subjected to examination of immunohistochemistry for β-catenin and *CTNNB1* mutation analysis. The following clinical data were collected: age at the diagnosis, gender, tumor location, and tumor size.

2.2. Mutation analysis

DNA was extracted from frozen tissue or formalin-fixed, paraffin-embedded tissue with the High Pure PCR Template Preparation Kit (Roche Molecular Diagnostics, Mannheim, Germany), according to the manufacturer's protocol. Extracted DNA was amplified by polymerase chain reaction (PCR) using LightCycler 480 System (Roche). To analyze the point mutations in codons 41 or 45 of *CTNNB1* exon 3, we designed two pairs of primers: forward 59- GATTTGATGGAGTTGGACATGG-39, reverse 59-TCTTCCTCAGGATTGCCTT-39, and forward 59-TGGAACCAGACAGAAAAGCG-39, reverse 59-TCAGGATTGCCTTTACCACTC-39. PCR products were isolated by gel electrophoresis, and amplified bands were extracted and purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA). Purified products were subjected to direct sequencing using the above primers (forward), with Applied Biosystems Big Dye Terminator V3.1, and Applied Biosystems 3730x DNA analyzer (Applied Biosystems, Foster City, CA) at FASMAC Co. Ltd. (Kanagawa, Japan). All sequencing results were compared with those in the databases of NCBI BLAST to confirm the mutation sites.

2.3. Immunohistochemistry

All tumor samples were obtained by needle or incisional biopsy. Biopsy specimens were fixed in 10% formaldehyde and embedded in paraffin. Immunohistochemistry was performed using a Ventana BenchMark® XT automated slide staining system (Roche). For antigen retrieval, slides were heated in an oven for 60 minutes. Endogenous peroxidase was blocked with the appropriate kit. Primary antibody against β-catenin (dilution 1:50; DAKO mouse monoclonal antibody, M3539) was then applied for 32 min at 37℃ in BenchMark® XT stainer and revealed with the DAB detection kit, yielding a brown reaction product. Nuclear or cytoplasmic positivity of β-catenin was evaluated by two independent observers (S.H., H.K.) without any knowledge of the clinicopathological information under the guidance of the experienced pathologists (Y.S. and K.K.), and the stainability was also validated by them. Staining positivity was divided into–four groups; absent (0%), weak (1% to 9%), moderate (10% to 50%), or strong (51% to 100%) by the numbers of positive stained cells (Fig. 1). Images of higher magnification are shown in Fig. 2.

2.4. Statistical analysis

Data are summarized by frequency and percentages for categorical variables and by mean and range for continuous variables. Differences between groups were assessed using Chi-squared test for categorical variables. Statistical analyses were performed using SPSS ver.24 (SPSS, Inc., Chicago, IL) and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

The mean age at the time of diagnosis was 42.3 years (range, 8-80). There were 32 males and 72 females. The location of tumors was the abdominal wall in 18 cases, an extremity in 28 cases, head and neck in 12 cases, and trunk in 46 cases. Shoulder and pelvic girdles were defined as trunk region in this study. The mean tumor size was 7.9 cm (range, 2-20) (Table 1).

3.2. Mutation status of *CTNNB1*

We performed genotyping of *CTNNB1* exon 3 for all the cases using tumor specimens and 5 cases of non-DF soft tissue tumors as a control group (Table S1). Replacement of threonine by alanine in codon 41 (T41A) was detected in 43 of the 104 cases (41%). Replacement of serine by phenylalanine in codon 45 (S45F) was identified in 10 of the 104 cases (10%). Fifty-one of the 104 cases (49%) did not have mutations in exon 3 (WT). There were no positive cases of hot spot mutation of *CTNNB1* in a control group, which was consistent with the previous report investigating 260 DF and 191 DF mimics that hot spot mutation of *CTNNB1* was specific for diagnosis of DF [7]. There were no significant correlations between mutation status of *CTNNB1* and age ($P = 0.84$), gender ($P = 0.58$), tumor size ($P = 0.29$), or tumor location ($P = 0.88$) (Table 2).

3.3. Immunohistochemical findings

Of the 104 cases evaluated, nuclear positivity of β-catenin staining was absent in 17, weak in 16, moderate in 47, and strong in 24, and cytoplasmic positivity was absent in 9, weak in 6, moderate in 25, and strong in 64. The ratio of the cases with nuclear stainability equal to or more than weak was 84%, and the percentage of the cases with cytoplasmic stainability equal to or more than weak was 91%. No significant

correlations were found between nuclear staining positivity and patient characteristics (Table 3), or cytoplasmic staining positivity (Table 4). Next, the relationship between staining positivity of β-catenin and *CTNNB1* mutation status was evaluated. There were no significant correlations between mutation statuses of *CTNNB1* and nuclear or cytoplasmic staining positivity dividing the positivity into strong/moderate/weak and absent groups (nuclear; $P = 0.24$, cytoplasmic; $P = 0.91$). However, dividing the positivity into strong and moderate/weak/absent groups, strong nuclear staining was significantly higher in cases with S45F than in cases with T41A or WT ($P < 0.01$, Table 5). The proportion of the cases with strong cytoplasmic staining was significantly higher in cases with T41A and S45F than in those with WT ($P < 0.05$; Table 5). Next, we evaluated whether stainability was more intense in nucleus or cytoplasm. The proportion of cases with staining predominant in cytoplasm was significantly higher in cases with T41A mutation than in those with S45F or WT ($P < 0.01$; Table 6).

Among 17 cases in which nuclear immunostaining were absent, hot spot *CTNNB1* mutation was observed in 5 cases (29.4%). On the other hand, among 51 cases negative for *CTNNB1* mutation, 39 cases (76%) showed positive nuclear immunostaining. Eighty-seven cases were nuclear positive for β-catenin, among them, CTNNB1 hot spot mutation was negative in 39 cases. Because positivity often differs according to the antibodies used for immunohistochemistry, we additionally evaluated the nuclear β-catenin stainability using a different antibody (dilution 1:50; Novocastra mouse monoclonal antibody, NCL-B-CAT). Ninety five cases were subjected to immunohistochemical staining using both antibodies. Thirty cases (32%) showed negative nuclear staining for β-catenin using Novocastra antibody, and among these cases, *CTNNB1* mutation was observed in 10 cases (33%) (Table 7). Identical results

were obtained with this different antibody (Novocastra) to those with DAKO antibody, including the relationship between staining pattern and CTNNB1 mutation status (Table S2-5).

4. Discussion

Res The results of this study revealed that not a few cases (16%) showed negative nuclear staining of β-catenin with a clinical and pathological diagnosis of DF. Moreover, among these cases, 29.4% had positive *CTNNB1* mutation. *CTNNB1* mutation has been reported to be highly prevalent in sporadic DF [7,8,12,17]. Le Guellec *et al.* identified the common *CTNNB1* mutations associated with sporadic DF by direct sequencing in 260 cases of typical DF and in 191 cases of spindle cell lesions, which can morphologically 'mimic' DF. They reported that *CTNNB1* mutations were observed in 88% of sporadic DF but not in any other lesions [7]. Amary *et al.* also analyzed 133 cases including 76 DF, and 18 superficial fibromatoses, in addition to a further 39 fibromatosis mimics. They reported that mutations were detected in 87% of 76 DF but in none of the other lesions studied [12]. Several reports investigated exon 3 *CTNNB1* mutations in carcinoma [18–25]. The same hot spot mutations as DF were observed in 6% of these carcinomas [7]. However, from a morphological point of view, differentiating histologically such neoplasms, carcinomas, from DF is not diagnostically problematic. Occasionally, spindle cell tumors and sarcomatous lesions need to be differentiated from DF. As reported by Guellec *et al*. and Amary *et al*., no *CTNNB1* mutations were observed in these suspiciously similar tumors, suggesting that *CTNNB1* mutation analysis is specific and useful for the diagnosis of DF. In the Japanese clinical setting, *CTNNB1* mutation analyses are not performed in most institutions, including

even specialist soft tissue tumor centers. Some of the institutions send specimens of cases with DF suspected to our institution (N.U.) to conduct *CTNNB1* mutation analysis in the present status. Considering that large pathology labs have already performed *CTNNB1* mutation analysis in US and European countries, centralized system for the analysis should be established.

There is also evidence that immunohistochemical detection of β-catenin can be helpful in the diagnosis of DF [6,10–16]. In these studies, in the cases diagnosed as DF, the positive rate of immunohistochemical staining of β-catenin was reported as 82% to 100%, which was consistent with the result of our study. Based on these results, immunohistochemistry is considered to show relatively high sensitivity for the diagnosis of DF. On the other hand, several spindle cell tumors other than DF exhibited positive staining for β-catenin. Ng TL *et al*. reported that a high level of positive nuclear β-catenin staining (>25% of cells) was observed in tumors including solitary fibrous tumor (6 of 15 cases), endometrial stromal sarcoma (4 of 10 cases), synovial sarcoma (16 of 58 cases), fibrosarcoma (1 of 3 cases), and clear cell sarcoma (1 of 3 cases) [10]. Amary *et al*. reported that 72% of the fibromatosis mimics showed nuclear positivity for β-catenin [12]. Several other studies also described that immunohistochemical detection of nuclear β-catenin was not a reliable test for distinguishing DF from benign or malignant fibroblastic lesions [7,11,12,26,27]. These reports indicate that immunohistochemistry for β-catenin is not a specific test for DF. In addition, considering that stainability for β-catenin is dependent on the antibody used and that the protocols of immunohistochemical staining differed in individual studies, the results of the immunohistochemical staining cannot be simply adopted for diagnosis. In the present study, nuclear accumulation of β-catenin was observed in 82% (DAKO) of the

cases, but only 67% of them showed positive β-catenin expression in the nucleus using the different anti β-catenin antibody (Novocastra). Furthermore, the presence of cases with negative nuclear β-catenin even in the cases with positive *CTNNB1* mutation suggested that immunohistochemical staining of β-catenin might be a less sensitive test for the diagnosis of DF than *CTNNB1* mutation analysis. Although immunohistochemical staining of β-catenin cannot be simply adopted for the diagnosis of DF, it would be helpful combined with characteristic findings of clinical features, images of magnetic resonance, and general microscopic findings.

Few reports have evaluated the immunohistochemical staining of β-catenin in the cytoplasm of DF. Andino *et al.* reported that all of 4 cases of pleuropulmonary DF showed strong nuclear and cytoplasmic staining [28]. Bhattacharya *et al.* reported that all 21 cases showed focal to diffuse cytoplasmic staining [11]. Ng TL *et al.* also reported that cytoplasmic β-catenin staining was seen commonly in 17 cases of DF [10]. Similarly, Abraham *et al.* described that almost all of 33 cases (97%) of fibromatoses of the breast showed prominent cytoplasmic β-catenin staining [13]. In the report of Andino *et al.*, the cytoplasmic staining property was evaluated in 4 cases, with all of them showing positive results in cytoplasm. In this study, the cytoplasmic staining was evaluated in 4 grades as well as the nucleus in all 104 cases, and we showed that the percentage of cases with strong cytoplasmic staining was significantly higher in T41A or S45F than in WT. The intracellular staining intensity was compared between nucleus and cytoplasm, indicating that a significantly higher number of cases in T41A showed stronger cytoplasmic staining than that of nucleus in T41A than compared to those in S45F or WT.

In normal fibroblasts, β-catenin staining is limited to the cytoplasm and/or cell

membranes. However, in DF, the Wnt signaling pathway is activated or the β-catenin degradation pathway is inactivated, leading to β-catenin accumulation in the cytoplasm and/or nucleus. In this study, the staining pattern of nucleus and cytoplasm differed between *CTNNB1* mutation statuses. Phosphorylation of S45 is thought to be the initial process in the degradation of β-catenin, suggesting that S45F mutation may block the first step of phosphorylation, thereby precluding subsequent phosphorylation [29]. Extent of phosphorylation may vary among cases depending on the specific codon in which mutation occurs. We speculate that this difference in phosphorylation may cause the differential staining pattern of β-catenin seen among cases.

There are few reports on the relationship between *CTNNB1* exon 3 mutation status and β-catenin staining property in sporadic DF. Amary *et al.* reported that all 76 cases of DF showed positive staining of β-catenin (66 cases of *CTNNB1* mutation positive DF included) [12]. However, they evaluated staining properties only as positive or negative. Hamada *et al.* evaluated immunohistochemical staining of β-catenin with positivity divided into 4 grades in 33 cases of DF, and demonstrated that the nuclear expression of β-catenin correlated significantly with *CTNNB1* mutation status [9]. However, their study analyzed only 33 cases, and cytoplasmic staining was not analyzed. A previous study from Lazar's lab reported the differential staining intensity and pattern in different *CTNNB1* mutation status. They analyzed 117 cases of DF with positive *CTNNB1* mutation [8]. Results showed that cases with T41A mutations had stronger staining intensity of β-catenin compared to those with S45F mutations, which is not consistent with the results of the present study. A possible reason of the discrepant results would be that they determined staining scores using tissue microarray, which was composed of small cores of samples, whereas specimens analyzed in the present study was obtained

mainly from incisional biopsy. Larger specimens would well reflect the characteristics of tumors. Thus, no definitive conclusion could be drawn. The present multi-institutional study focused on more than 3 times as many cases as in the previous study, making the present results more convincing.

The present study, however, had several limitations. It contained many wild-type cases. Recently, Crago *et al.* performed whole-exome sequencing on 8 cases of wild-type DF diagnosed with direct sequencing (Sanger method), and reported that 5 of 8 cases with wild-type actually had the mutation in the Wnt-related gene (3 in *CTNNB*, 2 in APC) [30]. Our present cases might similarly have included several with wild-type determined by the Sanger method, which would be shown to harbor mutations of Wnt-related genes if evaluated by whole exome sequencing. Another possible reason might be that inadequate fixation in formalin may cause a poor quality of extracted DNA, and false negative results of *CTNNB1* mutation status for the cases analyzed using FFPE samples. Actually, one of the participating six institutions, where specimens are generally fixed in formalin for a prolonged time, showed a considerable number of WT cases.

Conclusion

From the results of this study, staining pattern of β-catenin might be affected by *CTNNB1* mutation status. Although immunohistochemical staining of β-catenin is useful and intriguing for the diagnosis of DF, we should know the limitation of this tool (high sensitivity but not high specificity) for the diagnosis of DF. *CTNNB1* mutation analysis should be used in combination for the most accurate diagnosis of DF.

Acknowledgment

We thank Mr. Yoshiaki Inagaki for assistance with the immunohistochemical staining, Ms. Eri Ishihara for her secretarial assistance for this study, Dr. Junya Toguchida for his cooperation with this research, Drs. Hiroshi Urakawa and Eisuke Arai for collection of samples and Dr. Kunihiro Ikuta for assistance with the writing of this paper.

Reference

[1] Salas S, Dufresne A, Bui B, et al. Prognostic factors influencing progression-free survival determined from a series of sporadic desmoid tumors: A wait-and-see policy according to tumor presentation. J Clin Oncol 2011;29:3553–8. doi:10.1200/JCO.2010.33.5489.

[2] Clark SK, Murk S, Hosjitul YY, et al. Desmoids in familial adenomatous polyposis. Br J Surg 1996:1494–504.

[3] Mendenhall WM, Zlotecki RA, Morris CG, Hochwald SN, Scarborough MT. Aggressive fibromatosis. Am J Clin Oncol 2005;28:211–5. doi:10.1097/01.coc.0000144817.78549.53.

[4] Melis M, Zager JS, Sondak VK. Multimodality management of desmoid tumors: How important is a negative surgical margin? J Surg Oncol 2008;98:594–602. doi:10.1002/jso.21033.

[5] C.D.M. Fletcher, K. Unni FM. World Health Organization Classification of Tumours Pathology and Genetics of Tumours of Soft Tissue and Bone. Cancer 2002;177:1365–76. doi:10.1016/j.suronc.2004.03.001.

[6] Alman B, Li C, Pajerski ME, Diaz-Cano S, Wolfe HJ. Increased beta-catenin protein and somatic *APC* mutations in sporadic aggressive fibromatoses (desmoid tumors). Am J Pathol 1997;151:329–34.

[7] Le Guellec S, Soubeyran I, Rochaix P, et al. *CTNNB1* mutation analysis is a useful tool for the diagnosis of desmoid tumors: a study of 260 desmoid tumors and 191 potential morphologic mimics. Mod Pathol 2012;25:1551–8. doi:10.1038/modpathol.2012.115.

[8] Lazar AJF, Tuvin D, Hajibashi S, et al. Specific mutations in the beta-catenin gene (*CTNNB1*) correlate with local recurrence in sporadic desmoid tumors. Am J Pathol 2008;173:1518–27. doi:10.2353/ajpath.2008.080475.

[9] Hamada S, Futamura N, Ikuta K, et al. CTNNB1 S45F mutation predicts poor efficacy of meloxicam treatment for desmoid tumors: A pilot study. PLoS One 2014;9:5–10. doi:10.1371/journal.pone.0096391.

[10] Ng TL, Gown AM, Barry TS, et al. Nuclear beta-catenin in mesenchymal tumors. Mod Pathol 2005;18:68–74. doi:10.1038/modpathol.3800272.

[11] Bhattacharya B, Dilworth HP, Iacobuzio-Donahue C, et al. Nuclear beta-catenin expression distinguishes deep fibromatosis from other benign and malignant fibroblastic and myofibroblastic lesions. Am J Surg Pathol 2005;29:653–9. doi:10.1097/01.pas.0000157938.95785.da.

[12] Amary MFC, Pauwels P, Meulemans E, et al. Detection of beta-catenin mutations in paraffin-embedded sporadic desmoid-type fibromatosis by mutation-specific restriction enzyme digestion (MSRED): an ancillary diagnostic tool. Am J Surg Pathol 2007;31:1299–309. doi:10.1097/PAS.0b013e31802f581a.

[13] Abraham SC, Reynolds C, Lee JH, et al. Fibromatosis of the breast and mutations involving the *APC/β-Catenin* pathway. Hum Pathol 2002;33:39–46. doi:10.1053/hupa.2002.30196.

[14] Montgomery E, Lee JH, Abraham SC, Wu TT. Superficial fibromatoses are genetically distinct from deep fibromatoses. Mod Pathol 2001;14:695–701. doi:10.1038/modpathol.3880374.

[15] Saito T, Oda Y, Tanaka K, et al. β-catenin nuclear expression correlates with cyclin D1 overexpression in sporadic desmoid tumours. J Pathol 2001;195:222–8. doi:10.1002/path.942.

[16] Tejpar S, Nollet F, Li C, et al. Predominance of beta-catenin mutations and

beta-catenin dysregulation in sporadic aggressive fibromatosis (desmoid tumor). Oncogene 1999;18:6615–20. doi:10.1038/sj.onc.1203041.

[17] Dô Mont J, Salas S, Lacroix L, et al. High frequency of beta-catenin heterozygous mutations in extra-abdominal fibromatosis: a potential molecular tool for disease management. Br J Cancer 2010;102:1032–6. doi:10.1038/sj.bjc.6605557.

[18] Mirabelli-Primdahl L, Gryfe R, Kim H, et al. β-Catenin mutations are specific for colorectal carcinomas with microsatellite instability but occur in endometrial carcinomas irrespective of mutator pathway. Cancer Res 1999;59:3346–51.

[19] Fukuchi T, Sakamoto M, Tsuda H, Maruyama K, Nozawa S, Hirohashi S. β-Catenin Mutation in Carcinoma of the Uterine Endometrium. Cancer Res 1998;58:3526–8.

[20] Miyoshi Y, Iwao K, Nagasawa Y, et al. Activation of the β-Catenin Gene in Primary Hepatocellular Carcinomas by Somatic Alterations Involving Exon 3. Cancer Res 1998;3:2524–7.

[21] Palacios J, Camallo C. Mutations in the β-Catenin Gene (*CTNNBl*) in Endometrioid Ovarian Carcinomas. Cancer Res 1998;58:1344–7.

[22] Voeller HJ, Truica CI, Gelmann EP. β-Catenin Mutations in Human Prostate Cancer. 1998:2520–3.

[23] Oliva E, Sarrió EF, Brachtel EF, et al. High frequency of β-catenin mutations in borderline endometrioid tumours of the ovary. J Pathol 2006;208:708–13. doi:10.1002/path.1923.

[24] Koch A, Denkhaus D, Albrecht S, Leuschner I, Von Schweinitz D, Pietsch T. Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the β-catenin gene. Cancer Res 1999;59:269–73.

[25] Garcia-Rostan G, Tallini G, Herrero A, D'Aquila TG, Carcangiu ML, Rimm DL. Frequent mutation and nuclear localization of beta-catenin in anaplastic thyroid carcinoma. Cancer Res 1999;59:1811–5.

[26] Montgomery E, Folpe AL. The diagnostic value of beta-catenin immunohistochemistry. Adv Anat Pathol 2005;12:350–6.

[27] Carlson JW, Fletcher CDM. Immunohistochemistry for beta-catenin in the differential diagnosis of spindle cell lesions: analysis of a series and review of the literature. Histopathology 2007;51:509–14. doi:10.1111/j.1365-2559.2007.02794.x.

[28] Andino L, Cagle PT, Murer B, et al. Pleuropulmonary desmoid tumors: Immunohistochemical comparison with solitary fibrous tumors and assessment of β-catenin and cyclin D1 expression. Arch Pathol Lab Med 2006;130:1503–9. doi:10.1043/1543-2165(2006)130[1503:PDTICW]2.0.CO;2.

[29] Amit S, Hatzubai A, Birman Y, et al. Axin-mediated CKI phosphorylation of β-catenin at Ser 45: a molecular switch for the Wnt pathway. Genes Dev 2002;16:1066–76. doi:10.1101/gad.230302.somal.

[30] Crago AM, Caley M, Stephens P, et al. New Universal Detection of Alterations in *CTNNB1* and Wnt Pathway Regulators in Desmoid-Type Fibromatosis by Whole-Exome Sequencing and Genomic Analysis. Genes Chromosomes Cancer 2015;54:606–15. doi:10.1002/gcc.

Figure legend

Fig. 1

Representative images of immunohistochemical staining for β-catenin (original magnification, ×200). Differential staining positivity of nucleus and cytoplasm. Staining positivity; absent in nucleus (A) and cytoplasm (B), weak in nucleus (C) and cytoplasm (D), moderate in nucleus (E), and cytoplasm (F), strong in nucleus (G) and cytoplasm (H).

Fig. 2

Representative images of ×400 magnification. Staining intensity; absent in nucleus (A) and cytoplasm (B), weak in nucleus (C) and cytoplasm (D), moderate in nucleus (E), and cytoplasm (F), strong in nucleus (G) and cytoplasm (H).

Table 1. Patient characteristics

Variables	
Age	
Mean, years	42.3 (8-80)
Gender (%)	
male	32 (30%)
female	72 (70%)
Tumor size (cm)	
Mean	7.9 (2-20)
Tumor location (%)	
Abdominal wall	18 (17%)
Extremity	28 (27%)
Head and neck	12 (12%)
Trunk	46 (44%)

Table 2. Patient characteristics vs *CTNNB1* mutation status

Variables	*CTNNB1* mutation status			*p* value[a]
	T41A (n = 43)	S45F (n = 10)	WT (n = 51)	
Age				0.84
<40 ys	22 (46%)	4 (8%)	22 (46%)	
≧40 ys	21 (37%)	6 (11%)	29 (52%)	
Gender				0.58
Male	13 (41%)	5 (16%)	14 (43%)	
Female	30 (42%)	5 (7%)	37 (51%)	
Tumor size				0.29
<8 cm	25 (42%)	3 (5%)	32 (53%)	
≧8 cm	18 (41%)	7 (16%)	19 (43%)	
Tumor location				0.88
Abdominal wall	7 (39%)	1 (6%)	10 (55%)	
Extremity	12 (43%)	1 (4%)	15 (53%)	
Head and neck	4 (33%)	3 (25%)	5 (42%)	
Trunk	20 (44%)	5 (11%)	21 (45%)	

[a] Chi-square test

WT, Wild type

Table 3. Patient characteristics vs nuclear staining of β-catenin

Variables	Positivity				*p* value[a]
	absent (n = 17)	weak (n = 16)	moderate (n = 47)	strong (n = 24)	
Age					0.92
<40 ys	6 (12%)	8 (17%)	22 (46%)	12 (25%)	
≧40 ys	11 (20%)	8 (14%)	25 (45%)	12 (21%)	
Gender					0.83
Male	7 (22%)	5 (16%)	12 (37%)	8 (25%)	
Female	10 (14%)	11 (15%)	35 (49%)	16 (22%)	
Tumor size					0.85
<8 cm	11 (18%)	10 (17%)	24 (40%)	15 (25%)	
≧8 cm	6 (14%)	6 (14%)	23 (52%)	9 (20%)	
Tumor location					0.79
Abdominal wall	2 (11%)	1 (5%)	10 (56%)	5 (28%)	
Extremity	3 (11%)	4 (14%)	16 (57%)	5 (18%)	
Head and neck	1 (9%)	4 (33%)	4 (33%)	3 (25%)	
Trunk	11 (24%)	7 (15%)	17 (37%)	11 (24%)	

[a] Chi-square test

Table 4. Patient characteristics vs cytoplasmic staining of β-catenin

Variables	Positivity				*p* value[a]
	absent (n = 9)	weak (n = 6)	moderate (n = 25)	strong (n = 64)	
Age					0.79
<40 ys	6 (12%)	2 (4%)	10 (21%)	30 (63%)	
≧40 ys	3 (5%)	4 (7%)	15 (27%)	34 (61%)	
Gender					0.58
Male	2 (6%)	2 (6%)	11 (35%)	17 (53%)	
Female	7 (10%)	4 (6%)	14 (19%)	47 (65%)	
Tumor size					0.53
<8 cm	6 (10%)	5 (8%)	11 (19%)	38 (63%)	
≧8 cm	3 (7%)	1 (2%)	14 (32%)	26 (59%)	
Tumor location					0.87
Abdominal wall	2 (11%)	0 (0%)	3 (17%)	13 (72%)	
Extremity	2 (7%)	0 (0%)	10 (36%)	16 (57%)	
Head and neck	0 (0%)	1 (8%)	3 (25%)	8 (67%)	
Trunk	5 (11%)	5 (11%)	9 (19%)	27 (59%)	

[a] Chi-square test

Table 5. *CTNNB1* mutation status and strong staining of β-catenin

β-catenin positivity	*CTNNB1* mutation status			*p* value[a]
	T41A (n = 43)	S45F (n = 10)	WT (n = 51)	
Nuclear				P<0.01
strong	5	8	11	
absent, weak, moderate	38	2	40	
Cytoplasmic				P<0.05
strong	34	10	31	
absent, weak, moderate	9	0	20	

[a] Chi-square test

WT, Wild type

Table 6. *CTNNB1* mutation status and intracellular predominance of β-catenin staining

Intracellular predominance	*CTNNB1* mutation status			*p* value[a]
	T41A (n = 43)	S45F (n = 10)	WT (n = 51)	
Cytoplasmic	31	2	17	
Other	12	8	34	P<0.01

[a] Chi-square test

WT, Wild type

Table 7. *CTNNB1* mutation status and stainability of β-catenin in two different antibodies

nuclear β-catenin positivity	*CTNNB1* mutation status			*p* value[a]
	T41A (n = 42)	S45F (n = 9)	WT (n = 44)	
DAKO antibody				P<0.05
strong	5	7	8	
moderate	23	2	21	
weak	9	0	5	
absent	5	0	10	
Novocastra antibody				0.06
strong	2	3	5	
moderate	17	2	6	
weak	14	3	13	
absent	9	1	20	

[a] Chi-square test

WT, Wild type

Highlights

- Immunostaining of ß-catenin is not a definitive diagnostic tool for desmoid.
- Cytoplasmic staining for ß-catenin is darker in cases with T41A than others.
- *CTNNB1* mutation analysis had better be used for definitive diagnosis of desmoid.

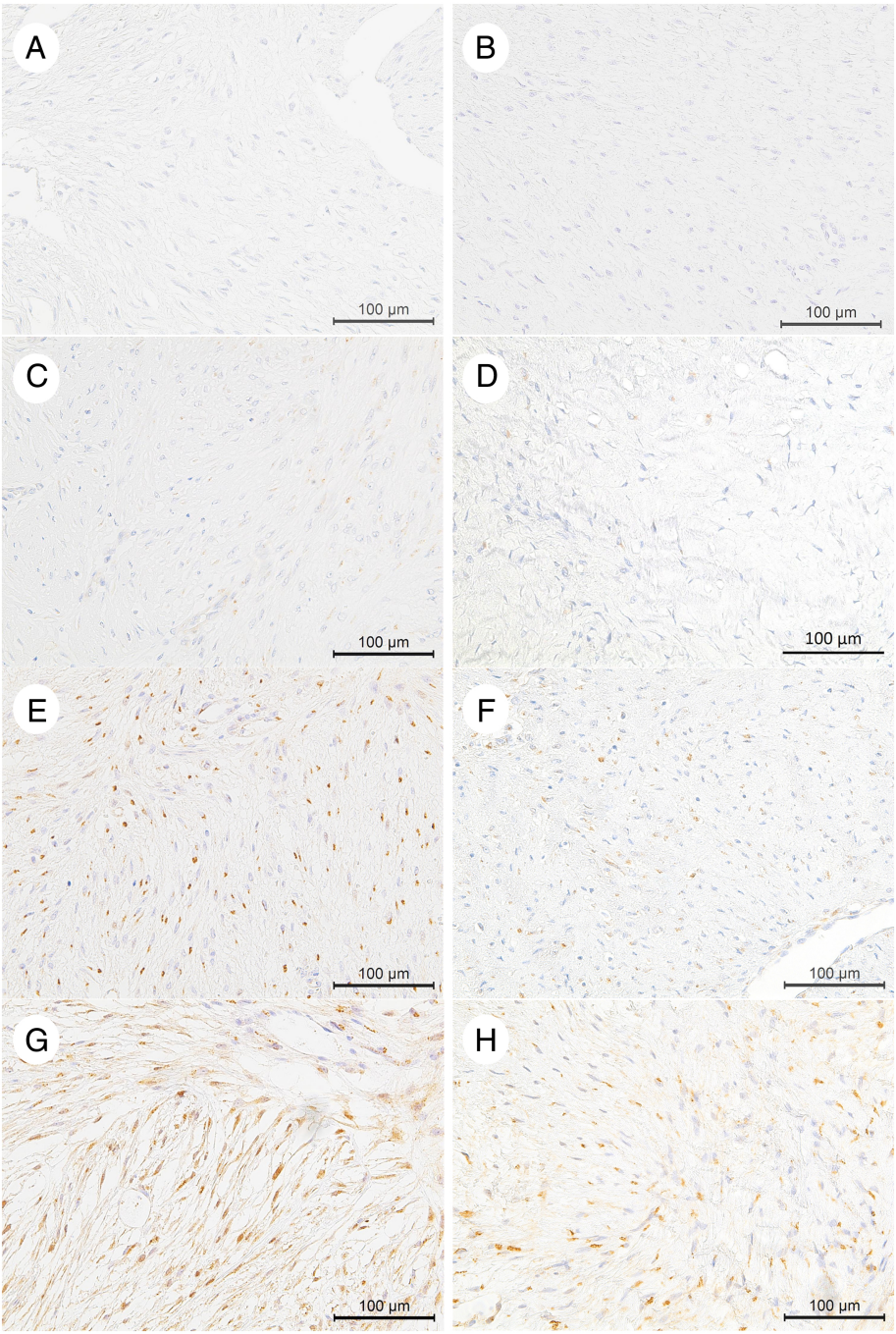

Figure 1

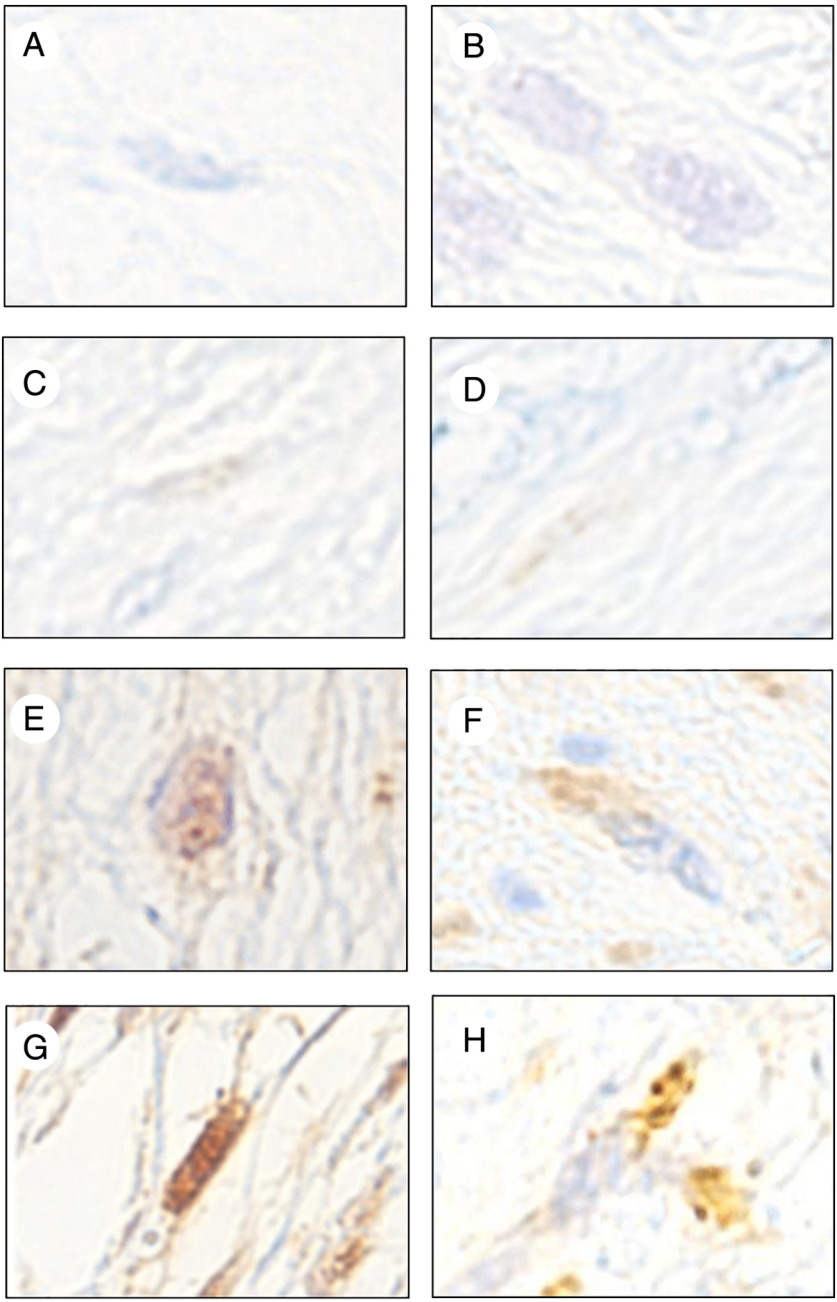

Figure 2