Differential protein immunoexpression profiles in appendiceal mucinous neoplasms: a special reference to classification and predictive factors

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Appendiceal mucinous neoplasms have been the focus of considerable debate in recent years. We histologically classified 70 appendiceal mucinous neoplasms into three categories: 32 mucinous adenoma, 23 mucinous neoplasm of uncertain malignant potential, and 15 mucinous adenocarcinomas. Immunohistochemistry was performed for 24 proteins in different functional categories, specifically, oncogenic proteins (bcl-2, β-catenin, CEA, C-erbB2, c-kit, Cox-2, Cyclin D1, EGFR, Ki-67, NF-κB, VEGF), tumor suppressors (E-cadherin, FHIT, hMLH1, p53, p63, smad4), cell-cycle regulators (p21, p27, p16), and mucin proteins (MUC1, MUC2, MUC5AC, MUC6). Our data showed that 9 out of the 24 proteins were more frequently altered in the mucinous adenocarcinoma group than in the mucinous adenoma group ($P < 0.05$), including β-catenin (13% in mucinous adenoma vs 60% in mucinous adenocarcinoma), CyclinD1 (44 vs 87%), Ki-67 (high labeling index: 31 vs 67%), NF-κB (19 vs 60%), VEGF (16 vs 87%), E-cadherin (0 vs 47%), p53 (6 vs 40%), MUC2 (9 vs 67%), and MUC5AC (3 vs 40%). The distinct immunoexpression profile of mucinous neoplasm of uncertain malignant potential was placed between those of mucinous adenoma and mucinous adenocarcinoma ($P < 0.05$). Moreover, the mucinous adenoma, mucinous neoplasm of uncertain malignant potential, and mucinous adenocarcinoma categories displayed differences in terms of the number of altered markers among the nine proteins ($P < 0.05$; mean 1.4 vs 2.6 vs 5.5, respectively). In mucinous adenocarcinoma, the p53 status was related to disease-free survival and overall survival of patients ($P < 0.05$, both). NF-κB status and the number of altered protein markers made statistically marginal impacts on disease-free survival; also β-catenin loss, on overall survival of patients. In conclusion, protein immunoexpression profiles may facilitate the classification of appendiceal mucinous neoplasms. In our study, the three tumor categories of mucinous adenoma, mucinous neoplasm of uncertain malignant potential, and mucinous adenocarcinoma exhibited distinct immunoexpression profiles. Five and more altered protein markers, p53 overexpression, NF-κB positivity, and β-catenin loss were predictive factors of adverse clinical outcomes in appendiceal mucinous adenocarcinomas.
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Appendiceal mucinous neoplasms are rare tumors, and their diagnostic classification is currently controversial.[1–12] Generally, in appendiceal tumors, the appendiceal wall is totally fibrotic, thus each layer in the wall cannot be discerned. Consequently, it is difficult to establish the tumor invasion status. In practice, the invasion front of appendiceal mucinous adenocarcinomas tends to display a broad pushing feature rather than an infiltrative pattern, imitating its benign counterpart. In addition, even tumors with bland-looking (mucinous adenoma-mimicking) cytoarchitecture can involve

other organs, such as ovary and spleen, as well as peritoneum (pseudomyxoma peritonei), and may behave in an aggressive manner.

In 1995, appendiceal mucinous neoplasm of uncertain malignant potential was introduced as a diagnostic entity.[2] Mucinous neoplasm of uncertain malignant potential was defined in detail, not to use mucinous neoplasm of uncertain malignant potential as the diagnostic term excessively. In 2005, the term 'mucinous neoplasm of uncertain malignant potential' was revisited by Pai *et al*[4] Under conditions where the muscularis mucosae was lost and invasion was unclear, appendiceal mucinous neoplasm displaying bland cytoarchitecture, but features of (1) epithelium pushing deeply into underlying tissue, (2) cystic gland-like structure in wall, or (3) uncertainty of complete excision, such as positive proximal margin or extensive mucin on the appendiceal serosa, were classified as mucinous neoplasm of uncertain malignant potential.[4] However, the issue of whether mucinous neoplasm of uncertain malignant potential progresses toward mucinous adenoma or mucinous adenocarcinoma in terms of biologic behavior remains to be established. A number of distinguished researchers have classified appendiceal mucinous neoplasm into two categories, specifically, low-grade appendiceal mucinous neoplasm or mucinous adenocarcinoma.[3]

Molecular changes, such as protein alterations, which may correspond with the biologic behavior of tumors, are helpful in classifying tumors and predicting clinical outcome. Following examination of the existing literature for immunoexpression data on proteins in mucinous neoplasms of various organs, including colorectum,[13–18] pancreas,[19,20] ovary,[21–23] and appendix,[24–26] we focused on 24 proteins. The immunoexpression profiles of different functional proteins in three groups—mucinous adenoma, mucinous neoplasm of uncertain malignant potential, and mucinous adenocarcinoma of the appendix—were analyzed. Additionally, the clinical implications of immunoexpression patterns were evaluated.

Materials and methods

Patient Samples

We retrieved database information on appendiceal mucinous neoplasms, which were resected at three affiliated hospitals to Seoul National University College of Medicine. In total, 70 cases were included in the study, of which 28 were resected at Seoul National University Hospital from 2003 to 2007, 25 at Seoul National University Boramae Hospital from 1998 to 2007, and 17 at Bundang Seoul National University Hospital from 2003 to 2007. Clinical information, including age, sex, survival data, metastasis to distant organs, recurrence, poor response to treatment or tumor-associated death, was obtained from medical records. The follow-up

period was 12–60 months in malignant cases, and 7–120 months in the remaining cases. The histology of all cases was reviewed by two pathologists (MSC and SOY).

Tissue Array

Two to five different representative areas per case were selected. Each tissue core (2.0 mm in diameter) was punched out from the original paraffin block of tumor samples using a trephine apparatus. The punched cores were arranged in a new tissue array paraffin block, containing 59 tissue cores and 1 ink core as a direction indicator. Five tissue array blocks (300 cores) were newly manufactured, which included 70 cases of appendiceal mucinous neoplasms and 32 nonneoplastic appendiceal tissues as a control.

Immunohistochemistry

Immunohistochemistry was performed on 4 μm thick paraffin-embedded tissue sections using the streptavidin–biotin peroxidase complex method after microwave or autoclave-based antigen retrieval. We selected 24 antibodies for immunostaining (Table 1), specific for oncogenic proteins (bcl-2, β-catenin, CEA, C-erbB2, c-kit, Cox-2, Cyclin D1, EGFR, Ki-67, NF-κB, VEGF), tumor suppressors (E-cadherin, FHIT, hMLH1, p53, p63, smad4), cell-cycle regulators (p21, p27, p16), and mucins (MUC1, MUC2, MUC5AC, MUC6). Immunohistochemistry was performed for the 24 proteins after a test procedure using human control slides for immunostaining (Superbiochips Laboratories, Seoul, Korea).

Data from immunohistochemical staining are presented in Table 2. In cases where more than 10% of tumor cells are immunoreactive, immunostaining is regarded as positive[27,28] or high labeling index for Ki-67.[29] MUC1, MUC2, MUC5AC, and MUC6 protein expression status is positive when more than 20% of the tumor cells display immunoexpression in the cytoplasm.[27] The immunoexpression profiles of protein markers were evaluated in each case. In cases displaying dissimilarities between appendix and extra-appendiceal organ lesions, changes in protein marker patterns within any individual organs were regarded as alterations in the case.

Statistical Analysis

Pearson's χ^2 and Fisher's exact tests were used to examine differences between the variables. Overall survival was measured from the date of diagnosis to death or last follow-up visit. Disease-free survival was calculated from the date of surgical resection to initial evidence of treatment failure. Patient survival

Table 1 Antibodies used for immunohistochemical analyses

Antibody	Retrieval methods	Dilution	Source	Nonneoplastic mucosa	Altered expression in tumors
Oncogenic proteins					
bcl-2	Microwave	1:100	DAKO	Negative	Nucleus
β-Catenin	Microwave	1:200	Transduction	Membrane	Loss[a]
CEA	Microwave	1:50	DAKO	Negative	Cytoplasm
c-erbB2	Microwave	1:75	DAKO	Negative	Membrane
c-kit	Microwave	1:250	DAKO	Negative	Cytoplasm
Cox-2	Microwave	1:400	Cayman	Negative	Cytoplasm
Cyclin D1	Microwave	1:300	DAKO	Nucleus[b]	Nucleus
EGFR	Autoclave	1:200	DAKO	Negative	Cytoplasm
Ki-67	Microwave	1:100	DAKO	Nucleus[b]	Nucleus
NF-κB	Autoclave	1:100	Santa Cruz	Negative	Nucleus
VEGF	Microwave	1:250	Santa Cruz	Negative	Cytoplasm
Tumor suppressor proteins					
E-cadherin	Microwave	1:200	Transduction	Membrane	Loss
FHIT	Microwave	1:250	Zymed	Nucleus	Loss
hMLH1	Microwave	1:50	Oncogene	Nucleus	Loss
p53	Microwave	1:100	DAKO	Negative	Nucleus
p63	Microwave	1:100	Santa Cruz	Negative	Nucleus
smad4	Microwave	1:50	Santa Cruz	Nucleus	Loss
Cell-cycle regulators					
p21	Microwave	1:100	Santa Cruz	Nucleus	Loss
p27	Microwave	1:100	Santa Cruz	Nucleus	Loss
p16	Autoclave	1:50	Pharmingen	Nucleus	Loss
Mucin proteins					
MUC1	Microwave	1:100	Novocastra	Negative	Cytoplasm
MUC2	Microwave	1:100	Novocastra	Cytoplasm[c]	Cytoplasm
MUC5AC	Microwave	1:100	Novocastra	Negative	Cytoplasm
MUC6	Microwave	1:100	Novocastra	Negative	Cytoplasm

[a]Negative immunostaining for β-catenin in the cytoplasmic membrane of tumor cells was an important finding, although nuclear accumulation is a well-known alteration.
[b]Nuclear staining in the base of the normal crypt was observed.
[c]MUC2 was positive in the base of the normal crypt. MUC2 was strongly and diffusely positive in the whole cytoplasm of tumor cells.

Table 2 Clinical information on 70 patients with appendiceal mucinous neoplasms and per tumor category

Variable	Total (n = 70)	Mucinous adenoma (n = 32)	Mucinous neoplasm of uncertain malignant potential (n = 23)	Mucinous adenocarcinoma (n = 15)
Age at diagnosis (years)				
Mean	64.2	63.1	64.8	64.7
Range	10–98	10–86	45–98	45–87
Sex				
Male	32	15	14	3
Female	38	17	9	12
Clinical outcome				
Dead of disease	4	0	0	4
Alive without disease	63	32	23	8
Alive with disease	3	0	0	3

rates were obtained using the Kaplan–Meier method, and differences in survival compared using the log-rank test. Multivariate analysis was performed using the Cox proportional hazards model. *P*-values of <0.05 were considered statistically significant. All statistical analyses were carried out using SPSS/PC version 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Classification of Appendiceal Mucinous Neoplasms

The clinicopathological features of 70 appendiceal mucinous neoplasm cases are listed in Table 2. The 70 resected tumors were categorized using a three-tiered system, specifically, 32 mucinous adenomas, 23 mucinous neoplasms of uncertain malignant potential, and 15 mucinous adenocarcinomas (Table 2). In terms of mucinous neoplasm of uncertain malignant potential, we followed the definition of Pai *et al*[4] (Figure 1). We adopted the
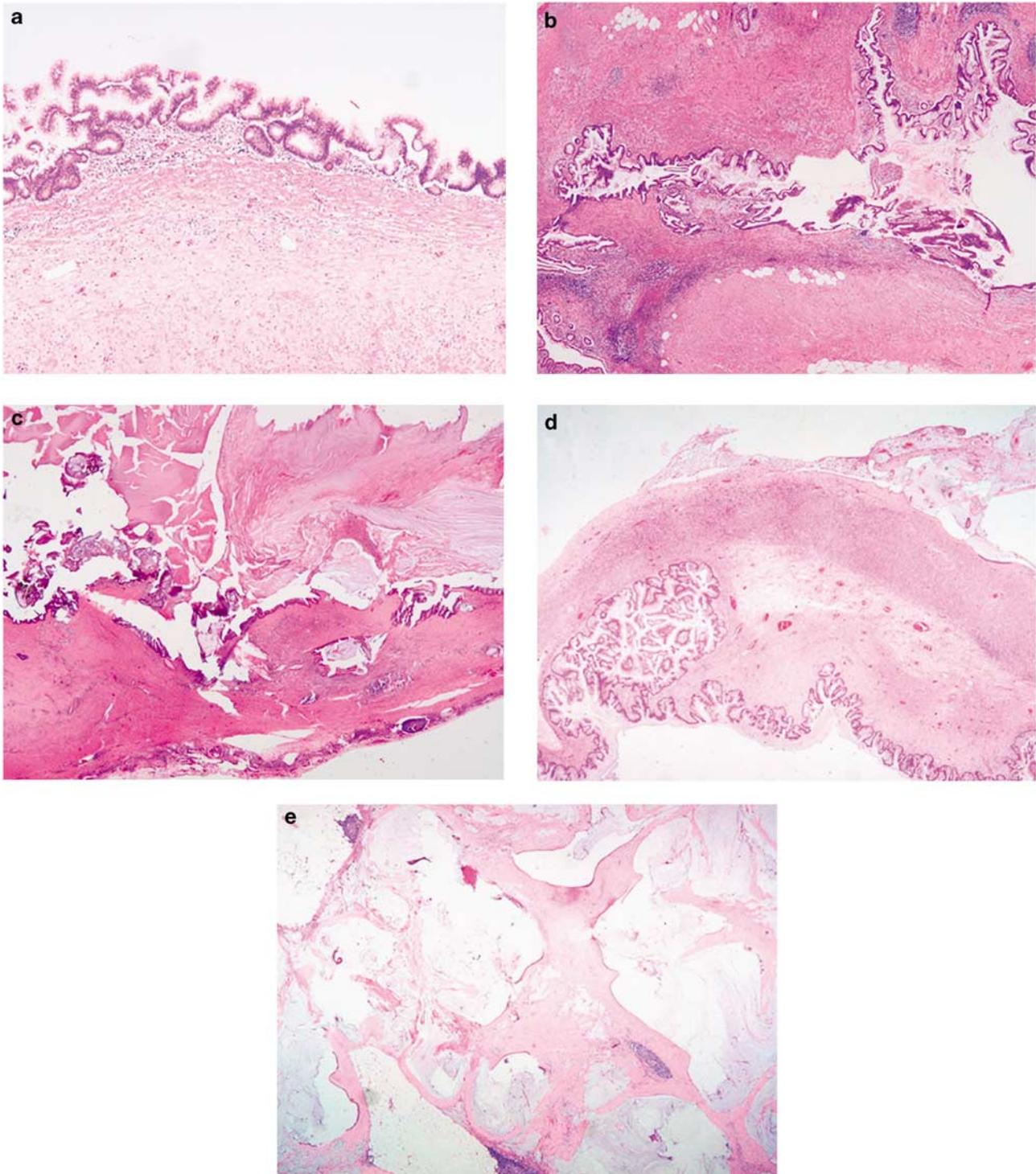
Figure 1 Histologic features of appendiceal mucinous neoplasms. (**a**) Mucinous adenoma. (**b**) Mucinous neoplasm of uncertain malignant potential. (**c–e**) Mucinous adenocarcinoma with low-grade atypia showing scarce epithelium in the appendix (**c**), involving ovary (**d**), and peritoneum (**e**).

mucinous adenocarcinoma definition of Carr *et al*[2,9] which included tumors displaying destructive invasion of the appendiceal wall, architectural complexity, cytologically high-grade atypia, or plump amount of epithelium outside the appendix (Figures 1 and 2). Interestingly, the proportions of tumor categories were strikingly different between tertiary-care and secondary-care hospitals. In the tertiary-care hospital (Seoul National University Hospital), 24, 42, and 34% mucinous adenoma, mucinous neoplasm of uncertain malignant potential, and mucinous adenocarcinoma cases were
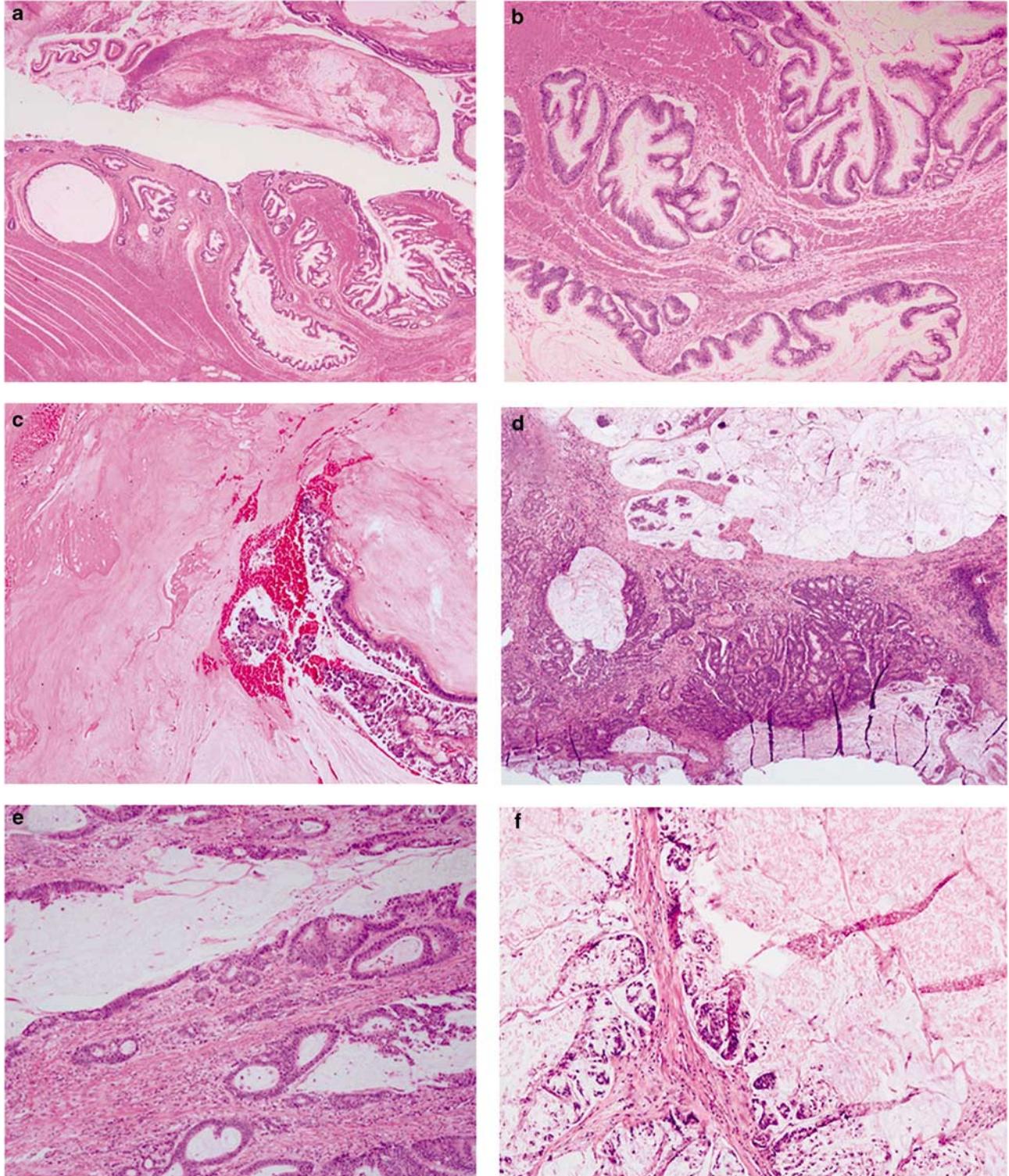
Figure 2 (**a–c**) Mucinous adenocarcinoma showing a broad invasion front in appendix (**a**, **b**) and involving peritoneum (**c**). (**d–f**) Mucinous adenocarcinoma with high-grade atypia in appendix (**d**, **e**) and involving peritoneum (**f**).

reported, respectively. Meanwhile, in two secondary-care hospitals, the proportions of mucinous adenoma, mucinous neoplasm of uncertain malignant potential, and mucinous adenocarcinoma were 76, 16, and 8% (Seoul National University Boramae Hospital) and 82, 12, and 6% (Bundang Seoul National University Hospital), respectively.

Differential Immunoexpression Profiles of Proteins in Mucinous Adenoma, Mucinous Neoplasm of Uncertain Malignant Potential and Mucinous Adenocarcinoma

Among the 24 protein markers, 9 were more frequently altered in mucinous adenocarcinoma, compared to the mucinous adenoma group (Figure 3). Immunoexpression profiles of nine proteins (β-catenin, Cyclin Di, Ki-67, NF-κB, VEGF, E-cadherin, p53, MUC2, and MUC 5AC) according to tumor category are presented in Figure 4 and Table 3.

The alteration frequencies of β-catenin, CyclinD1, Ki-67, E-cadherin, MUC2, and MUC5AC in mucinous neoplasm of uncertain malignant potential approached those in mucinous adenoma, whereas frequencies of NF-κB, VEGF and p53 were comparable to those in mucinous adenocarcinoma.

Discriminative Numbers of Altered Protein Markers in Mucinous Adenoma, Mucinous Neoplasms of Uncertain Malignant Potential, and Mucinous Adenocarcinoma Groups

The number of altered protein markers per case was determined. Mean numbers were significantly different among the three groups. Mucinous neoplasm of uncertain malignant potential was placed in the middle spectrum between mucinous adenoma and mucinous adenocarcinoma, with respect to the mean number of altered protein markers, estimated as 1.4 (median 1.0, range 0–5) in mucinous adenoma, 2.6 (median 2.0, range 0–6) in mucinous neoplasms of uncertain malignant potential, and 5.5 (median 5.0, range 3–8) in mucinous adenocarcinoma ($P < 0.05$; Figure 5).

There were several cases, which unexpectedly showed marker alteration (Figure 5; Supplementary Information-1). A mucinous adenoma case (No. 27) displayed alterations in five markers. In this case, small mucinous adenoma (0.8 cm in tumor size) in appendix was observed incidentally, in the specimen undergone right hemicolectomy due to synchronous adenocarcinomas of the tubular type in ascending colon and cecum. In the mucinous neoplasm of uncertain malignant potential group, cases 34 and 38 displayed five altered marker proteins, and six altered markers were detected in case 44. Additionally, cases 37, 45, and 52 showed one altered marker, whereas case 47 presented no marker alterations. However, these cases displayed no specific features that could allow differentiation from other mucinous neoplasms of uncertain malignant potential patients, except that case 37 displayed mucinous adenoma-mimicking microscopic features, but a positive proximal resection margin.

Immunoexpression Status in the Appendix and Extra-appendiceal Organ in Mucinous Adenocarcinoma

In this study, extra-apendiceal organ involvement was pathologically confirmed in 14 out of 15 mucinous adenocarcinoma cases as follows: 8 cases in peritoneum, 8 in ovary, 7 in omentum, 3 in other intestinal segments, 3 in uterus, 2 in salpinx, 2 in spleen capsule, and 2 in regional lymph nodes.

In 10 cases, alterations in protein markers in the appendix and extra-appendiceal organ were slightly dissimilar. Moreover, the immunoexpression status within the mucinous adenocarcinoma group was not related to cytologic atypia, architecture complexity, or extent of extra-appendiceal organ involvement. For instance, case 68 displayed mucinous neoplasm of uncertain malignant potential-mimicking cytoarchitecture in the appendix per se, and more complex architecture in the ovary. However, this patient presented six altered markers in the appendix and four in the ovary. In two mucinous adenocarcinoma cases with high cytologic atypia, one displayed five and six altered markers in the appendix and extra-appendiceal lesion, whereas five altered markers each in the appendix and extra-appendiceal lesion were observed in the other case.

Clinical Significance of Immunoexpression Patterns in Mucinous Adenocarcinoma

In the mucinous adenocarcinoma group, the number of altered protein markers had an impact on clinical response. The mean number of altered protein markers in the mucinous adenocarcinoma group was 5.5, and median number, 5. The median number was rated as a cutoff, and clinical significance of the number of altered protein markers was evaluated. Eleven mucinous adenocarcinoma cases displayed alteration of five and more proteins. Treatment failure was reported for seven (64%) of these cases. Treatment failure was defined as metastasis to distant organs, recurrence, poor response to treatment or tumor-associated death after initial treatment at any time during the follow-up period. Meanwhile, four cases displayed alteration of four and less proteins, none of whom exhibited treatment failure. In univariate analysis, 11 cases with five and more protein alteration demonstrated a shorter period of disease-free survival than the 4 cases with four and less protein alteration, although the statistical significance was marginal ($P = 0.087$; Figure 6a).
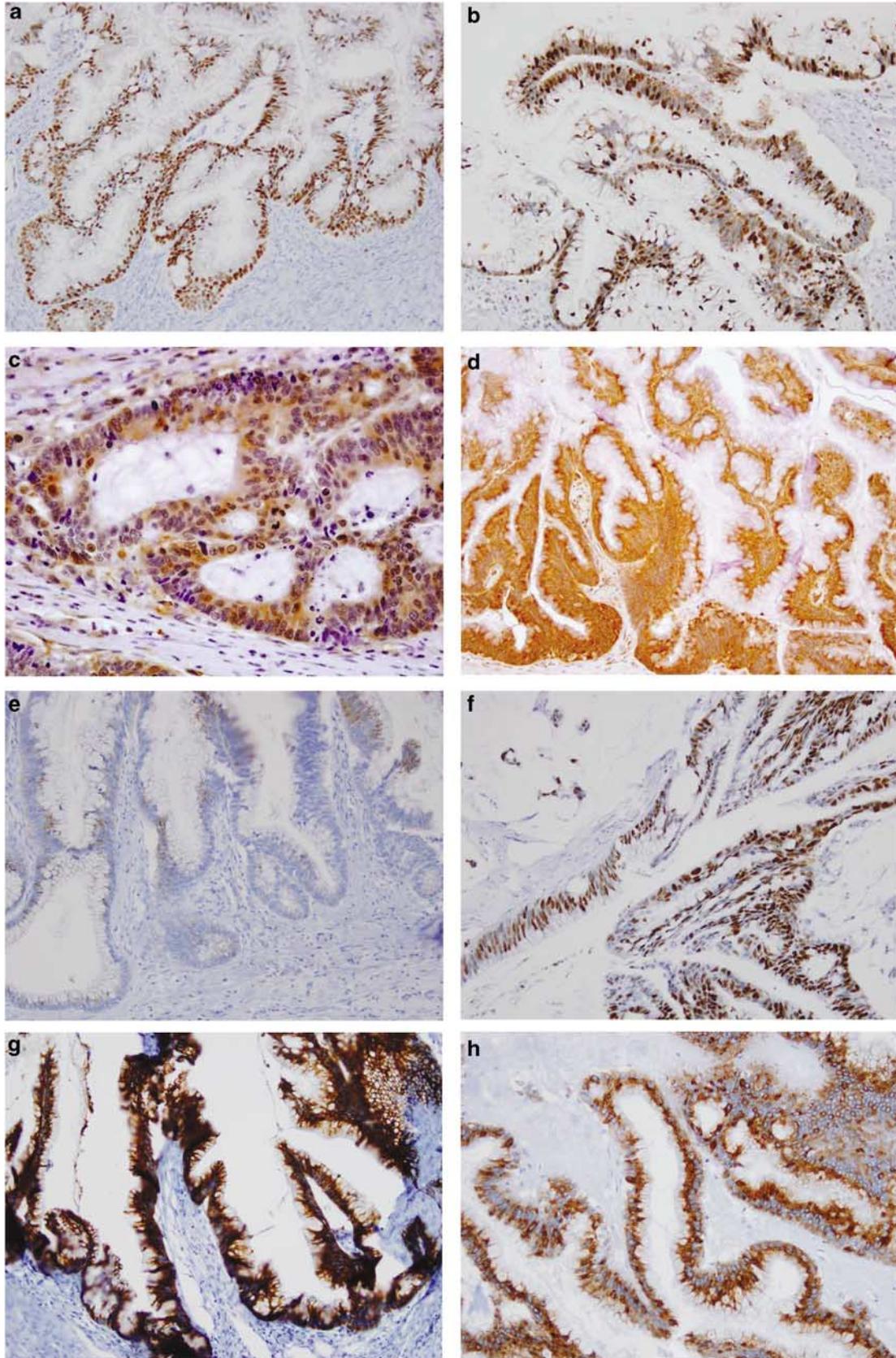
Figure 3 Representative immunohistochemical features of nine protein markers, which are more frequently altered in mucinous adenocarcinoma than mucinous adenoma ($P < 0.05$). (**a**) Cyclin D1, nuclear positive. (**b**) Ki-67 (high labeling of nuclear positive). (**c**) NF-κB, nuclear positive. (**d**) VEGF, cytoplasmic positive. (**e**) E-cadherin, cytoplasmic membranous loss. (**f**) p53 overexpression (nuclear accumulation). (**g**) MUC2, cytoplasmic positive. (**h**) MUC5AC, cytoplasmic positive.
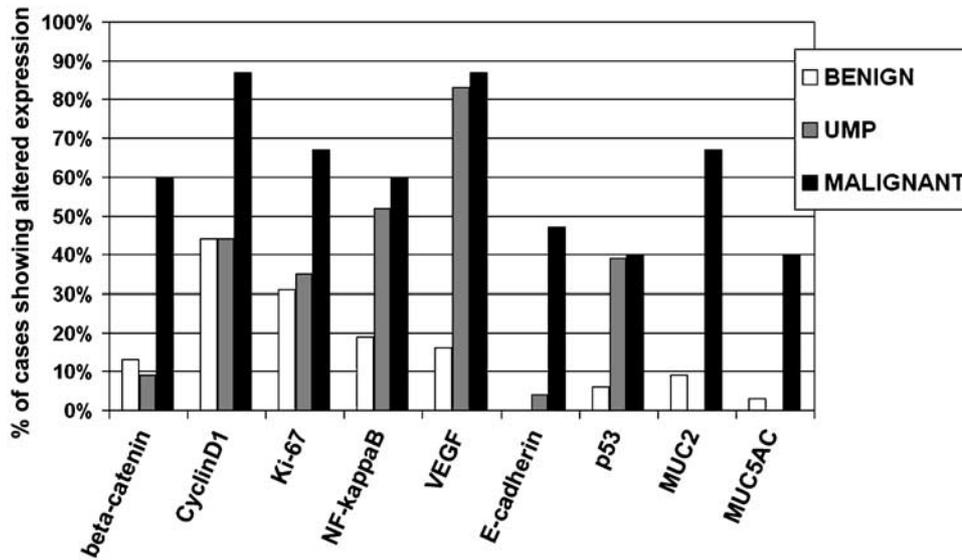
Figure 4 Differential immunoexpression patterns of proteins in appendiceal mucinous neoplasms according to tumor category. Immunoexpression of nine protein markers in mucinous adenoma, mucinous neoplasm of uncertain malignant potential, and mucinous adenocarcinoma groups, reveals significant differences ($P < 0.05$). BENIGN, mucinous adenoma; UMP, mucinous neoplasm of uncertain malignant potential; MALIGNANT, mucinous adenocarcinoma.

Table 3 Altered immunoexpression of proteins per tumor category

|	Mucinous adenoma (n = 32); percent (no.)	Uncertain malignant potential (n = 23); percent (no.)	Mucinous adenocarcinoma (n = 15); percent (no.)
β-Catenin, loss	13 (4)	9 (2)	60 (9)
CyclinD1, positive	44 (14)	44 (10)	87 (13)
Ki-67, high index	31 (10)	35 (8)	67 (10)
NF-κB, positive	19 (6)	52 (12)	60 (9)
VEGF, positive	16 (5)	83 (19)	87 (13)
E-cadherin, loss	0	4 (1)	47 (7)
p53, overexpression	6 (2)	39 (8)	40 (6)
MUC2, positive	9 (3)	0	67 (10)
MUC5AC, positive	3 (1)	0	40 (6)

In terms of clinical implications of individual markers, p53 alterations were associated with treatment failure in mucinous adenocarcinoma ($P = 0.041$). Of the 15 mucinous adenocarcinoma cases, 6 displayed p53 overexpression, and treatment failure was reported for 5 (83%) of these cases. Meanwhile, only two (22%) of the nine p53-negative mucinous adenocarcinoma cases resulted in treatment failure. In univariate analysis, p53-overexpressing mucinous adenocarcinoma cases ($n = 6$) showed a shorter period of disease-free survival than p53-negative cases ($n = 9$; $P = 0.011$; Figure 6b). In addition, NF-κB-positive mucinous adenocarcinoma cases ($n = 9$) displayed a shorter period of disease-free survival than NF-κB- negative cases ($n = 6$; $P = 0.083$; Figure 6c). Regarding overall survival of patients, p53 overexpression was related to lower rate of overall survival ($P = 0.040$), and β-catenin loss tended to show lower rate of overall

survival ($P = 0.080$; Figures 7a and b). Multivariate analysis disclosed no independent prognostic factors among protein markers.

Treatment failure was not reported for any of the mucinous adenoma and mucinous neoplasm of uncertain malignant potential cases during the follow-up period.

Discussion

We suggest that immunoexpression profiles of the nine protein markers are representative for appendiceal mucinous adenocarcinoma. Although appendiceal mucinous neoplasm accompanying peritoneal lesions are cytoarchitecturally benign in appearance, the molecular status of the tumor is consistent with features of mucinous adenocarcinoma, which are significantly different from those of
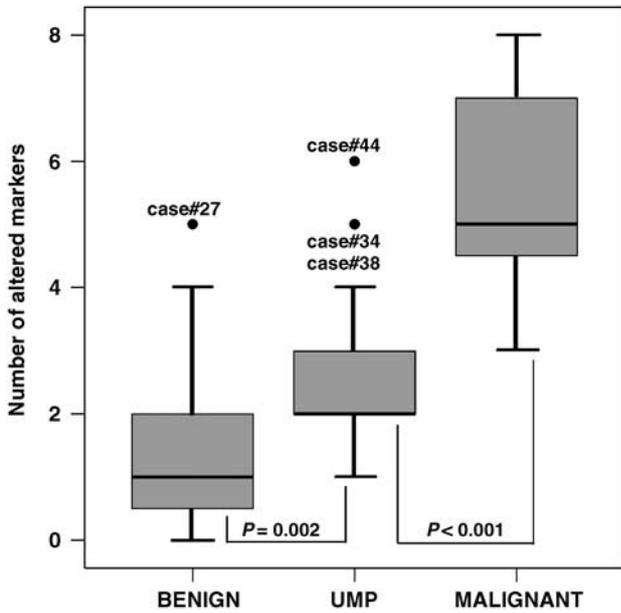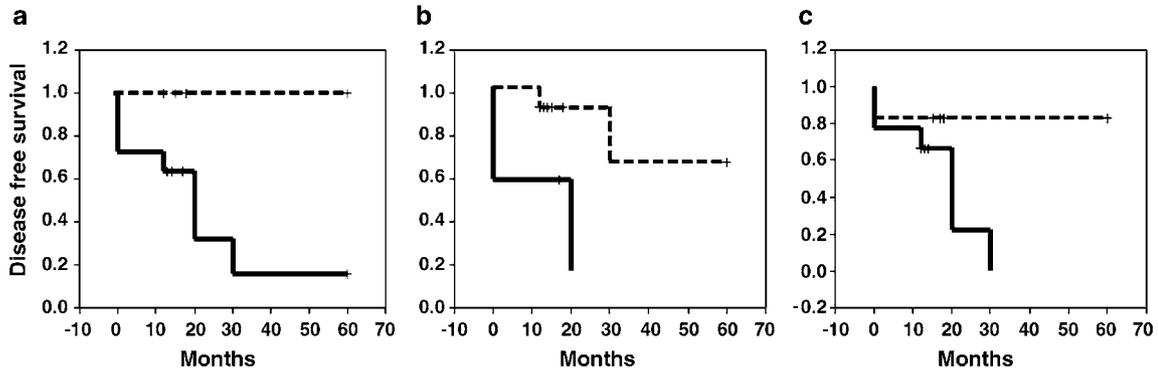
Figure 5 Number of altered protein markers among nine proteins in appendiceal mucinous neoplasms. Mean numbers of altered markers in mucinous adenoma, mucinous neoplasm of uncertain malignant potential and mucinous adenocarcinoma groups were 1.4, 2.6, and 5.5, respectively ($P < 0.05$). BENIGN, mucinous adenoma; UMP, mucinous neoplasm of uncertain malignant potential; MALIGNANT, mucinous adenocarcinoma.

mucinous adenoma or mucinous neoplasms of uncertain malignant potential. Meanwhile, once cases are classified as mucinous adenocarcinoma, the number of altered markers or individual marker alterations are not related to the cytoarchitecture grade of appendiceal and extra-appendiceal lesions, or extent of extra-appendiceal involvement. To date, few molecular studies on appendiceal mucinous neoplasms and even more limited immunohisto-chemistry analyses have been performed. To the best of our knowledge, limited information is available on mucin, E-cadherin, and p53 immunoexpression patterns focusing on both benign and malignant tumors.[26]

We propose that the number of altered protein markers, p53 status, NF-κB status, and β-catenin status are of clinical significance in appendiceal mucinous adenocarcinomas. In other words, p53 overexpression may signify shorter periods of disease-free survival and overall survival of patients. In addition, five and more altered markers and NF-κB positivity or β-catenin loss may be a sign of shorter periods of disease-free survival or overall survival, respectively.

In this study, a three-tiered classification of appendiceal mucinous neoplasms is supported by an analysis based on altered protein markers and

Figure 6 Disease-free survival plots in mucinous adenocarcinoma patients according to (**a**) number of altered protein markers; five and more altered markers (solid line) *vs* four and less altered markers (dotted line; $P = 0.087$); (**b**) p53 status; overexpression (solid line) *vs* negative (dotted line; $P = 0.011$); and (**c**) NF-κB status; nuclear positive (solid line) *vs* negative (dotted line; $P = 0.083$).
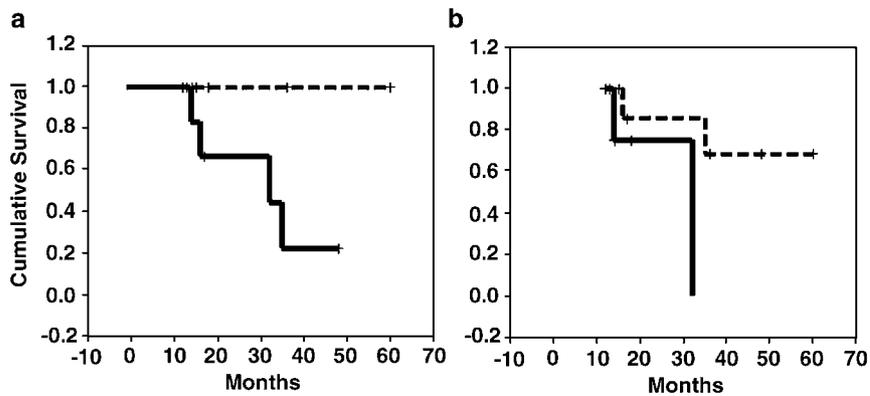
Figure 7 Overall survival plots in mucinous adenocarcinoma patients, according to (**a**) p53-status; overexpression (solid line) *vs* negative (dotted line; $P = 0.004$) and (**b**) β-catenin status; loss (solid line) *vs* preservation (dotted line; $P = 0.080$).

clinical outcome. We adopted the three-tiered system suggested by Carr et al,[2] and applied the strict guidelines of Carr et al[2] and Pai et al[4] to define mucinous neoplasms of uncertain malignant potential. In fact, Pai et al[4] suggested a four-tiered system, specifically, mucinous adenoma, mucinous neoplasm of uncertain malignant potential, low malignant potential, and mucinous adenocarcinoma. Pai et al[4] proposed the term low malignant potential for tumors that were cytoarchitecturally similar to mucinous adenoma, but neoplastic cells penetrated appendiceal wall and were present in peritoneal implants, and there was no metastasis. Taking into account this four-tiered system, 6 out of 15 mucinous adenocarcinoma cases in our study may be defined as low malignant potential. However, we cannot categorically discriminate between six cases of so-called low malignant potential and nine cases of higher grade atypia-mucinous adenocarcinoma, in terms of altered protein markers and clinical outcome (Supplementary Information-1 and -2). The above-described six cases may belong to low-grade mucinous neoplasm in two-tiered categorization system (low-grade mucinous neoplasm and mucinous adenocarcinoma) suggested by Misdraji et al.[3] Like the preceding, so-called low-grade mucinous neoplasm and higher grade atypia-mucinous adenocarcinoma were not separated, based on altered protein markers and clinical outcome (Supplementary Information-1 and -2). The tumors of low-grade cytologic atypia (nucleomegaly, nuclear stratification, rare mitotic figures, single cell necrosis) and minimal architectural complexity (viliform, flat epithelial proliferation, small papillary excrescence) was designated as low-grade mucinous neoplasm by Misdraji et al,[3] thus the concept of low-grade mucinous neoplasm encompasses mucinous adenoma through to low-grade atypia-mucinous adenocarcinoma. In daily practice, the term 'low-grade mucinous neoplasm' may be employed in ambiguous cases. However, we preferred a three-tiered categorization, along with a descriptive explanation, for instance, mucinous adenocarcinoma with high-grade or low-grade atypia.

In this study, 21 patients classified as mucinous neoplasm of uncertain malignant potential appeared close to mucinous adenoma, because no adverse clinical events were observed such as recurrence, metastasis, or high-grade transformation after initial treatment. In the series of Carr et al,[2] 2 out of 18 patients with mucinous neoplasm of uncertain malignant potential displayed evidence of recurrence, one at 6 months of follow-up and the other at 52 months after appendectomy. However, the first patient had a coexisting 'ovarian mucinous tumor with low malignant potential', which may have been an appendiceal mucinous adenocarcinoma involving ovary rather than a double organ primary tumor. In the study by Pai et al,[4] late recurrences occurred in several patients with mucinous neoplasm of uncertain malignant potential during long-term follow-up. A small percentage of mucinous neoplasm of uncertain malignant potential tumors can develop into late recurrence. In our analysis, mucinous neoplasm of uncertain malignant potential cases exhibited differential protein immunoexpression profiles, which were distinguishable from those of benign and malignant appendiceal mucinous neoplasms. Hence, mucinous neoplasm of uncertain malignant potential is justified as a separate diagnostic entity in terms of clinical behavior and molecular changes.

Extra-appendiceal involvement (including pseudomyxoma peritonei) was additionally noted in 14 (18%) out of 70 patients, but only 3 (7%) out of 42 patients from secondary-care hospitals among our data. According to a 10-year nationwide study from the Netherlands, 9% of patients with appendiceal lesions developed pseudomyxoma peritonei.[30] The proportion according to tumor category in a tertiary-care hospital was comparable to that in an AFIP series by Carr et al,[2] ie. mucinous adenoma/mucinous neoplasm of uncertain malignant potential/mucinous adenocarcinoma was 24/42/34% in our data, and 28/17/55% in AFIP series.

In conclusion, immunoexpression profiling may be an effective complementary strategy in the classification of appendiceal mucinous neoplasms. Appendiceal mucinous adenoma, mucinous neoplasm of uncertain malignant potential, and mucinous adenocarcinoma are distinct in terms of protein marker alterations. In appendiceal mucinous adenocarcinoma cases, five and more altered protein markers, p53 overexpression, NF-κB positivisty, and β-catenin loss are factors associated with unfavorable clinical events, such as treatment failure, shorter period of disease-free survival, and shorter period of overall survival.

Acknowledgements

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Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)