Differential Expression of Mimecan and Thioredoxin Domain–Containing Protein 5 in Colorectal Adenoma and Cancer: A Proteomic Study

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Adenoma is the major precursor lesion of colorectal cancer, one of the most common cancers worldwide. The elucidation of the molecular mechanism underlying adenoma is essential for early detection, prevention, and intervention of colorectal cancer. Using a combination of two-dimensional gel electrophoresis and mass spectrometry, we identified 27 differentially expressed proteins in adenoma, compared with matched normal mucosa and cancer tissue. Seventeen proteins were upregulated and six downregulated in adenoma when compared with the same proteins in individual-matched normal mucosa. Four were downregulated, but none upregulated in adenoma when compared with the same proteins in matched cancer tissue. Two novel proteins, mimecan and thioredoxin domain–containing protein 5 (TXNDC5), were further validated by Western blot in 8 colorectal adenomas and 19 cancers that were matched with normal mucosa. All adenoma and cancer tissues did not express mimecan, but all normal mucosa did ($P < 0.01$). In contrast, TXNDC5 was significantly upregulated in colorectal adenoma and cancer tissues as compared with that in normal mucosa ($P < 0.05$). This study clearly demonstrated that absence of mimecan and upregulation of TXNDC5 are involved in the early development of colorectal cancer. Thus, the differentially expressed proteins might serve as potential biomarkers for colorectal cancer detection and intervention. Exp Biol Med 232:1152–1159, 2007

Key words: differentially expressed proteins; colorectal adenoma; mimecan; thioredoxin domain–containing protein 5; proteomic study

Introduction

Colorectal cancer is one of the most common causes of cancer-related deaths worldwide. In 2004, there were an estimated 100,000 new cases and 57,000 deaths due to this disease in the United States (1). In China, the incidence and mortality of colorectal cancer have been rising rapidly in the past two decades. Colorectal carcinogenesis is a well-known multistep process. The central paradigm for the initiation and development of colorectal carcinoma is the adenoma-carcinoma sequence (2). Colorectal cancer might be effectively controlled if the main premalignant lesion—adenoma—is detected and removed before invasion occurs. Therefore, it is crucial to elucidate the molecular mechanism underlying colorectal adenomas.

To make progress in cancer biology, it is important to explore the differential protein expression pattern between tumor and paired normal tissue. Such proteomic study not only contributes to elucidating the underlying molecular carcinogenesis but also provides a robust tool to identify novel biomarkers in cancer. At present, two-dimensional gel electrophoresis (2-DE) remains one of the most common approaches for proteomic studies. Previous studies in colorectal cancer have identified many valuable biomarkers (3–7). However, specifically regulated proteins in colorectal adenoma are exceedingly rare (7). Particularly, the differential protein expression pattern among colorectal mucosa, adenoma, and cancer from a single patient has not been reported yet.

The tumor suppressor gene *p*53 plays essential roles in colorectal cancer initiation and progression. However, its downstream targets are not well understood. Mimecan was first isolated from bovine cornea (8) and is a novel *p*53 target gene (9). Mimecan expression was lost in several cancer cell lines, implicating its potential role as a tumor suppressor gene in cancer biology, although its function remains unknown. The thioredoxin family of endoplasmic reticulum proteins is a family whose members contain a disulfide isomerase–like (PDI) domain. They are closely

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associated with many important biological activities, such as antihypoxia-induced apoptosis, cell proliferation, differentiation, and angiogenesis (10, 11). A growing body of studies has indicated their roles in cancers and their precursors (12, 13). Thioredoxin domain–containing protein 5 (TXNDC5) is a newly identified member of this family and may serve as a biomarker for hepatocellular carcinoma as suggested by recent studies (14, 15). However, its role in colorectal cancer has not been shown yet.

To determine differentially expressed proteins in colorectal adenoma, we subjected 10 individual-matched specimens of normal, adenoma, and adenocarcinoma to proteomic study. We found a specific protein-expression pattern of adenoma as compared with that of matched normal or cancer tissue. Western blot analysis confirmed the loss of mimecan expression and upregulation of TXNDC5 in colorectal tumors. To our knowledge, the current study is the first detailing the differential expression of mimecan or TXNDC5 in colorectal tumors.

Materials and Methods

Sample Collection. Samples were taken from 10 patients with colorectal cancer from the First Affiliated Hospital, School of Medicine, Zhejiang University. Normal colonic mucosa was defined as that adjacent to the tumor-free distant resection margin. Concurrent adenomas were cut into two parts: one underwent pathological diagnosis, and the other was saved for future study. The patients were well informed in accordance with the disciplines of the Ethics Committee of Biomedicine, Zhejiang University, China.

All samples were snap-frozen in liquid nitrogen and stored in a deep freezer (–80°C) until they were used. Tissues (50–100 mg) were crushed in liquid nitrogen and lysed in 1 ml of 7 *M* urea, 2 *M* thiourea, 4% 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonate (CHAPS), 65 m*M* dithiothreitol (DTT), and 0.2% Bio-Lyte (pH 5–8, Bio-Rad, Hercules, CA) with sonication on ice. The lysates were centrifuged at 18,000 *g* for 1 hr at 4°C. Supernatants were removed and concentrations were determined by the Bio-Rad AC DC protein assay kit (Bio-Rad). The protein samples were stored in aliquots at –80°C. Before 2-DE was performed, the protein samples were purified by using the Readyprep 2-D cleanup kit (Bio-Rad) according to the manufacturer's instructions.

Clinical Data of Samples. Detailed clinical and pathological data from the health care information center were reviewed. None of the patients had received adjuvant chemotherapy or radiotherapy prior to surgery. Of the 10 patients, seven were men, and three were women; the mean age at the time of the operations was 57 years (range, 44–80 years). Nine patients suffered colorectal cancer with concurrent adenoma, of which five were colonic and four were rectal. All adenomas had definite histologic diagnoses: tubular ($n = 8$), tubulovillous ($n = 1$), and villous ($n = 1$). All showed low-to-moderate dysplasia.

2-DE. Individual paired samples of normal colon mucosa, adenoma, and cancer were analyzed by 2-DE as described previously (6). Briefly, linear gradient 24-cm (pH 5–8) readystrip (Bio-Rad) was rehydrated overnight at 17°C with 300 μg of protein samples in 500 μl of rehydration buffer (7 *M* urea, 2 *M* thiourea, 4% CHAPS, 65 m*M* DTT, and 0.2% Bio-Lyte). Isoelectric focusing (IEF) was performed by using PROTEAN IEF Cell (Bio-Rad). After IEF, the immobilized pH gradient (IPG) strip was immediately equilibrated for 15 mins in equilibration buffer I (6 *M* urea, 2% sodium dodecyl sulfate (SDS), 0.375 *M* Tris-HCl pH 8.8, 20% glycerol, and 2% DTT) and then for 15 mins in equilibration buffer II (6 *M* urea, 2% SDS, 0.375 *M* Tris-HCl pH 8.8, 20% glycerol, and 2.5% iodoacetamide). SDS-polyacrylamide gel electrophoresis (PAGE) was carried out on 12% SDS-polyacrylamide gels (25 cm × 20.5 cm × 1.0 mm) by using the PROTEAN Plus Dodeca Cell (Bio-Rad) at a constant voltage of 200 V at 20°C. After electrophoresis, the gels were stained by using the Silver Stain Plus Kit (Bio-Rad). The above processes were performed in triplicate for each sample.

Image Analysis. The silver-stained 2-DE gels were scanned on a GS-800 Calibrated Imaging Densitometer (Bio-Rad) at a resolution of 300 dots per inch (dpi). Intensities of protein spots were analyzed with PDQuest software ver7.1 (Bio-Rad). Protein spots that showed more than 5-fold differential expression and had the same directional change in at least five cases were taken as differentially expressed candidates, in agreement with our previous report (6). The gray values of the differentially expressed protein candidates were statistically analyzed by the nonparametric Wilcoxon test.

Spot Cutting and In-Gel Digestion. The 10-sample pool was used for spot cutting. Equal protein masses of each of the 10 samples (normal, adenoma, and cancer tissue) were pooled, and 300 μg of the mixture was loaded for 2-DE. Some differentially expressed protein spots identified as described in the preceding text were excised from gels by Proteomeworks Spot Cutter (Bio-Rad), destained for 20 mins in 30 m*M* potassium ferricyanide/100 m*M* sodium thiosulfate (1:1 [v/v]), and washed in Milli-Q water until the gels shrank and were bleached. The gel pieces were incubated in 0.2 *M* NH_4HCO_3 for 20 mins and dried by lyophilization. To each gel piece, 20 μl of 20 μg/ml trypsin (proteomics grade, Sigma, St. Louis, MO) was added and incubated at 37°C overnight. The peptides were extracted three times with 50% acetonitrile (ACN) and 0.1% trifluoroacetic acid (TFA) and dried in a vacuum centrifuge.

Mass Spectrometry (MS). Samples were analyzed by a Voyager-DE STR MALDI-TOF mass spectrometer (Applied Biosystems, Foster City, CA) with delayed extraction in which α-cyano-4-hydroxycinnamic acid was exploited as the matrix (in 50% ACN in 0.05% MS). The total 2-μl solution was applied onto a target disk and allowed to air-dry. Mass-to-charge ratios were measured in a reflector/delayed extraction mode with an accelerating

Table 1. Differentially Expressed Proteins in Colorectal Adenoma

ID no.	Protein name[a]	Swissprot ID	m/z (charge)[b]	Cover rate (%)[c]	Sequence of amino acid (LTQ-MS/MS)
Downregulated proteins in adenoma compared with the same proteins in normal mucosa					
1	Intelectin-1 precursor	Q8WWA0	646.69 (2)	10.45	K.TASYYSPYGQR.E
2	Mimecan precursor	P20774	530.59 (2)	24.16	K.DFADIPNLR.R
3	Heterogeneous nuclear ribonucleoprotein A0	Q13151	845.96 (2)	5.25	K.LFIGGLNVQTSESGLR.G
4	Splice isoform 3 of itric-oxide synthase	P29475	1052.70 (2)	1.64	−.MGSIMHPSQHARRPEDVR.T
5	F_c fragment of IgG-binding protein	O95784	640.70 (2)	2.68	K.AGCVAESTAVCR.A
6	Serine (or cysteine) proteinase inhibitor	Q53H28	42743 (1)	30	—
Upregulated proteins in adenoma compared with the same proteins in normal mucosa					
7	*Endoplasmin precursor*	P14625	645.18 (2)	33.75	K.DISTNYYASQK.K
8	Serum amyloid P-component precursor	P02743	679.26 (2)	19.28	K.IVLGQEQDSYGGK.F
9	*Ferritin light chain*	P02792	804.39 (2)	5.91	R.LGGPEAGLGEYLFER.L
10	Pituitary adenylyl cyclase–activating protein	Q53BH1	711.83 (2)	36.51	R.AVAYQMWQNLAK.A
11	Putative sialic acid–specific acetylesterase II	Q8A932	669.72 (2)	2.29	R.ELSNTAAYQSVR.I
12	Glutathione peroxidase-gastrointestinal	P18283	631.22 (2)	33.68	K.FLIGPEGEPFR.R
13	Thioredoxin domain–containing protein 5	Q8NBS9	43642 (1)	22	—
14	*Albumin*	P02768	52048 (1)	45	—
15	*α-enolase*	P06733	47139 (1)	23	—
16	MHC class I antigen	P30443	31585 (1)	36	—
17	*Tubulin α6*	Q9BQE3	49863 (1)	40	—
18	Human leukocyte antigen	P08575	10730 (1)	75	—
19	*Heat shock 70-kDa protein 7 isoform 2*	P48741	53484 (1)	24	—
20	*Capping protein α*	P52907	32854 (1)	21	—
25	Apolipoprotein A-I binding protein	Q5T3I2	31668 (1)	51	—
26	Tubby protein	P50607	30340 (1)	24	—
27	*78-kDa glucose-regulated protein precursor*	P11021	72331 (1)	5.3	—
Downregulated proteins in adenoma compared with the same proteins in cancer tissue					
21	Citrate lyase β-like protein	Q8N0X4	1074.16 (2)	6.54	R.VNSVSSGLAEEDLETLLQSR.V
22	N-myc downstream-regulated gene 1	Q92597	42808 (1)	35	—
23	Guanine deaminase	Q9Y2T3	50971 (1)	34	—
24	EVI-5 homolog	O60447	92789 (1)	18	—

[a] The proteins identified by previous proteomic studies of colorectal cancer are in italic type.
[b] The m/z ratio is the ratio of the mass to the charge of each peptide.
[c] The cover rate is the percentage of amino acids in a particular peptide that were identified by mass spectrometry and matched those of the corresponding protein.
[d] The theoretical mass of each intact protein is given.
[e] The intensities of candidate protein spots in silver-stained 2-DE gels, as analyzed with PDQuest software, are given as values × 10^3 (mean ± SD).

voltage of 20 kV, a grid voltage of 63%–65%, positive polarity, and a delay time of 200 nanoseconds. Laser shots at 300 per spectrum were used to acquire the spectra with a range from 800 to 4000 Daltons. Trypsin autolysis products were used for internal mass calibration. Database searching was performed by using Mascot software (http://www.matrixscience.com). The search parameters were the nrNCBI database, human, 10–150 kDa, trypsin (1 missed enzymatic cleavage), and 100-ppm mass tolerance. The best match was the one with the highest score, and a significant match was typically a score of the order of 70 ($P < 0.05$) (16).

To increase the reliability of some low-abundance protein spots, those protein digests were further analyzed by a Finnigan LTQ ion-trap microelectrospray mass spectrometer (ThermoQuest, San Jose, CA) coupled with a Surveyor high performance liquid chromatography (HPLC) system (ThermoQuest). For LC separation, a microbore RP column (RP-18, 0.15 mm × 120 mm id, ThermoHypersil, San Jose, CA) was used. Solvent A was 0.1% (v/v) formic acid, and solvent B was 0.1% formic acid in 100% ACN. The gradient was held at 2% solvent B for 40 mins and increased linearly to 98% solvent B in 110 mins. The digests were applied to the RP18 column by an autosampler and eluted at a flow rate of 3 μl/min. The eluted peptides were introduced online to the electrospray source. Data collections were performed automatically. The ion source instrumental parameter settings were as follows: ESI voltage, 3.2 kV; capillary voltage, 5 V; and capillary temperature, 170°C. In the full scan mode, ions were collected in three microscans with a maximum ion injection time of 200 milliseconds, covering the range of m/z 400-1800. MS/MS was performed in a data-dependent mode. The MS/MS collision energy was 35%. Selected data were appended to a query and submitted to a nonredundant database search.

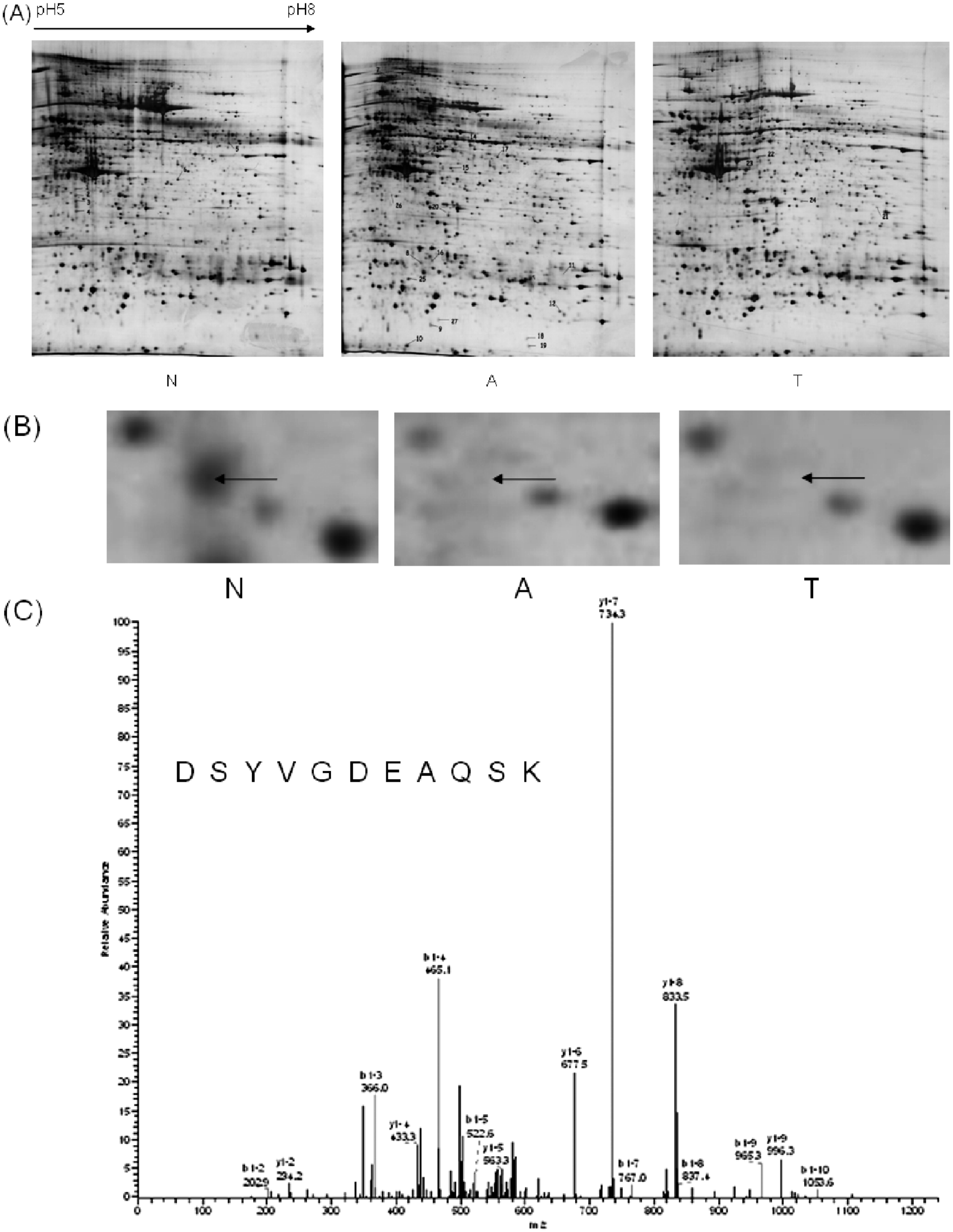
Table 1. Extended.

Theoretical MW[d]	Mascot scores (MALDI-TOF)	Intensity of candidate protein spots[e]		
		Normal	Adenoma	Cancer
34962	—	34.7 ± 8.7	0	0
33922	—	82.9 ± 5.7	0	0
30841	—	73.2 ± 21.9	10.5 ± 18.8	56.8 ± 19.0
160970	—	86.2 ± 17.2	1.1 ± 0.9	21.2 ± 9.3
572068	—	8.6 ± 1.7	0	0
44535	66	43.0 ± 19.1	8.5 ± 4.1	13.5 ± 2.6
92469	—	4.4 ± 3.9	44.6 ± 12.1	41.6 ± 7.9
25387	—	2.6 ± 1.5	20.3 ± 14.2	20.4 ± 7.6
20020	—	5.2 ± 2.1	88.0 ± 16.1	19.8 ± 5.7
14955	—	3.5 ± 1.5	39.5 ± 20.9	41.1 ± 5.8
53065	—	2.6 ± 1.0	18.5 ± 6.6	16.2 ± 4.4
21907	—	3.7 ± 2.5	20.6 ± 4.4	17.5 ± 7.4
47629	66	20.1 ± 9.7	81.5 ± 12.8	95.2 ± 6.7
69367	176	5.9 ± 2.6	31.3 ± 7.8	41.0 ± 13.5
47169	79	7.8 ± 3.8	37.7 ± 26.4	37.1 ± 19.1
40846	65	8.5 ± 6.1	44.4 ± 13.2	36.7 ± 14.3
49895	78	6.4 ± 2.3	39.2 ± 26.2	33.3 ± 11.9
147254	67	10.9 ± 6.8	64.9 ± 17.5	76.9 ± 23.1
26907	65	10.2 ± 7.1	64.8 ± 16.7	41.6 ± 8.6
32923	67	7.2 ± 4.3	42.0 ± 21.4	45.7 ± 15.8
31675	97	17.6 ± 5.7	70.5 ± 27.8	58.8 ± 22.9
55651	84	2.2 ± 1.1	11.9 ± 2.5	8.5 ± 3.5
72333	98	4.1 ± 2.3	14.1 ± 2.8	8.3 ± 2.3
37359	—	3.6 ± 2.8	5.4 ± 1.9	18.3 ± 4.3
42835	77	5.0 ± 2.2	9.4 ± 3.7	48.2 ± 16.2
51003	68	5.0 ± 4.8	5.9 ± 2.8	29.8 ± 11.5
92935	68	3.9± 1.7	4.1 ± 1.5	26.2 ± 11.7

Western Blot Analysis. To confirm the 2-DE results, additional samples, including 19 colorectal cancers and 8 colorectal adenomas with individual-matched normal mucosa, were used for Western blot analysis. Tissue samples were lysed as described above for use in 2-DE. Protein extracts (40 μg) were separated on a 12% SDS-polyacrylamide gel. Proteins were transferred to a polyvinylidene difluoride (PVDF) membrane ((Bio-Rad). After blocking, the membranes were incubated with a polyclonal goat antimimecan antibody (dilution of 1:2000; R&D Systems, Minneapolis, MN) and polyclonal goat anti-TXNDC5 antibody (dilution of 1:1000; Cell Signaling Technology, Danvers, MA); subsequently the membranes were incubated in horseradish peroxidase–anti-goat IgG (Abcam, Cambridge, UK). The specific proteins were visualized with chemiluminescent reagent (Pierce Biotechnology, Rockford, IL). As a control for equal protein loading, blots were restained by using antiactin antibody (dilution of 1:4000; Santa Cruz Biotechnology, Santa Cruz, CA). The band intensity was analyzed by PDQuest software ver7.1. The relative expression amount was calculated as the intensity ratio of mimecan or TXNDC5 to that of actin. The association between categorical data was analyzed by the nonparameter Wilcoxon test or Friedman test, when appropriate, by using the SPSS13.0 software package (SPSS Inc, Chicago, IL). The threshold was established at 0.05 (two-tailed).

Results

Proteomic Study of Colorectal Adenoma. The 2-DE protein patterns were studied in nine patients with individual-matched normal mucosa, adenoma, and cancer tissue and in one patient with individual-matched normal and adenoma tissue. There were about 1100 proteins in each gel. The proteins expressed in adenoma tissue were compared with those in matched normal and cancer tissue. The protein spots that showed 5-fold greater differential expression in at least five cases were considered to be differentially expressed candidates. The exact peak density values of the differentially expressed proteins are shown in Table 1. The quantities of all detected spots were analyzed by the nonparametric Wilcoxon test. In this way, we found 33 significantly different candidate protein spots, which were also present in the 2-DE gels of the sample pools (Fig. 1A). We analyzed these candidate spots by using mass spectrometry and identified a total of 27 proteins (Fig. 1B


(A)
pH5
pH8
5
6
7
14
17
15
26
20
8
16
25
11
12
27
9
10
18
19
22
23
24
21
N
A
T
(B)
N
A
T
(C)
D S Y V G D E A Q S K
Relative Abundance
m/z
y1-7 734.3
b1-4 465.1
y1-8 833.5
b1-3 366.0
y1-6 677.5
y1-4 433.3
b1-5 522.6
y1-5 963.3
b1-2 202.9
y1-2 294.2
b1-7 767.0
b1-8 837.4
b1-9 965.3
y1-9 996.3
b1-10 1053.6

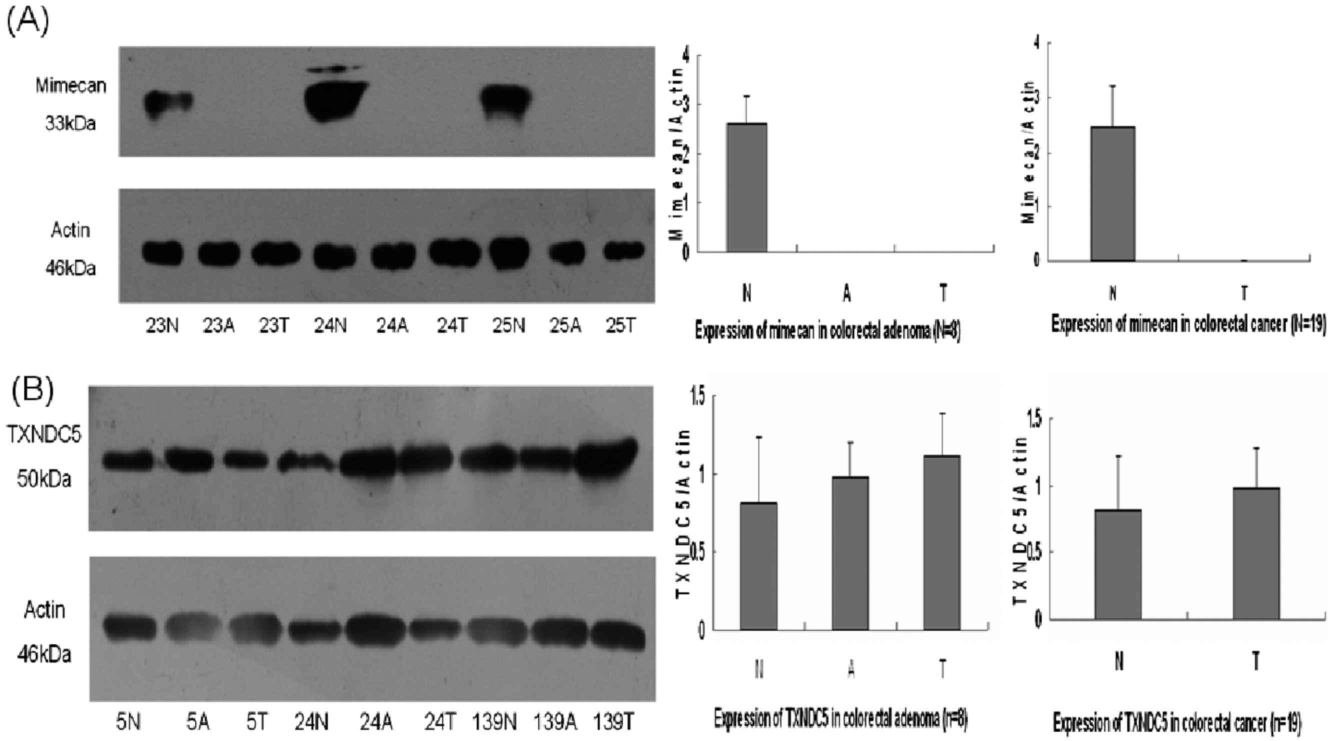


Figure 2. Western blot analysis of mimecan and TXNDC5. (A) Marked downregulation of mimecan in normal (N) tissues, colorectal adenoma (A) tissues, and cancer (T) tissues. Protein extracts (60 μg) were separated on a 12% SDS-polyacrylamide gel. Proteins were transferred to a PVDF membrane. After blocking, the membranes were incubated with polyclonal goat antimimecan antibody (dilution of 1:2000) and subsequently incubated with HRP–anti-goat IgG. The specific proteins were visualized with chemiluminescent reagent. As a control for equal protein loading, blots were restained with antiactin antibody. Immunosignals were quantified by densitometry scanning. The relative quantification was calculated as the ratio of mimecan expression to actin expression as shown in the right chart. (B) Upregulation of TXNDC5 in colorectal adenoma (A) and cancer (T) tissue in comparison with that in normal (N) mucosa. The same experimental process was performed, except that the membranes were incubated with polyclonal goat anti-TXNDC5 antibody (dilution of 1:1000).

and C and Table 1). The Mascot scores for the protein identified by MALDI-TOF, the amino acid sequence for the protein identified by LTQ-MS, and other parameters are given in Table 1. Six proteins were downregulated and 17 upregulated in adenoma when compared with the same proteins in normal mucosa. Four proteins were downregulated in adenoma when compared with the same proteins in colorectal cancer, but none was upregulated. We failed to detect six protein spots. There might be several reasons leading to this result, such as lower abundance, lower reliability of the MS results, and some characteristics of these proteins.

Validation of Mimecan and TXNDC5 by Western Blot. We found two novel candidate proteins, mimecan and TXNDC5, among the differentially expressed proteins. Their expression profiles in colorectal tumors have not been reported previously. Western blot analysis showed that mimecan was not expressed in any colorectal adenomas and cancers but was expressed in matched normal tissue (mean ± SD: 2.614 ± 0.537) (Fig. 2A). Mimecan was significantly downregulated in colorectal adenomas and cancers as compared with that in normal mucosa ($P < 0.01$, the Wilcoxon test or the Friedman test). TXNDC5 was upregulated in 6 of 8 colorectal adenomas and 16 of 19

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Figure 1. Differentially expressed proteins in colorectal adenoma. (A) Representative 2-DE images of matched colorectal normal (N), adenoma (A), and cancer (T) tissue. A linear gradient 24-m (pH 5–8) readystrip was rehydrated overnight with 300 μg of protein samples in 500 μl of rehydration buffer. After IEF, the proteins were further separated by electrophoresis in a 12% polyacrylamide gel. The proteins expressed in adenoma tissue were compared with those expressed in matched normal and cancer tissue. The protein spots that showed more than 5-fold differential expression in at least five cases were taken as differentially expressed candidates. Of 33 differentially expressed protein spots, 27 were identified by MS (numbered arrows; for protein nomenclature, see Table 1). (B) The magnified region of the 2-DE gel of downregulated mimecan (arrow) in colorectal adenoma (A) or cancer (T) tissue, compared with normal tissue (N). (C) MS of in-gel trypsin digests of this protein and analysis of the depicted peptide spectrum resulted in the identification of mimecan. The differentially expressed proteins were excised from the gel by Proteomeworks Spot Cutter and digested in the gel. Some protein digests were analyzed by a Finnigan LTQ ion-trap microelectrospray mass spectrometer coupled with a Surveyor HPLC system. Selected data were appended to a query and submitted to a nonredundant database search.

cancers as compared with that in normal mucosa (Fig. 2B). Compared with TXNDC5 in the matched normal tissue (mean ± SD: 0.815 ± 0.414), TXNDC5 was significantly upregulated in adenoma (mean ± SD: 0.978 ± 0.218) and cancer (mean ± SD: 1.112 ± 0.273) ($P < 0.05$, the Wilcoxon test or the Friedman test).

Discussion

Elucidating the molecular events underlying colonic adenoma will help us to understand the early stages in the genesis of colorectal cancer and has the potential to aid in the prevention of and intervention for colorectal cancer. In this study, we utilized the common approach of 2-DE coupled with mass spectrometry to study the differentially expressed proteins in individual-matched cases of normal mucosa, adenoma, and cancer and confirmed the differential expression of mimecan and TXNDC5 by Western blot.

We identified 27 differentially expressed proteins in colorectal adenoma. However, only 8 proteins (Table 1) had been found in nine previous proteomic studies (two studies used two-dimensional difference gel electrophoresis (2-DIGE), and seven used 2-DE) (4–7, 17–20). Initially, we postulated that the low coverage of differentially expressed proteins between our study and others may largely be due to our stringent standard of a 5-fold change. Most of the previously mentioned studies had a cut-off of 2-fold (7, 19) or were simply based on statistical assessment (3, 17, 18, 20). Presumably, our high cut-off may have missed other potentially important changes, although it can minimize the possibility of individual difference and ensure the fidelity of the change. However, as we reviewed the total of 213 differentially expressed proteins identified in all proteomic studies, including the current study, we only found 44 proteins (21%) in at least two studies and 6 (7%) between the 2-DIGE studies (4, 5). We believe that the major underlying interpretations for the discrepancy between different studies are the inherent heterogeneity of colorectal cancer and methodologic differences. A long-standing concept in cancer biology is that tumors arise and grow as a result of "tumor stem cells" or "stem cells" with multiple additional mutations (21). Consistent with this idea is the possibility that a defined minority of these cells might be able to proliferate, differentiate, dedifferentiate, and transdifferentiate *via* different pathways, showing heterogenous gene expression patterns and morphologic features. A differential protein expression profile is no more than a snapshot of the proteomics composition of a specific tissue at a specific time. There is also a strong methodologic contribution here to the underlying discrepancies between different studies, and the methodologic contribution impenetrates all processes of proteomic studies, including the sample collection and the separation and identification of proteins. Among these methods and technique parameters, we consider some factors to be especially associated with the inconsistent results; for instance, the pH ranges of IPG strips and the defined fold-change for the differentially expressed protein spots, as well as ethnic background and individual matched samples.

Strikingly, mimecan expression was strong in normal mucosa but lost in adenoma and cancer tissue, a finding in keeping that of with previous studies (22, 23). Mimecan has an extensive expression pattern in many normal tissues, including cornea, ventricle, intestine, kidney, skeletal muscle and skin, and its expression is lowest in brain, liver sternum, and amniotic membrane. Differential expression of mimecan is associated with many human diseases, such as primary open-angle glaucoma and pituitary tumors (24, 25). In cancer, mimecan expression is absent in different cancer cell lines such as HeLa and Saos-2, in which p53 is frequently inactivated/mutated (9). A conserved consensus p53-binding DNA sequence in the first intron of the human mimecan gene was validated. Wild-type p53 can activate mimecan expression *via* this binding sequence (9). Thus, mimecan has been accepted as a direct target gene of p53. Our recent preliminary study showed that mimecan could inhibit cell growth and induce cell apoptosis *in vitro* (data not shown). Collectively, these studies suggest that the mimecan gene, as a direct target of p53, may be a potential tumor suppressor gene involved in cancer initiation and progression. We postulate that mimecan might be a potential biomarker for future gene therapy.

TXNDC5 was significantly upregulated in colorectal adenoma and cancer tissue although its expression remained in normal mucosa. TXNDC5 was first detected by 2-DE analysis of the luminal environment of the endoplasmic reticula of hepatic tissue in 2003 (14). Upregulation of TXNDC5 was found in tumors of the cervix, uterus, stomach, and lung (10). A recent study showed that a variant of the TXNDC5 gene, *Hcc*-2, was upregulated in poorly differentiated hepatocellular carcinoma (HCC) but unchanged in well-differentiated HCC (15). A bioinformatics assessment suggested that TXNDC5 is a human secreted and transmembrane protein (26). Hence, we believe that TXNDC5 may be a promising serum biomarker for cancer diagnosis, although much work should be done in the future. TXNDC5 has a PDI domain with high sequence similarity to that of thioredoxin, a catalyst of the rate-limiting reaction of disulfide-bond formation, isomerization, and reduction within the endoplasmic reticulum, which plays important roles in antioxidative injury, antianoxia-induced apoptosis, and promotion of proliferation (27, 28). Emerging evidence has shown that TXNDC5 has similar biological effects. Yeast complementation tests showed that TXNDC5 can replace PDI function *in vivo* (14). TXNDC5 expression is upregulated by hypoxia and has a protective effect on endothelial cells under hypoxia by folding and chaperone activity on hypoxia-induced antiapoptotic molecules (10). However, the detailed biological roles of TXNDC5 in cancer remain to be elucidated.

In summary, the present study showed a differential protein expression profile of colorectal adenoma and cancer compared with that of matched normal mucosa. The candidate proteins may serve as potential biomarkers for early detection of colorectal cancer or may be of use in the therapeutic intervention for colorectal cancer in the future.

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