SPANDIDOS PUBLICATIONS

Differential expression in normal-adenoma-carcinoma sequence suggests complex molecular carcinogenesis in colon

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Abstract. The majority of colon cancers develop from pre-existing adenomas. We analyzed the expression profiles in the sequence of normal colon crypts, adenomas and early-stage carcinomas using microdissected cells from tubular adenomas with foci of malignant transformation. Differentially expressed genes were detected between normal-adenoma and adenoma-carcinoma, and were grouped according to the patterns of expression changes in the sequence. Down-regulated genes in the sequence included *PLA2G2A*, *TSPAN1*, *PDCD4*, *FCGBP*, *AATK*, *EPLIN*, *FABP1*, *AGR2*, *MTUS1*, *TSC1*, *galectin 4* and *MT1F*. *PLA2G2A* has been shown to suppress colon tumorigenesis in mice, but the pathobiological role in humans has been controversial. Our data showed continuous down-regulation of *PLA2G2A* in the sequence supporting an implication in human colon cancer. Tumor suppressor and/ or proapoptotic activities have also been reported in other genes. Up-regulated genes included ribosomal proteins, *IER3* and *TPR*. TGF-ß2 and matrix metalloproteinase 23B were up-regulated in carcinoma but not in adenoma, supporting the pathobiological roles in malignant transformation. Differentially expressed genes partly coincided with those in the adenoma-carcinoma sequence of the stomach, which was published previously, suggesting a partial overlap between the adenoma-carcinoma sequences of the colon and stomach.

Introduction

Colon cancers arise in pre-existing adenomas frequently, although alternative pathways are known to exist (1). Multiple genetic alterations have been implicated in the adenoma-carcinoma sequence (2). Many studies have suggested a role for uncharacterized genetic factors in predisposition to the majority of colon cancers (3-5). Thus, expression changes in the sequence might give us an insight into the molecular carcinogenesis. Nonetheless, surprisingly little expression profiling data of the adenoma/carcinoma sequence has been reported so far. The available data mostly report the differential expression between whole tissue samples of adenoma and carcinoma from multiple individuals (6-10). However, DNA microarray data using whole tissue samples of gastrointestinal tracts is particularly difficult to interpret because of considerable data noise from various inflammatory infiltration and reactive stromal changes (11). Furthermore, potential individual variations should be considered. To avoid those problems, it would be advantageous to investigate the sequential expression changes in a given adenoma with foci of malignant transformation and adjacent normal crypts using microdissection.

Formalin-fixation and paraffin-embedding is usually required for the delineation of subtle lesions such as malignant foci developing in adenoma. It also makes retrospective studies possible using paraffin blocks in pathology files. A problem of formalin-fixation is that it causes extensive base modification of nucleic acids (12), which makes it difficult to recover RNAs for the expression profiling. However, expression profiling of formalin-fixed tissue samples has been improved considerably (13,14). Recently, we reported a reproducible procedure of RNA extraction and amplification which showed differential expression in the adenoma-carcinoma sequence of the stomach (15).

The adenoma-carcinoma sequence may also be found in a group of stomach cancers (16,17), although it does not seem to be as frequently shown as in colon. Gastric tubular adenomas almost always develop in the background of intestinal metaplasia and often show the adenoma-carcinoma sequence reminiscent of that in the colon (Lee I, unpublished data), suggesting that similar molecular sequences of carcinogenesis might be implicated in both type of cancers.

In this study, we analyzed the expression changes in the normal-adenoma-carcinoma sequence in the colon using microdissected cells from formalin-fixed, paraffin-embedded tissues. Differentially expressed genes were categorized according to the patterns of change in the sequence. They partly coincided with those published in the adenoma-carcinoma sequence of the stomach, suggesting a partial

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overlap between the adenoma-carcinoma sequences in the colon and stomach. The pathobiological implications of *PLA2G2A* and other differentially expressed genes in colon carcinogenesis are discussed.

Materials and methods

Tissue samples and microdissection. Two colon biopsies of tubular adenomas with focal malignant changes were selected from the surgical pathology file of Asan Medical Center, Seoul, Korea, following the guidelines of the Institutional Review Board. Polyps were incidentally detected in the sigmoid colon of 48- and 54-year old males who underwent colonoscopic check-up without clinical symptoms. They measured 3.0 and 1.4 cm in diameter, and were completely resected at the stalk.

Polyps were fixed immediately in 10% buffered-formalin and processed routinely. After the histopathological diagnosis, additional 6-μM sections were taken from the paraffin blocks. For the sectioning and H&E staining, all the solutions were freshly made using DEPC-treated water, and the slides and instruments were autoclaved. Normal crypts, adenoma and carcinoma cells were microdissected using an AutoPix laser capture microscope system (Arcturus, Mountain View, CA).

RNA extraction and amplification. Deparaffinized sections were removed from the slides by applying 200 μl proteinase K buffer [2% SDS, 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA]. Samples were transferred into a microcentrifuge tube and incubated at 70˚C for 1 h. Then, 3 μl proteinase K (30 μg/μl, Intron Biotechnology, Songnam, Korea) was added, and incubated again at 55˚C for 1 h. RNAs were extracted with TRIzol reagents (Invitrogen, Carlsbad, CA) as described previously (15).

First- and second-strand DNA synthesis was performed using T7dT primers and RNase H/T3N6 primers, as described previously (15). Then the double-stranded DNA was applied to PCR amplification using T7 promoter primers (100 pM/μl, 5'-CGGCCAGTGAATTGTAATACGACTCACTATAG GCG-3') and T3 promoter primers (100 pM/μl, 5'-GCGCG AAATTAACCCTCACTAAAGGGAGAGGG-3'). The PCR reaction was performed in a GeneAmp PCR 9600 system (PE Biosystems, Foster City, CA) for 1 min at 95˚C; 20 cycles of 30 sec at 95˚C, 40 sec at 65˚C and 5 min at 68˚C; and 7 min at 68˚C. PCR products were purified using a MinElute™ PCR purification kit (Qiagen, Valencia, CA). *In vitro* transcription was performed using AmpliScribe™ T7 or T3 high yield transcription kit (Epicenter, Madison, WI) at 37˚C for 5 h in 40 μl of reaction volume. Synthesized aRNA was purified using an RNeasy Mini kit (Qiagen).

cDNA microarray analysis. GenePlorer TwinChip Human-8K (Digital Genomics, Seoul, Korea) microarray chips were applied. The chips had two identical microarrays per slide, total gene spots of 8170 each. Probe labeling and hybridization were performed using the amine-modified random primer aminoallyl method as described previously (15,18). Either adenoma or carcinoma was labeled with Cy3 and hybridized against the same normal control labeled with Cy5. Then, the hybridization was performed as described previously (15).

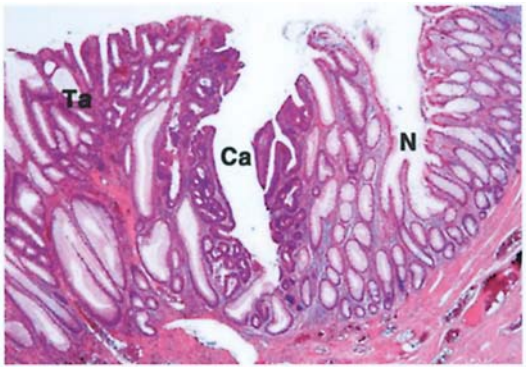

Figure 1. Histopathology of tubular adenoma (Ta) with focal malignant transformation (Ca). The right hand side shows the stalk consisting of normal crypts (N). (H&E stain x30).

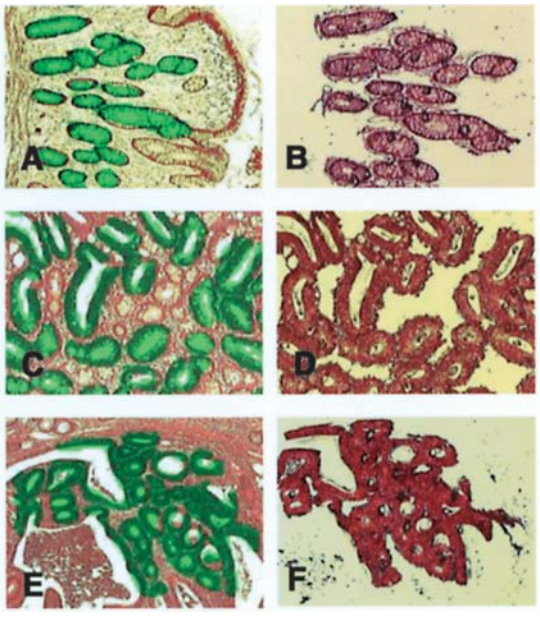

Figure 2. Crypts marked as green circles under the laser capture microscope before microdissection (left) and microdissected cells (right) of the normal gastric mucosa (A and B), adenoma (C and D), and carcinoma (E and F). (H&E stain x30).

Arrays were scanned with a GenePix 4000B scanner (Axon, Foster City, CA), and images were analyzed using the GeneSight program (BioDiscovery, Los Angeles, CA). Gene spots with threshold R-value <2.0 were omitted, and a total of 7087 normalized spots (86.7%) were selected for analysis. Differentially expressed genes were analyzed using the Significance Analysis of Microarray (SAM) method, which provides the serial lists of both up-, and down-regulated genes at a given false-significance rate of choice (19).

Results

cDNA microarray analysis of normal-adenoma-carcinoma sequence. Tubular adenomas had foci of malignant transformation with early-stage stromal invasion (Fig. 1). They were completely resected at the stalk, which consisted of normal colon mucosa. Crypts from normal mucosa, adenoma, and carcinomas were microdissected without stromal or

Up-regulated genes in colon polyps.

Symbol	Title	Unigene ID	GenBank ID
RPL23	Ribosomal protein L23	Hs.406300	AI147195
PPAP2C	Phosphatidic acid phosphatase type 2C	Hs.24879	AF047760
IER3	Immediate early response 3	Hs.76095	AI185199
DDX21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21	Hs.169531	U41387
RPL35	Ribosomal protein L35	Hs.182825	AA305945
RECQL4	RecQ protein-like 4	Hs.31442	AB006532
RPL13	Ribosomal protein L13	Hs.410817	AI382216
GTF3A	General transcription factor IIIA	Hs.445977	AI686944
AHCY	S-adenosylhomocysteine hydrolase	Hs.388004	M61831
OLFM4	Olfactomedin 4	Hs.508113	AI923293
TBC1D16	TBC1 domain family, member 16	Hs.458300	AI683825
FLJ38753	Hypothetical protein FLJ38753	Hs.406913	AI820608
TNNC2	Troponin C2, fast	Hs.182421	NM_003279
RPS12	Ribosomal protein S12	Hs.380956	AA314429
PAIP1	Poly(A) binding protein interacting protein 1	Hs.374614	AF013758
SORD	Sorbitol dehydrogenase	Hs.878	U07361
GPX2	Glutathione peroxidase 2 (gastrointestinal)	Hs.2704	X68314
KIAA0582	KIAA0582	Hs.146007	AI637917
DTX2	Deltex homolog 2 (Drosophila)	Hs.89135	AW001329
RPS7	Ribosomal protein S7	Hs.444012	AA315981
CTSZ	Cathepsin Z	Hs.252549	AI913006
TOP2A	Topoisomerase (DNA) II α 170 kDa	Hs.156346	NM_001067
TMEM9	Transmembrane protein 9	Hs.181444	AI281733
SMARCC1	SWI/SNF related, matrix associated, regulator of chromatin	Hs.162086	AI333650
DACH1	Dachshund homolog 1 (Drosophila)	Hs.63931	AJ005670
TNFRSF12A	Tumor necrosis factor receptor superfamily, member 12A	Hs.355899	AI827127
IFRD1	Interferon-related developmental regulator 1	Hs.7879	Y10313
RPS14	Ribosomal protein S14	Hs.381126	AI928982
MCLC	Mid-1-related chloride channel 1	Hs.93121	AI139458
HOXB2	Homeo box B2	Hs.290432	X16665
DDAH2	Dimethylarginine dirnethylaminohydrolase 2	Hs.247362	AI971320
GSTO1	Glutathione S-transferase ω 1	Hs.11465	AI752707
PAICS	Phosphoribosylaminoimidazole carboxylase	Hs.444439	AI524157
GPC4	Glypican 4	Hs.58367	AA887423
NHP2L1	NHP2 non-histone chromosome protein 2-like 1	Hs.182255	AI338639
AUH	AU RNA binding protein/enoyl-Coenzyme A hydratase	Hs.81886	X79888
KIAA1434	Hypothetical protein KIAA1434	Hs.145509	AI187171
RPL9	Ribosomal protein L9	Hs.412370	AI625598
TKT	Transketolase (Wernicke-Korsakoff syndrome)	Hs.89643	AI378884
HHEX	Hematopoietically expressed homeobox	Hs.118651	X67235
ZNF204	Zinc finger protein 204	Hs.8198	AF033199
CAMLG	Calcium modulating ligand	Hs.13572	AF068179
ZNF581	Zinc finger protein 581	Hs.82482	AA280033
TPR	Translocated promoter region (to activated MET oncogene)	Hs.170472	AA779660
C6orf11	Chromosome 6 open reading frame 11	Hs.436930	AI745013

inflammatory cells (Fig. 2). From 10000 microdissected cells, approximately 70-80 μg aRNA was obtained after *in vitro* transcription. The expression profiles were analyzed using cDNA microarrays. After normalization, 7087 gene spots (86.7%) were selected for analysis. The complete data set is publicly available in the NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) through the accession number GSE3880.

Table II. Down-regulated genes in colon polyps.

Symbol	Title	Unigene ID	GenBank ID
PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)	Hs.76422	H00742
SLC26A2	Solute carrier family 26 (sulfate transporter), member 2	Hs.302738	AA194161
MYLK	Myosin, light polypeptide kinase	Hs.386078	AF069604
TSPAN1	Tetraspan 1	Hs.38972	AF065388
PDCD4	Programmed cell death 4 (neoplastic transformation inhibitor)	Hs.257697	N92498
PEX1	Peroxisome biogenesis factor 1	Hs.164682	AL046043
FCGBP	Fc fragment of IgG binding protein	Hs.111732	D84239
AATK	Apoptosis-associated tyrosine kinase	Hs.514575	AB014541
CLDN7	Claudin 7	Hs.513915	AJ011497
SLC26A2	Solute carrier family 26 (sulfate transporter), member 2	Hs.302738	U14528
HLA-DQA1	Major histocompatibility complex, class II, DQ α 1	Hs.387679	AI214199
C1orf34	Chromosome 1 open reading frame 34	Hs.112949	AF007170
EPLIN	Epithelial protein lost in neoplasm ß	Hs.10706	AL048161
APPL	Adaptor protein, PTB domain and leucine zipper motif 1	Hs.27413	AA493310
SEMA6A	Sema domain, transmembrane domain and cytoplasmic domain	Hs.443012	AB002438
SULT1A3	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 3	Hs.460587	L25275
FABP1	Fatty acid binding protein 1, liver	Hs.380135	M10050
SLC26A3	Solute carrier family 26, member 3	Hs.1650	L02785
NEK3	NIMA (never in mitosis gene a)-related kinase 3	Hs.2236	Z29067
KIAA0703	KIAA0703 gene product	Hs.6168	AB014603
MATN2	Matrilin 2	Hs.153647	AA953246
AGR2	Anterior gradient 2 homolog (Xenopus laevis)	Hs.226391	AI800451
IMPA1	Inositol(myo)-1(or 4)-monophosphatase 1	Hs.334022	NM_005536
MTUS1	Mitochondrial tumor suppressor 1	Hs.7946	AI028661
ITM2C	Integral membrane protein 2C	Hs.111577	AA989167
MECP2	Methyl CpG binding protein 2 (Rett syndrome)	Hs.3239	NM_004992
PVRL3	Poliovirus receptor-related 3	Hs.436602	AL050071
C9orf19	Chromosome 9 open reading frame 19	Hs.302766	AI359275
PROC	Protein C (inactivator of coagulation factors Va and VIIIa)	Hs.2351	NM_000312
ENPP1	Ectonucleotide pyrophosphatase/phosphodiesterase 1	Hs.213840	M57736
IGLJ3	Immunoglobulin λ joining 3	Hs.449601	BF238115
IGJ	Immunoglobulin J polypeptide	Hs.381568	AA507911
TSC1	Tuberous sclerosis 1	Hs.69429	AF013168
FXYD3	FXYD domain containing ion transport regulator 3	Hs.301350	AA826766
HDLBP	High density lipoprotein binding protein (vigilin)	Hs.427152	M64098
LGALS4	Lectin, galactoside-binding, soluble, 4 (galectin 4)	Hs.5302	AA130458
NUCB2	Nucleobindin 2	Hs.423095	X76732
CD63	CD63 antigen (melanoma 1 antigen)	Hs.445570	NM_001780
MGC31967	Hypothetical protein MGC31967	Hs.534579	AI300939
DDX5	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	Hs.279806	X15729
AKAP9	A kinase (PRKA) anchor protein (yotiao) 9	Hs.58103	AB019691
ZYX	Zyxin	Hs.75873	X94991
MUC1	Mucin 1, transmembrane	Hs.89603	AI922289
DNCL12	Dynein, cytoplasmic, light intermediate polypeptide 2	Hs.369068	AF035812
MT1F	Metallothionein 1F (functional)	Hs.438737	AI814448

Differential expression in the sequence. Hybridization data of 4 normal-adenoma and normal-carcinoma sets were analyzed using the SAM method, respectively (19). At the expected false significance rate of 0.88%, 54 up-regulated and 471 down-regulated genes were detected in combined adenoma/carcinoma (Fig. 3). Up-regulated genes included ribosomal

Genes up-regulated in cancer but not in adenoma.

Symbol	Title	Unigene ID	GenBank ID
TGFB2	Transforming growth factor, ß 2	Hs.133379	M19154
RERG	RAS-like, estrogen-regulated, growth inhibitor	Hs. 199487	AI971219
RPESP	RPE-spondin	Hs.439040	AA939100
DPP4	Dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2)	Hs.368912	X60708
LRPPRC	Leucine-rich PPR-motif containing	Hs.368084	AA663581
ISL1	ISL1 transcription factor, LIM/homeodomain, (islet-1)	Hs.505	U07559
ITGA4	Integrin, α 4 (antigen CD49D, α 4 subunit ofVLA-4 receptor)	Hs.553495	X16983
SLC35F5	Solute carrier family 35, member F5	Hs.292509	N25427
PDE4B	Phosphodiesterase 4B, cAMP-specific	Hs.198072	L12686
MMP23B	Matrix metalloproteinase 23B	Hs.211819	AB010961
ZNF217	Zinc finger protein 217	Hs.155040	AA460802
MPP6	Membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6)	Hs.533355	AI979249
MAP3K4	Mitogen-activated protein kinase kinase kinase 4	Hs.390428	AI299943
CHML	Choroideremia-like (Rab escort protein 2)	Hs.170129	N66913
FBX07	F-box protein 7	Hs.5912	AI360050

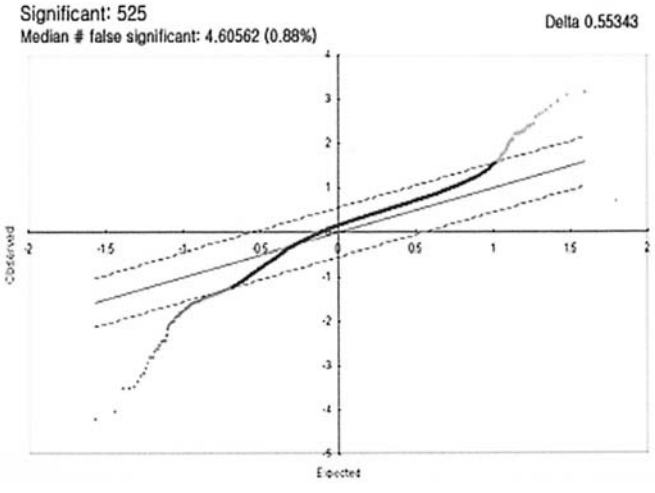

Figure 3. SAM analysis of colon polyps microdissected from formalin-fixed biopsies. At the expected false significance rate of 0.88%, 54 up-regulated and 471 down-regulated genes were detected in colon polyps compared to normal mucosa crypts.

proteins, *IER3*, *DDX21*, *RECQL4*, *AHCY*, *SORD*, *DDAH2*, transketolase and *TPR* (Table I). TPR was reported to re-arrange with MET oncogene upon chromosomal translocation in gastric cancers and precursor lesions (20).

Down-regulated genes included *PLA2G2A*, solute carrier proteins, *TSPAN1*, *PDCD4*, *AATK*, *FCGBP*, *EPLIN*, *FABP1*, *AGR2*, *MTUS1*, *TSC1* and *2*, *galectin 4* and *MT1F* (Table II). *PLA2G2A* has been shown to suppress APC-induced tumorigenesis in mice (21,22). *PDCD4* and *AATK* have been implicated in apoptosis (23,24). *PDCD4* has been reported to down-regulate in lung, breast, colon, and prostate cancers (23), and also in stomach cancers (11). *FCGBP* is a mucin-like, non-Fc receptor protein, normally expressed in colon goblet cells (25). Mitochondrial tumor suppressor 1 (*MTUS1*) and metallothionein 1F (*MT1F*) have been implicated in tumor suppressor activity and cancer growth inhibition (26,27). *TSC1* and *2* are tumor suppressor genes implicated in the tuberous sclerosis complex (28). Galectin 4 has been reported to down-regulate in colon cancers, suggesting an implication in early colorectal carcinogenesis (29). *DDX5* is an RNA helicase which functions as a transcriptional repressor, being implicated in organ differentiation (30).

Pattern analysis of differentially expressed genes in the sequence. We then analyzed the step-wise changes in normal-adenoma-carcinoma sequence. In comparison with adjacent normal crypts, tubular adenoma showed 422 up-, and 16 down-regulated genes at the expected false significance rate 0.66%. In comparison with adenoma, carcinoma showed 20 up-, and 455 down-regulated genes at the expected false significance rate 0.16%. Genes with differential expression were categorized into 9 groups according to the patterns in the normal-adenoma-carcinoma sequence as described in Materials and methods.

The group of genes that show significant up-regulation in carcinoma but not in adenoma might be implicated in the process of malignant transformation. This group included TGF-ß2 and matrix metalloproteinase 23B (Table III). TGF-ß signaling controls diverse cellular responses and has been implicated in various cancers (31). Matrix metalloproteinases have been associated with cancer invasiveness (32).

Twenty-one genes showed continuous down-regulation in the normal-adenoma-carcinoma sequence, including *PLA2G2A*, *FCGBP*, *SLC26A2*, *EPLIN*, *TSPAN1*, *PDCD4*, *PEX1*, *MTUS1*, *FABP1*, *DDX5*, *HLA-DQA1*, *AGR2*, *MT1F* and *galectin 4*. Many genes have been reported to have tumor suppressor/proapoptotic activities as described above. It was of interest that down-regulation of *HLA-DQA1* has also been described by Nosho *et al* using fresh tissue samples of colon polyps without signal amplification (10). It has been implicated in

Table IV. Overlapping genes between the adenoma/carcinoma sequences in the colon and stomach.

Symbol	Up-regulated genes
RPL23	Ribosomal protein L23
IER3	Immediate early response 3
DDX21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21
RECQL4	RecQ protein-like 4
AHCY	S-adenosylhomocysteine hydrolase
SORD	Sorbitol dehydrogenase
RPS7	Ribosomal protein S7
DDAH2	Dimethylarginine dimethylaminohydrolase 2
TKT	Transketolase (Wernicke-Korsakoff syndrome)
TPR	Translocated promoter region
RPS23	Ribosomal protein S23
EEF1B2	Eukaryotic translation elongation factor 1 ß 2
RPLP0	Ribosomal protein, large, P0
RPL37	Ribosomal protein L37
RPLP1	Ribosomal protein, large, P1
RPS29	Ribosomal protein S29
MRPL3	Mitochondrial ribosomal protein L3
KPNB1	Karyopherin (importin) ß 1
RPL10A	Ribosomal protein L10a
HMGB2	High-mobility group box 2
TRA1	Tumor rejection antigen (gp96) 1
CHD4	Chromodomain helicase DNA binding protein 4
RPS27A	Ribosomal protein S27a
NCL	Nucleolin
RPL11	Ribosomal protein L11
ETS2	v-ets erythroblastosis virus E26 oncogene homolog 2
HNRPK	Heterogeneous nuclear ribonucleoprotein K
TPT1	Tumor protein, translationally-controlled 1
TCF3	Transcription factor 3
MAGOH	Mago-nashi homolog, proliferation-associated
SFRS1	Splicing factor, arginine/serine-rich 1
MCM3	MCM3 minichromosome maintenance deficient 3
YWHAQ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, θ polypeptide
HNRPU	Heterogeneous nuclear ribonucleoprotein U
SIVA	CD27-binding (Siva) protein
CXCL16	Chemokine (C-X-C motif) ligand 16
BARD1	BRCA1 associated RING domain 1
POGZ	Pogo transposable element with ZNF domain
LBR	Lamin B receptor
PRKAG1	Protein kinase, AMP-activated
TMPO	Thymopoietin
LIG1	Ligase I, DNA, ATP-dependent
BLVRB	Diliverdin reductase B [flavin reductase (NADPH)]
GLB1	Galactosidase, ß 1
GBAS	Glioblastoma amplified sequence
HSPCB	Heat shock 90 kDa protein 1, ß
C14orf170	Chromosome 14 open reading frame 170
PSMD11	Proteasome (prosome, macropain) 26S subunit
ZDHHC3	Zinc finger, DHHC domain containing 3
ATP6V1G1	ATPase, H^+ transporting, lysosomal 13 kDa
ATP5L	ATP synthase, H^+ transporting
FLJ14299	Hypothetical protein FLJ14299
FBX07	F-box only protein 7
Symbol	**Down-regulated genes**
TSPAN1	Tetraspan 1
FABP1	Fatty acid binding protein 1, liver
AGR2	Anterior gradient 2 homolog
IGLJ3	Immunoglobulin λ joining 3
PIGR	Polymeric immunoglobulin receptor
SLC12A2	Solute carrier family 12
RPS4Y	Ribosomal protein S4, Y-linked

gastritis and gastric cancer (33), although the pathobiological role in colon carcinogenesis is not clear.

Overlapping in the sequences of the colon and stomach. We then compared the differentially expressed genes with those in the adenoma-carcinoma sequence of the stomach similarly studied in our previous study (the complete data set is publicly available at NCBI GEO, accession numbers GSM20670-5) (15). Numerous up-regulated genes overlapped in the colon and stomach sequence, including ribosomal proteins and *TPR* (Table IV). Overlapping down-regulated genes included *TSPAN1*, *FABP1* and *AGR2*, the pathobiological roles of which were not clear.

Discussion

We have shown the sequential expression change in the normal-adenoma-carcinoma sequence in the colon using microdissected cells from polyps with foci of malignant transformation. To our knowledge, this is the first report of the expression profiling in the colon adenoma-carcinoma sequence using microdissected cells. Differentially expressed genes were grouped according to the patterns of expression change, which might suggest pathobiological roles in the sequence.

expression profiling of microdissected cells has evident advantages in the analysis of subtle pathologic changes in continuity such as the adenoma-carcinoma sequence excluding the contamination of other cell types. On the other hand, a potential risk of the procedure-induced data noise should be weighed against the advantage. Nonetheless, several lines of evidence support the general applicability of our approach. Previously, we showed good correlation coefficients with fresh tissue counterparts using the same procedure (15). Also, the data recovery rate, 86.7% hybridization in all gene spots, was reasonably high. Furthermore, coinciding data was shown in other reports in which fresh samples were used without amplification. For instance, *HLA-DQA1* was also reported to be down-regulated in colon adenomas (10). Similarly, genes such as *PDCD4*, *SEPP1*, *TSNAX* and *IGLJ3* were also down-regulated in our previous expression profiling study of stomach cancer without amplification (11).

PLA2G2A is one of the most extensively studied genes for tumorigenesis in the colon. It was first identified in mice as a chromosomal deletion mutant which modified the tumorigenesis caused by germ-line mutation of the Apc gene, *Mom-1* (modifier of min-1) (34). *Mom-1* was soon revealed to be *PLA2G2A* (21), which played a central role in the cytokine-induced release of arachidonic acid (35). Later, *PLA2G2A* was shown to suppress APC-induced tumorigenesis in mice (22,36). However, the pathobiological role in human colon cancer has been controversial as no coding sequence mutation was reported (37,38). On the other hand, *PLA2G2A* has been shown to down-regulate in the majority of colon cancers (39,40), suggesting its implication in human colon cancers. Our report of the continuous down-regulation of *PLA2G2A* in the adenoma-carcinoma sequence strongly supports its implication in the tumorigenesis and carcinogenesis of the colon. It is suggested that the transcriptional regulation of *PLA2G2A* might be of pathobiological significance in the adenoma-carcinoma sequence. *PLA2G2A* has been associated with prolonged survival in stomach cancers (41).

One of the advantages of microdissection-expression profiling is the sequential analysis at multiple transition points of disease progression. In this study, we analyzed 3 phases (2 transition points), resulting in grouping of differentially expressed genes into 9 groups according to the patterns of expression change. Such grouping based on sequential expression changes would facilitate the sorting of candidates for 'meaningful' genes in the functional genomics of cancer *in vivo*. More phases could be analyzed such as different grades of dysplasia, stromal invasion, or metastasis in various organs. This unique opportunity could also be applied to the analysis of pathogenetic steps and/or progression of other diseases *in vivo*.

The detection of multiple differentially expressed genes in the sequence suggests a complex molecular carcinogenesis in the colon. It is of interest that the sequential expression changes in the colon partly overlapped with those in the stomach (15). Our data suggests that the adenoma-carcinoma sequences in the colon and stomach might share certain common characteristics of carcinogenesis. Further analyses of differential expression in detailed pathological steps of carcinogenesis and cancer progression are required.

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