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BRIEF ARTICLE

Identification of differential gene expressions in colorectal cancer and polyp by cDNA microarray

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Abstract

AIM: To screen the differential expressed genes in colorectal cancer and polyp tissue samples.

METHODS: Tissue specimens containing 16 cases of colorectal adenocarcinoma and colorectal polyp *vs* normal mucosae were collected and subjected to cDNA microarray and bioinformatical analyses. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to confirm some of the cDNA microarray data.

RESULTS: The experimental data showed that eight genes were differentially expressed, most of which were upregulated in adenomatous polyp lesions. Forty-six genes expressions were altered in colorectal cancers, of which 29 were upregulated and 17 downregulated, as compared to the normal mucosae. In addition, 18 genes were similarly altered in both adenomatous polyps and colorectal cancer. qRT-PCR analyses confirmed the cDNA microarray data for four of those 18 genes: *MTA1*, *PDCD4*, *TSC1* and *PDGFRA*.

CONCLUSION: These differentially expressed genes likely represent biomarkers for early detection of colorectal cancer and may be potential therapeutic targets after confirmed by further studies.

Key words: Colorectal polyp; Colorectal cancer; cDNA microarray; Quantitative reverse transcription-polymerase chain reaction

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INTRODUCTION

Colorectal polyp (CRP) is considered as a premalignant lesion for development of colorectal cancer (CRC)[1]. Although the mechanism underlying colorectal cancer de-

velopment remains to be defined, a series of genetic and epigenetic events are thought to play important roles in colorectal carcinogenesis, including oncogene activation and tumor suppressor gene inactivation[2,3].

By attaining a detailed understanding of the altered gene expression profile of colorectal cancer novel strategies may be developed for earlier detection and more effective prevention and treatment, thereby reducing colorectal cancer incidence and increasing survival rates.

In this study, we performed a cDNA microarray analysis to profile differential gene expressions in tissue specimens of polyps and colorectal carcinoma and compared the expression profiles to that in corresponding normal tissues. We chose the genes with marked differential expressions for verification by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). These data provide insightful information into the genetic mechanisms of colorectal cancer and identify genes that may be useful as biomarkers for early disease detection.

Table 1 Clinical characteristics of patients with colorectal cancer or polyps

Case	Sex	Age	Size in cm/*n*		Differentiation	Depth	Dukes
			CRC	CRP	CRC	Polyps	
1	F	41	1.7/2	0.6/2	High	S	B1
2	M	37	0.9/1	1.2/3	Poor	Ss	C1
3	M	68	2.9/2	0.7/1	Poor	Sm	D1
4	F	27	2.8/1	1.4/1	Poor	Ss	C2
5	M	59	1.8/1	0.5/3	Moderate	S	C2
6	F	52	2.9/1	1.9/4	Poor	Ss	B2
7	M	71	3.3/2	0.4/1	High	Sm	B1
8	M	46	2.7/1	3.1/1	Moderate	Ss	C1
9	M	28	0.9/1	2.8/2	Poor	Sm	B1
10	F	36	2.6/1	0.4/1	Poor	Ss	C2
11	F	30	2.4/1	2.8/2	Moderate	S	C1
12	M	46	3.1/1	1.3/2	Poor	Sm	B1
13	M	62	1.5/1	2.0/3	Poor	Ss	C2
14	F	24	0.8/1	2.4/2	Moderate	Sm	C2
15	M	70	2.5/1	4.3/1	Poor	S	B2
16	M	43	3.2/2	3.7/1	High	Ss	B2

S: Serosa; Ss: Subserosa; Sm: Submucosa.

MATERIALS AND METHODS

Ethics

This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and approved by the Medical Ethics Committee of Fujian Province, China. All patients read and signed an informed consent form prior to surgery and sample collection.

Patient tissue

A total of 16 patients with colorectal adenocarcinoma and adenomatous polyp lesions were collected from The 174th Hospital of the Chinese PLA between May 2006 and December 2010. Diagnosis of these patients was confirmed by surgical pathology. None of the patients received any pre-surgical chemo- or radiation-therapy. All tissue specimens were immediately taken from the operation room upon excision from the patient, snap frozen in liquid nitrogen, and stored at -80 ℃ until use. The tissue specimens from these patients were divided into two groups: adenomatous polyp lesions *vs* proximal non-cancerous colorectal mucosae (Group A) or colorectal cancer *vs* proximal non-cancerous colorectal mucosae (Group B). The clinicopathological characteristics of these patients are summarized Table 1.

RNA isolation and cDNA microarray analysis

Total cellular RNA was extracted from the tissue samples by using the Trizol reagent (Sigma-Aldrich Inc., Germany)[2,4]. mRNA isolation was then carried out with Qiagen Oligotex beads, (Valencia, CA, United States) according to the manufacturer's instructions. The final concentration of mRNA was measured by spectrophotometer.

Next, the mRNAs from colorectal cancer or adenomatous polyp lesions were reverse transcribed into cDNA by means of Cy5-dUTP labeling, while the mRNAs from the normal mucosae were processed with Cy3-dUTP labeling by following the manufacturer's protocols (NEN Company, Boston, MA, United States). The labeled probes were then hybridized to the cDNA microarray (Chipscreen, Shenzhen, China), which contained 8064 human genes.

Microarray scanning and data analysis

Hybridized cDNA microarrays were scanned using a Gene PIX 4000 microarray fluorescence scanner (Axon Instruments, Foster City, CA, United States). Accompanying bioinformatical software was used convert the output images to data form and perform analysis. Ratios of Cy5: Cy3 were normalized to the median ratio value of all the microarray spots detected. Spots with intensities in both channels that were 0.5 to 2.0-fold higher than the local background were excluded from further analysis. SPSS v13.0 statistical software (Chicago, IL, United States) was used to carry out Student's *t*-test statistical analysis to determine significant intergroup differences of gene expression. *P*-values < 0.05 were considered statistically significant.

Quantitative reverse transcription-polymerase chain reaction

Differentially expressed genes between the adenomatous polyp lesions and colorectal cancer samples detected by the cDNA microarray, as compared to non-cancerous tissues, were verified by using qRT-PCR. The primers for these mRNAs used for qRT-PCR is listed in Table 2.

RESULTS

Qualification of the isolated total RNA and mRNA from the tissue samples

Quality of the isolated total RNA and mRNA from the tissue samples was found to have high correlation coefficients (Figure 1). The fluorescence signal was consistent

Table 2 Primers used for quantitative reverse transcription polymerase chain reaction analysis

Gene tag	Sequences	Tm (℃)	Cycle	Product (bp)
MTA1	5′-AGCCGTGCTTCGGTATCTT-3′ 5′-CCCGTTGTGCTGCTCGTA-3′	57	30	580
PDCD4	5′-GCTGAATTCGGATGGATG-TAGAAAATGAGCAGA-3′ 5′-CTGCTCGAGTCAG-TAGCTCTCTGGTTTAAGA-3′	54	27	470
TSC1	5′-ATCGCCTTTATGGAATGT-3′ 5′-GCTTGTGGTGGTTCAGTT-3′	49	29	510
PDGFRA	5′-ACCATAAGGCTCTTACTCT-3′ 5′-TTCTGGCACTTACCTACA-3′	45	31	490

with the expectations and standards (Figure 2); for example, the good 28S to 18S RNA subunit ratio in these samples indicated that there was no significant degradation (data not shown).

Identification of differential gene expression profile between colorectal cancer and adenomatous polyp lesions

cDNA microarray analysis of these tissue mRNAs revealed that eight genes were differentially expressed between adenomatous polyp lesions and the normal mucosae, and most of these were upregulated in the polyps (Table 3, $P < 0.05$). Meanwhile, 46 genes were differentially expressed between colorectal cancer and normal tissues, of which 29 were up-regulated in the cancer samples (Table 3, $P < 0.05$). A total of 18 genes were found to be similarly altered in both adenomatous polyp lesions and colorectal cancer samples (Table 4). qRT-PCR confirmed the observed patter for four of those 18 genes: *MTA1*, *PDCD4*, *TSC1* and *PDGFRA* (Table 5).

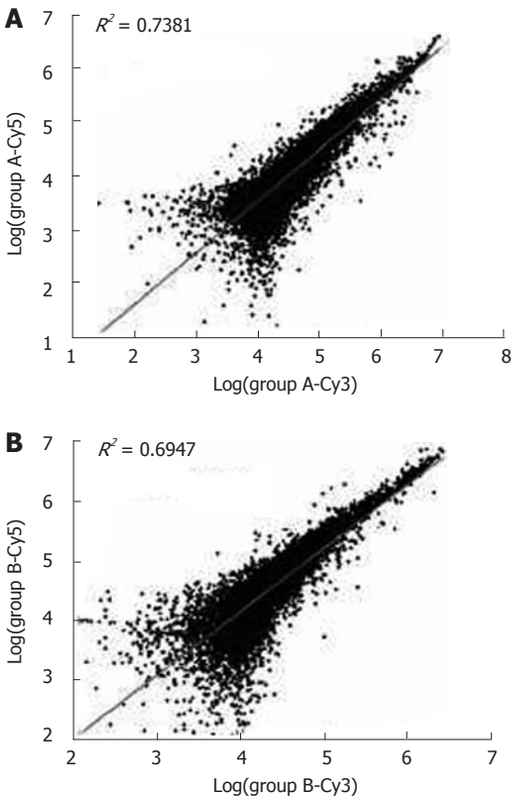

Figure 1 Correlation of CY5/CY3 hybridization signal intensity of all gene spots in Group A (polyps *vs* normal) and B (colorectal cancer *vs* normal) (*R* = 0.8591 and 0.8335, respectively). For visualization of these gene distributions, dispersion plots containing the log2 (normal tissues) and log2 (polyps or colorectal cancer) values were constructed.

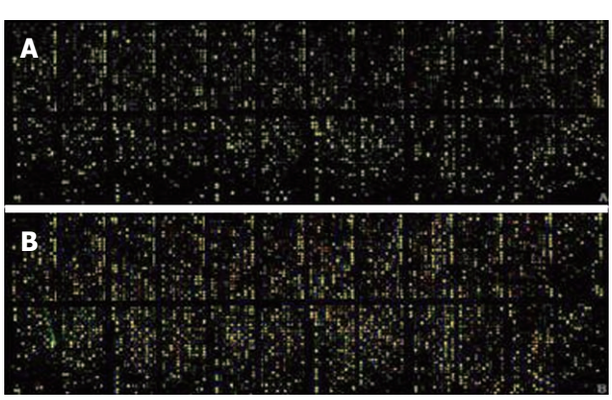

Figure 2 Microarray scanned data from Group A and B.

DISCUSSION

Colorectal carcinoma is a major cause of cancer-related deaths in China[5,6]. Unfortunately, little is known about the gene expression profiles between colorectal cancer and adenomatous polyp lesions. Genes that are differentially expressed in adenomatous polyp lesions may represent useful biomarkers for risk of colorectal cancer development, while altered genes in cancerous tissues may be used as therapeutic targets for colorectal cancer treatment[7].

Adenomatous polyp is the premalignant lesion of colorectal cancer[8-12]. Therefore, we conducted the current study to profile the differential gene expressions between normal mucosae and adenomatous polyp lesions, between normal mucosae and colorectal cancer, and between adenomatous polyp lesions and colorectal cancer. We found that eight genes were differentially expressed in adenomatous polyp lesions, as compared to normal mucosae. In addition, 46 genes were differentially expressed in colorectal cancer, as compared to the normal mucosae; twenty-nine of which were up-regulated. A total of 18 genes were significantly upregulated in both colorectal cancer and adenomatous polyp lesions, further indicating that adenomatous polyp is a precancerous lesion. However, some genes were downregulated in adenomatous polyp lesions (such as *MGST1* and *PDGFRA*) or upregulated in colorectal cancers only (*PLAGL1*). These genes encode proteins that are known to be involved in cell growth, apoptosis, and metastasis and are likely to contribute to colorectal carcinogenesis, as purported by previous studies[13,14].

The *MGST1* gene is located in 12p13.1-13.2 and was previously considered to be a "housekeeping" gene[15].

Table 3 Identification of differential gene expression profile between colorectal cancer and adenomatous polyp lesions

Accession	Gene function	Gene tag	Ratio
AY421086	Programmed cell death 4	*PDCD4*	6.41 ± 0.10
AA630800	Interferon, γ-inducible protein 30	*IFI30*	1.31 ± 0.18
AA400973	Lipocalin 2 (oncogene 24p3)	*LCN2*	3.16 ± 0.22
AI817942	Zeta-chain associated protein kinase (70 kD)	*ZAP70*	3.29 ± 0.31
AA447515	Mad4 homolog	*MAD4*	2.35 ± 0.20
W47350	Retinoic acid receptor responder 3	*RARRES3*	0.18 ± 0.13
AA436401	TU3A protein	*TU3A*	5.40 ± 0.27
AI650283	Serum/glucocorticoid regulated kinase 2	*SGK2*	1.29 ± 0.13
NM003542	H4 histone family, member G	*H4FG*	7.04 ± 0.17
NM205510	Fibroblast growth factor receptor 1	*FGFR1*	2.94 ± 0.21
NM204434	Cyclin-dependent kinase inhibitor 2A	*CDKN2A*	3.16 ± 0.28
NM005438	FOS-like antigen-1	*FOSL1*	1.93 ± 0.25
NM005439	Myeloid leukemia factor 2	*MLF2*	2.17 ± 0.24
BC08072	v-raf murine sarcoma 3611 viral oncogene homolog 1	*ARAF1*	0.98 ± 0.15
NM020531	Chromosome 20open reading frame 3	*C20ORF3*	2.23 ± 0.21
NM033158	Hyaluronoglucosaminidase 2	*HYAL2*	4.16 ± 0.28
NM001950	E2F transcription factor 4,p107/p130-binding	*E2F4*	5.26 ± 0.28
BC059522	Ribosomal protein S30	*FAU*	2.58 ± 0.13
NM008583	Multiple endocrine neoplasia I	*MEN1*	3.89 ± 0.11
NM011492	Serine/threonine kinase 11	*STK11*	5.12 ± 0.27
NM133862	Fibrinogen, gamma polypeptide	*FGG*	3.0 4 ± 0.28
BC162533	GRO2 oncogene	*GRO2*	1.39 ± 0.30
NM000612	Insulin-like growth factor 2 receptor	*IGF2*	5.06 ± 0.29
NM005343	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	*HRAS*	4.74 ± 0.27
NM002634	Prohibitin	*PHB*	2.98 ± 0.15
BC046375	p53-induced protein	*PIG11*	6.67 ± 0.29
NM004448	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2	*ERBB2*	3.15 ± 0.17
NM010658	v-maf musculoaponeurotic fibrosarcoma oncogene family	*MAFG*	5.61 ± 0.04
NM022012	Mitogen-activated protein 3 kinase 11	*MAP3K11*	3.32 ± 0.06
NM023983	Melanoma adhesion molecule	*MCAM*	7.38 ± 0.14
NM014567	Breast cancer anti-estrogen resistance 1	*BCAR1*	5.12 ± 0.23
NM000535	Postmeiotic segregation increased 2	*PMS2*	5.17 ± 0.25
NM183243	Inosine monophosphate dehydrogenase 1	*IMPDH1*	7.14 ± 0.10
NM005380	Neuroblastoma, suppression of tumorigenicity 1	*NBL1*	5.62 ± 0.16
NM002429	Matrix metalloproteinase 19	*MMP19*	3.90 ± 0.22
NM002466	v-myb avian myeloblastosis viral oncogene homolog-like 2	*MYBL2*	9.70 ± 0.21
NM022588	Metastasis associated 1	*MTA1*	10.41 ± 0.37
NM017045	Retinoblastoma 1	*RB1*	2.81 ± 0.14
NC006104	SET translocation	*SET*	2.69 ± 0.11
NM002439	Phosphatase and tensin homolog	*PTEN*	3.94 ± 0.21
NM053455	Fibrinogen-like 2 GTPase activating	*FGL2*	3.40 ± 0.27
NM005638	ADP-ribosylation factor protein 1	*ARFGAP*	5.14 ± 0.25
NM032415	Mucosa associated lymphoid tissue lymphoma translocation gene 1	*MALT1*	4.84 ± 0.21
NM006283	Transforming acidic coiled-coil containing protein 1	*TACC1*	3.41 ± 0.13
NM00288	v-ral simian leukemia viral oncogene homolog B	*RALB*	4.12 ± 0.18
NM003766	Myosin-like BCL2-interacting protein	*BECN1*	5.05 ± 0.14
NG027821	TRK-fused gene	*TFG*	4.33 ± 0.28
NM005805	Cadherin 1,type 1,E-cadherin (epithelial)	*CDH1*	4.11 ± 0.26
NM001982	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3	*ERBB3*	5.82 ± 0.15
NM133250	MutS (*Escherichia coli*) homolog 2	*MSH2*	2.13 ± 0.21
AY805747	Ras homolog gene family, member E	*ARHE*	3.61 ± 0.22
NM002884	RAP1A, member of RAS oncogene family	*RAP1A*	5.18 ± 0.27
NM000368	Tuberous sclerosis 1	*TSC1*	6.02 ± 0.14
L36953	Mothers against decapentaplegic homolog 4	*MADH4*	4.93 ± 0.26

However, it has been frequently observed as upregulated in various cancers. A recent *in vitro* study has implicated the role of MGST1 in development of multiple drug resistance during breast cancer chemotherapy with several cytostatic drugs (such as cisplatin)[16]. Polymorphisms in MGST1 have also been associated with colorectal cancer risk in Chinese[17]. In this study, we found that MGST1 mRNA levels were upregulated in colorectal cancer, as compared to those detected in normal mucosae (2.90 ± 0.16). Intriguingly, MGST1 was down-regulated in adenomatous polyps, as compared to the normal mucosae (2.14 ± 0.23), but further study is necessary to fully understand the implications of this finding.

PDGFRA gene mutation is commonly observed in tissues of gastrointestinal stromal tumors[18]. Mutated PDGFRA proteins demonstrate constitutively elevated tyrosine

Table 4 Differentially expressed genes between polyps and colorectal cancer

Accession	Gene function	Gene tag	Ratio	
			A	B
AA191692	Stratifin	*SFN*	2.36 ± 0.25	3.86 ± 0.11[a]
H15456	Calpain 1, (mu/I) large subunit	*CAPN1*	8.17 ± 0.20	10.25 ± 0.24[a]
AA043501	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene homolog	*MAF*	1.36 ± 0.08	4.85 ± 0.27[a]
AA495936	Microsomal glutathione S-transferase 1	*MGST1*	-2.14 ± 0.23	2.90 ± 0.16[a]
H23235	Platelet-derived growth factor receptor	*PDGFRA*	-0.15 ± 0.31	4.81 ± 0.14[a]
AA430032	Pituitary tumor-transforming 1	*PTTG*	4.91 ± 0.23	11.46 ± 0.18[a]
N94468	Jun B proto-oncogene	*JUNB*	1.27 ± 0.20	11.09 ± 0.14[a]
T61948	FBJ murine osteosarcoma viral oncogene B homolog	*FOSB*	1.37 ± 0.31	10.21 ± 0.20[a]
AA460168	Growth arrest and DNA damage inducible 34	*GADD34*	1.07 ± 0.15	4.15 ± 0.20[a]
L36870	MAP kinase kinase 4	*MKK4*	4.01 ± 0.23	6.93 ± 0.24[a]
AA426216	Malignant cell expression-enhanced gene	*LENG4*	7.03 ± 0.16	8.81 ± 0.23[a]
AA486219	SRp25 nuclear protein	*LOC51329*	2.09 ± 0.12	6.18 ± 0.23[a]
AA457705	Immediate early response 3	*IER3*	1.94 ± 0.25	9.17 ± 0.20[a]
AA485377	v-fos FBJ murine osteosarcoma viral oncogene homolog	*FOS*	4.03 ± 0.27	7.21 ± 0.05[a]
AA463204	Pleiomorphic adenoma gene-like 1	*PLAGL1*	7.11 ± 0.24	-4.16 ± 0.06[a]
AA434373	E74-like factor 3 (epithelial-specific)	*ELF3*	1.09 ± 0.29	7.03 ± 0.15[a]
AI677994	Fms-associated tyrosine kinase 3 ligand	*FLT3LG*	1.94 ± 0.32	4.35 ± 0.02[a]
AA464600	v-myc avian myelocytomatosis viral oncogene homolog	*MYC*	3.27 ± 0.17	8.01 ± 0.13[a]

[a]$P < 0.05$. "-" indicates down-regulation. A: Polyps *vs* normal mucosae; B: Colorectal cancer *vs* normal mucosae.

Table 5 cDNA microarray data confirmed by quantitative reverse transcription-polymerase chain reaction

Gene function	Gene tag	Ratio	
		cDNA microarray	qRT-PCR
Metastasis associated 1	*MTA1*	8.01 ± 0.47	6.72 ± 0.20[a]
Programmed cell death 4	*PDCD4*	6.41 ± 0.10	5.35 ± 0.01[a]
Tuberous sclerosis 1	*TSC1*	6.02 ± 0.14	4.83 ± 0.26[a]
Platelet-derived growth factor receptor	*PDGFRA*	-0.15 ± 0.31	0.03 ± 0.07[a]

[a]$P < 0.05$. "-" indicates down-regulation.

kinase activity and possess transforming ability, which can be reversed through PDGFR blockade[19]. Thus, mutants of PDGFRA protein behave as oncogenes, as has been demonstrated in glioma samples[20]. Here, we observed high expression of PDGFRA in colorectal cancers, as compared to that in normal tissues (4.81 ± 0.14). This observation suggests that PDGFRA may contribute to cancer development or maintenance of the tumor phenotype, possibly by supporting properties of tumor cell growth and invasiveness. However, to the reason why PDGFRA was down-regulated in adenomatous polyps remains unclear.

Finally, *PLAGL1*, a tumor suppressor gene, is localized on the chromosome 6q24-25 and is the target of several types of chromosomal rearrangement, including one identified in pleomorphic adenomas and malignant tumors. PLAGL1 is ubiquitously expressed in many human tissues where it regulates normal physiological functions; however, it has also been demonstrated to functionally contribute to complex pathologies such as cancer[21-23]. Our current study showed that PLAGL1 mRNA was down-regulated in colorectal cancer, as compared to adenomatous polyps, suggesting that PLAGL1 protein may also play a role in suppressing colorectal cancer development.

The functional roles for each of these genes in colorectal tumorigenesis remain to be verified. Nonetheless, our data provide insightful information into their potential roles in this complex and diverse disease[24]. Future studies will aim to verify these differentially expressed genes as biomarkers for early detection and/or therapeutic targets for treatment of colorectal cancers.

COMMENTS

Background

The mechanism of colorectal carcinogenesis remains to be defined and this study aims to obtain the gene expression profiles between colorectal cancer and adenomatous polyp lesions.

Research frontiers

Many researchs on genetic and epigenetic events which are thought to play important roles in colorectal carcinogenesis, such as oncogene activation and tumor suppressor gene inactivation.

Innovations and breakthroughs

The study firstly screened the differential expressed genes in colorectal adenocarcinoma and colorectal polyp *vs* normal mucosae.

Applications

These differentially expressed genes maybe as biomarkers for early detection and/or therapeutic targets for treatment of colorectal cancers.

Peer review

Study was well designed and performed methodologically.

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