

Received: 2015.08.06
Accepted: 2015.09.01
Published: 2015.12.17

Aberrant Gene Expression Profile of Unaffected Colon Mucosa from Patients with Unifocal Colon Polyp

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: This work was supported by National Natural Science Foundation of China (Lili Xu, Grant No. 81370510) and Shanghai Committee of Science and Technology (Lili Xu, Grant No. 13140902000)

Background: The aim of this study was to evaluate gene expression profiles in unaffected colon mucosa and polyp tissue from patients with unifocal colon polyp to investigate the potential mucosa impairment in normal-appearing colon mucosa from these patients.





Material/Methods: Colon polyp patients were prospectively recruited. We obtained colon biopsies from the normal-appearing sites and polyp tissue through colonoscopy. Gene expression analysis was performed using microarrays. Gene ontology and clustering were evaluated by bioinformatics.

Results: We detected a total of 711 genes (274 up-regulated and 437 down-regulated) in polyp tissue and 256 genes (170 up-regulated and 86 down-regulated) in normal-appearing colon mucosa, with at least a 3-fold of change compared to healthy controls. Heatmapping of the gene expression showed similar gene alteration patterns between unaffected colon mucosa and polyp tissue. Gene ontology analyses confirmed the overlapped molecular functions and pathways of altered gene expression between unaffected colon mucosa and polyp tissue from patients with unifocal colon polyp. The most significantly altered genes in normal-appearing tissues in polyp patients include immune response, external side of plasma membrane, nucleus, and cellular response to zinc ion.

Conclusions: Significant gene expression alterations exist in unaffected colon mucosa from patients with unifocal colon polyp. Unaffected colon mucosa and polyp tissue share great similarity and overlapping of altered gene expression profiles, indicating the potential possibility of recurrence of colon polyps due to underlying molecular abnormalities of colon mucosa in these patients.

MeSH Keywords: **Gene Expression Profiling • Intestinal Polyps • Microarray Analysis • Recurrence**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/895576>

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Background

Colon polyp is a nodule or mass which protrudes from the mucosa surface toward the lumen. It is a common lesion in the gastrointestinal tract and has been reported in 25% of people aged over 60 years of age [1–5]. Polyps may result from inflammation or genetic disorders and most are asymptomatic [1,6]. Investigations revealed that inflammation triggered by Toll-like receptors against commensal bacteria normally present in the intestinal microflora could affect cell proliferation and polyp formation. The most important complication of colon polyps is colon cancer [7]. Colorectal cancer is the second most common cancer in females and the third most common cancer in males. It was reported that more than 1 million people are diagnosed with colorectal cancer every year [8]. In general, the prevalence of carcinoma in colorectal polyps increases along with polyp size. It was reported that the percentage of carcinoma among colon polyps larger than 10 mm was 25.2%, while the percentage of carcinoma among colon polyps smaller than 5 mm was only 0.4% [9]. The progression from adenoma to carcinoma is a slow phenomenon [4]; therefore, early detection and removal of the polyps with malignant potential under colonoscopy could be possible.

During clinical practice we noticed that some colon polyp patients had recurrent polyps within 1 year at a different part of the colon after complete removal of the original polyps. It was reported that the early recurrence rate of polyps was around 16% and the late recurrence rate was around 4% after endoscopic mucosal resection [10–12]. Larger size and villous pathology might indicate higher possibility of recurrence [10,11]. It was reported that recurrence of advanced adenoma was associated with the size of primary polyps (>1 cm) and their location in the colon. However, the mechanism associated with polyp recurrence has not been clarified [13,14]. It is possible that underlying impairment could exist in normal-appearing colon mucosa in colon polyp patients; therefore, recurrent polyps may appear after complete removal of the original colon polyp.

In the current study we evaluated the gene expression profiles of unaffected colon mucosa and polyp tissue from unifocal colon polyp patients to compare the gene expression profiles and investigate the potential mucosa impairment in normal-appearing colon mucosa from these patients.

Material and Methods

Patients

A total of 20 patients with unifocal colon polyp (13 men and 7 women, mean age 51±15 years) visiting the outpatient clinic of Zhongshan Hospital between July 2014 and March 2015 were

Table 1. Clinical characteristics of the participants.

Clinical feature	N (%)	
Gender		
Male	13	(65)
Female	7	(35)
Anatomical sites		
Rectum	10	(50)
Sigmoid	8	(40)
Descending colon	2	(10)
Size		
<10 mm	15	(75)
10–15mm	5	(25)
Histologic classification		
Tubular	8	(40)
Villous	7	(35)
Tubulovillous	5	(25)

enrolled in this study. The size of all polyps was less than 15 mm. The clinical characteristics of the polyp patients are listed in Table 1. All polyp specimens were from polypectomy through colonoscopy and normal-appearing colon mucosa specimens were obtained from biopsy 5–10 cm apart from the polyps of the same patients. As a control group, 9 healthy subjects without colon polyp history were enrolled and colon biopsies were obtained through colonoscopy. The study was approved by Ethics Committee of Medical Research, Zhongshan Hospital, Fudan University and all the participants gave their informed consent.

RNA extraction and purification

Colon biopsy specimens were homogenized and total RNA was extracted using TRIZOL Reagent (Life technologies, Carlsbad, CA, USA) following the manufacturer's instructions and were checked for a RIN number to inspect RNA integrity by an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Qualified total RNA was further purified by use of an RNeasy micro kit (QIAGEN, GmBH, Germany) and RNase-Free DNase Set (QIAGEN, GmBH, Germany). Sample quality was assessed by a photospectrometer (Nano drop technologies, ThermoScientific, Wilmington, Delaware, USA). The 260/280 ratios in all samples were >1.8.

Microarray experiment

DNA microarrays (Affymetrix GeneChip® Human Transcriptome Array 2.0, Affymetrix, Santa Clara, California, USA) were performed according to the manufacturer's instructions ([This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License](http://</p>
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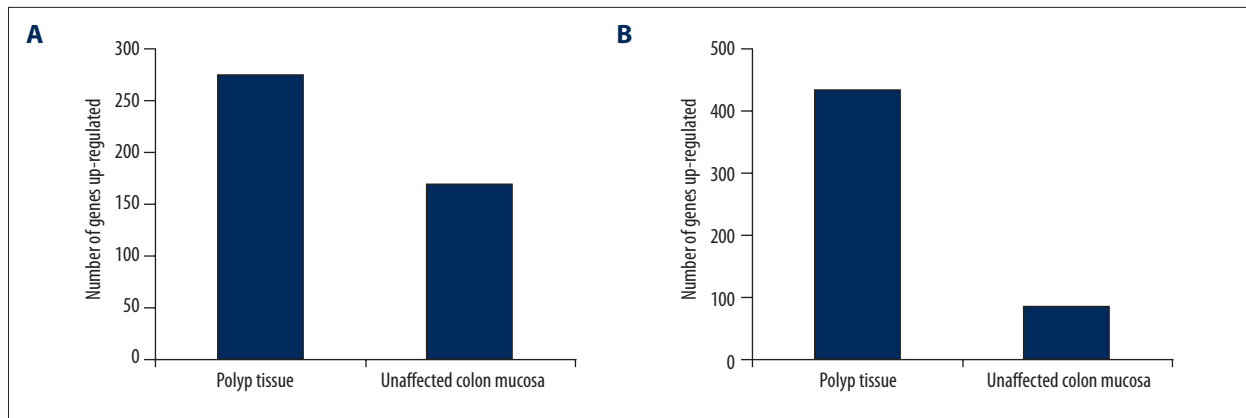


Figure 1. Number of genes expressed with alterations in unaffected colon mucosa and polyp tissue from unifocal colon polyp patients. Only genes expressed with at least 3-fold change compared to healthy controls are shown. **(A)** Number of genes up-regulated in unaffected colon mucosa and polyp tissue. **(B)** Number of genes down-regulated in unaffected colon mucosa and polyp tissue.

www.affymetrix.com). The GeneChip® Human Transcriptome Array 2.0 array interrogates 44 699 well-annotated genes with more than 6 million distinct probes. The design of the GeneChip® Human Transcriptome Array 2.0 was based on Hg19 with comprehensive coverage of all RefSeq, Ensembl, and UCSC (known genes and lincRNA transcripts) in an unbiased manner.

Statistical methods and bioinformatics

Microarray analysis was performed on CEL files using Affymetrix® Expression Console™ software (<http://www.affymetrix.com>) and data were normalized and summarized by this software. Alterations of gene expression were defined using a fold change cutoff of $\geq \pm 2$. Cluster analysis of differentially expressed genes was visualized by Affymetrix® Transcriptome Analysis Console (TAC) 2.0 software. Post-processing analysis of significant transcripts was performed to determine their functionality using gene ontology (GO) analyses performed using David (<http://david.abcc.ncifcrf.gov/>). A p-value < 0.05 was considered as significant.

Results

Many genes with altered expression were present in unaffected colon mucosa and colon polyp tissue from unifocal colon polyp patients

We detected 711 genes with more than 3-fold altered expression (274 up-regulated and 437 down-regulated) compared to healthy controls in colon polyp biopsies. Surprisingly, we found 256 genes with at least 3-fold altered expression (170 up-regulated and 86 down-regulated) compared to healthy controls in unaffected mucosa of colon biopsies from the same colon polyp patients (Figure 1). These results indicate that the

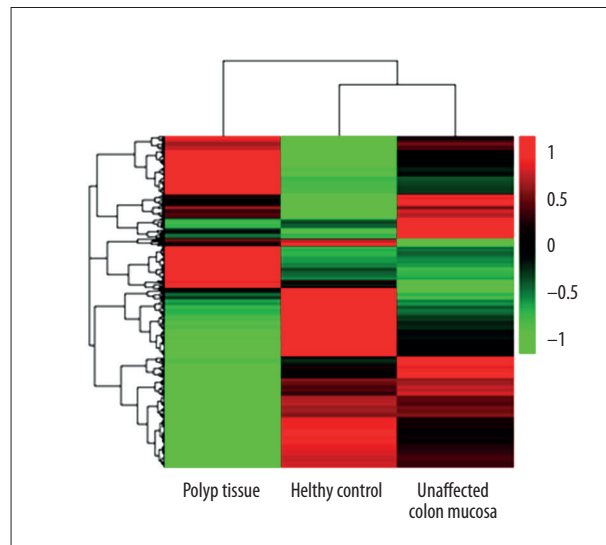


Figure 2. Heatmap of gene expression alterations in unaffected colon mucosa and polyp tissue from unifocal colon polyp patients compared to healthy controls.

abnormalities at the molecular level were present at normal-appearing sites away from polyps.

Similarity of gene expression alterations in unaffected colon mucosa and colon polyp tissue from colon polyp patients

To further compare the gene expression pattern between unaffected colon mucosa and colon polyp tissue from the same colon polyp patients, we compared the intensity of genes with altered expression between unaffected colon mucosa and colon polyp tissue. The heatmap showed that the expression profiles detected in unaffected colon mucosa and colon polyp tissue were overlapped (Figure 2).

Table 2. Molecular functions of most associated genes with altered expression in polyps and unaffected colon mucosa from unifocal colon polyp patients.

Name of molecular function	Polyp tissue p Value	Unaffected colon mucosa p Value
Immune response (GO: 0006955)	3.39E-10	3.85E-12
External side of plasma membrane (GO: 0009897)	1.30E-09	1.89E-08
Cellular response to zinc ion (GO: 0071294)	1.04E-08	3.03E-06
Transmembrane transport (GO: 0055085)	1.40E-06	4.68E-06
Nucleus (GO: 0005634)	5.33E-06	9.87E-08
Cellular response to cadmium ion (GO: 0071276)	1.67E-05	4.16E-06
Establishment of T cell polarity (GO: 0001768)	4.78E-05	5.42E-06
Negative regulation of leukocyte apoptotic process (GO: 2000107)	4.78E-05	5.42E-06

Table 3. Pathways of most associated genes with altered expression in polyps and unaffected colon mucosa from unifocal colon polyp patients.

Name of pathway	Polyp tissue p Value	Unaffected colon mucosa p Value
Mineral absorption (hsa04978)	2.67E-09	0.000522
Protein digestion and absorption (hsa04974)	2.61E-05	0.00169
Cell adhesion molecules (CAMs) (hsa04514)	5.88E-05	0.023461
Primary immunodeficiency (hsa05340)	0.001939	0.239157
Starch and sucrose metabolism (hsa00500)	0.008457	0.005474
Chemokine signaling pathway (hsa04062)	0.00993	0.082939
<i>Staphylococcus aureus</i> infection (hsa05150)	0.028444	0.005898
Intestinal immune network for IgA production (hsa04672)	0.038537	0.019216

Altered transcripts are present in unaffected colon mucosa and colon polyp tissue from colon polyp patients

Next, we did gene ontology assessments of the alteration detected within the unaffected colon mucosa and colon polyp tissue. The most prominent molecular functions detected in colon polyp tissue were immune response, external side of plasma membrane, and cellular response to zinc ion, while in unaffected colon mucosa they were immune response, external side of plasma membrane, and nucleus, indicating the overlapping alteration of molecular function of the transcripts between unaffected colon mucosa and colon polyp tissue (Table 2). We further analyzed the pathways associated with these transcripts and found that the common pathways involved in polyp tissue were mineral absorption, protein digestion, and absorption

and cell adhesion molecules (CAMs), while the common pathways involved in unaffected colon mucosa were mineral absorption, protein digestion and absorption, and starch and sucrose metabolism (Table 3).

Among the most significantly altered genes detected in this study, we found many common genes involved in both unaffected colon mucosa and colon polyp tissue (Table 4). The immune response-related genes IGHA1, IGJ, IGKV1D-33, IGKV1-5, and IGKV4-1 were up-regulated, while C3, MS4A1, CCR7, and CR2 were down-regulated in both unaffected colon mucosa and colon polyp tissue. External side of plasma membrane-related genes ANPEP, CR2, MS4A1, CD22, CCR7 were down-regulated in both unaffected colon mucosa and colon polyp tissue. The nucleus-related genes ANXA1, GPX2, and DDX3Y were

Table 4. Comparison of up- and down-regulated genes with altered expression in polyps and unaffected colon mucosa from unifocal colon polyp patients.

Name of molecular function	Polyp tissue		Unaffected colon mucosa	
	Up-regulated gene name	Down-regulated gene name	Up-regulate gene name	Down-regulated gene name
Immune response	CXCL1, IGHA1, IGJ, TLR4, TNFSF15, CCL20, IGKV1-5, IGKV1D-33I, GKV4-1	C3, MS4A1, CCR7, CR2	IGHA1, IGJ, IGKV1-5, IL1R2, IGKV1D-33, IGKV4-1	C3, MS4A1, CCR7, CR2
External side of plasma membrane	ALCAM, CD44, ITGA2, TLR4, TMEM123, FCRL6	ANPEP, CD2, CD3E, MS4A1, CD22, CCR7, CR2, CD69	MUC17	ANPEP, CR2, CD22, MS4A1, CCR7, CTLA4, CXCR4, ICOS
Nucleus	ANXA1, DDX3Y, GPX2, HOXA7, HOXA9, APEX1, CCND1, CDK4, DAB2	MOCS1, PRKCB, TTN, AIM3, STAP1, CMPK2, PARP15, MIER3	ANXA1, GPX2, HOXD10, HOXD13, DDX3Y, RFX6	PRKCB, TTN, MIER3, AIM2, STAP1, PARP15, CMPK2
Cellular response to zinc ion	N/A	MT1A, MT1B, MT1F, MT1G, MT1H, MT1X	N/A	MT1F, MT1G, MT1H, MT1X

up-regulated, while PRKCB, TTN, STAP1, CMPK2, PARP15, and MIER3 were down-regulated in both unaffected colon mucosa and colon polyp tissue. Cellular response to zinc ion-related genes MT1F, MT1G, MT1H, and MT1X were down-regulated in both unaffected colon mucosa and colon polyp tissue.

Discussion

The causes of recurrence in some colon polyp patients after endoscopic resection are not clear. Some studies suggested that incomplete resection of colon mucosa may result in recurrence, especially in piecemeal EMR of large colon polyps [6,7]. However, in our clinical practice we noticed recurrence even in small colon polyps after mucosa resection. It is possible that the underlying intrinsic abnormality might be present in colon polyp patients; therefore, colon polyps could recurrently appear after the complete removal of the original polyp. The necessity of colon polyp, especially diminutive polyp removal, is debatable. It was reported that only 56% of endoscopists routinely remove all adenomatous polyps in Japan. Some endoscopists leave small adenomatous polyps unresected after detailed observation and close follow-up because they think many polypectomies are unnecessary and add risks during colonoscopy [9].

In this study, we found many genes were abnormally expressed (either up-regulated or down-regulated) in unaffected colon mucosa compared to normal controls, indicating that underlying impairments are present in normal-appearing colon mucosa. Furthermore, our study revealed the great similarity of gene expression alteration between polyp tissue and unaffected mucosa in colon polyp patients. These findings suggest that

abnormality of colon mucosa might not be limited to polyps in the gut, and that molecular abnormalities might reflect the preceding impairment of intestinal homeostasis and subclinical intestinal inflammation with the potential to undergo apparent pathological changes.

The molecular functions of altered genes associated with polyps and unaffected colon mucosa observed in this study were mainly related to 3 categories: inflammation, nutrition absorption, and cell structure. We speculate that altered expression of inflammation-related genes might be the initial abnormality, but it is possible that abnormal cell structure might trigger the impairment of barrier function before the induction of unbalanced immune response to gut microbiota [15,16]. In this study we found that genes related to immunoglobulin formation IGHA1 and IGKV family members were up-regulated, while complement component and receptor C3 and CR2, as well as chemokine receptor CCR7, were down-regulated, indicating the unbalanced immune response in colon mucosa in colon polyp patients. It has been demonstrated that CCR7 is a central regulator in the maintenance of cellular homeostasis of mucosal tissues and CCR7 deficiency resulted in chronic diarrhea and enhanced formation of intestinal lymphoid follicles associated with activated colonic T cells and increased production of the cytokine interleukin-1 β [17]. We speculated that alteration of CCR7 expression in these patients might induce chronic inflammation, cell proliferation, and polyp development in colon mucosa. We found that glutathione peroxidase 2 was up-regulated, while metallothionein family members MT1A, MT1F, MT1G and MT1H, protein kinase C, beta PRKCB, signal transducing adaptor family member 1 STAP1, and cytidine monophosphate kinase 2, as well as mitochondrial CMPK2, were down regulated in colon mucosa from colon

polyp patients, indicating the complexity of genes involved in the development of colon polyps. Studies suggested that nutrition absorption pattern might affect the gut barrier function and polyp formation [18,19]. The precise relationships among inflammation, nutrition absorption, and cell structure need to be further investigated. We noticed that besides nutrition digestion, absorption, and metabolism, *Staphylococcus aureus* infection was another common pathway involved both in polyp tissue and unaffected colon mucosa. However, none of the patients recruited in this study was diagnosed with *Staphylococcus aureus* infection. The significance and specificity of *Staphylococcus aureus* infection pathway detected by microarray in our study need to be further determined.

The mechanisms underlying colon polyp formation and recurrence are not clear. Some studies suggested that chronic inflammation in the colon mucosa against commensal bacteria in gut lumen might be the initial factor [15,16]. Our study confirmed that immune response-related genes were expressed with most significant alteration in unaffected colon mucosa and polyp tissue in these patients. More importantly, our study revealed that abnormalities of nutrition absorption and cell structure in colon mucosa were present in colon polyp

patients. These findings highlight the importance of nutrition absorption and cell structure in colon polyp formation and the possible role in colon polyp recurrence.

Conclusions

In the current study we evaluated the gene expression profiles of unaffected colon mucosa and polyp tissue from unifocal colon polyp patients. We found significant alterations of gene expression profile of unaffected colon mucosa in these patients. There were great similarity and overlapping of gene expression alterations between unaffected colon mucosa and colon polyp tissue. Our study demonstrates the underlying impairment of unaffected colon mucosa in unifocal colon polyp patients and provides evidence of the potential recurrence of colon polyps.

Acknowledgements

We thank Shanghai South Gene Technology (Shanghai, China) for assistance with the microarray study and bioinformatics analysis.

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