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Bioinformatical analysis of gene expressions and pathways in human colorectal cancer tissues.

Mitsuru Chiba*

Department of Bioscience and Laboratory Medicine, Graduate School of Health Sciences, Hirosaki University, Japan

Abstract

Introduction: Colorectal cancer (CRC) is a major disease and a leading cause of mortality and morbidity worldwide. The expression of various genes changes in CRC. This study aimed to investigate differentially expressed genes (DEGs) in CRC tissues and to predict important pathways associated with CRC.

Methods: DEGs between cancerous and non-cancerous tissues from 17 patients with CRC were screened using Affymetrix HG-U133 Plus 2.0 microarray data downloaded from the Gene Expression Omnibus (GSE32323). Next, functional and pathway analysis of DEGs was performed by Gene Ontology (GO) analysis and WikiPathways analysis using GeneSpring 14.5 software.

Results and discussion: Compared with non-cancerous tissues, 3,856 DEGs were identified in the CRC samples, which included 2,015 upregulated DEGs and 1,841 downregulated DEGs, with the selection criteria of $p < 0.05$ and a two-fold change. The GO analysis identified 448 and 606 GO terms using the upregulated and downregulated DEGs, respectively. Interestingly, exosomes and extracellular vesicles were associated with the downregulated DEGs. A total of 240 pathways were associated with DEGs in CRC tissues. Taken above, I have identified candidate DEGs in CRC tissues and pathways. These candidate genes and pathways could be therapeutic targets for CRC.

Keywords: Bioinformatics, Colorectal cancer, Gene expression, Pathway, Gene ontology.

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Introduction

Colorectal cancer (CRC) is the fourth most common type of cancer in the United States and the second leading cause of death due to cancer [1]. Recently, the incidence and mortality rates of colorectal cancer have increased in Japan. It is estimated that about 10% of men and 8% of women in Japan will be diagnosed with CRC during their lifetime [2]. CRC affects all racial and ethnic groups and is the most common in people aged 50 years and older. The stage of the cancer is indicative of how far it has spread, and determining the stage helps in choosing the most appropriate treatment. However, CRC remains a prominent global health problem, which may be attributed to a lack of comprehensive and systemic understanding of the underlying molecular mechanisms of carcinogenesis and progression.

CRC was one of the first solid tumours that was molecularly characterized, with several genes and pathways implicated in tumour initiation and growth [3]. CRC carcinogenesis is the result of a stepwise accumulation of genetic events occurring in oncogenes and tumour suppressor genes that deregulate the key signaling pathways that drive progression, such as *Wnt/β-catenin* [4], transforming growth factor beta (TGF-β) [5], epidermal growth factor receptor (EGFR) [6], mitogen-activated protein kinase and phosphoinositide 3-kinase (PI3K)-Akt pathways [7,8]. Therefore, CRC carcinogenesis and progression involve the activation or suppression of a complex network of molecules. However, the understanding of CRC carcinogenesis and progression networks is insufficient.

Khamas et al. examined gene expression profiles of cancerous and non-cancerous tissues from 17 patients with CRC using Affimetrix GeneChip Human Genome U133 Plus 2.0 Array [9]. In the present study, a bioinformatical analysis was performed to identify genes and pathways involved in CRC using these data sets (GSE32323).

Materials and Methods

Data source

The gene expression profile of GSE32323 was obtained from the Gene Expression Omnibus database. This dataset contains 17 pairs of cancer and non-cancerous tissues from CRC patients [9]. Microarray analysis was performed using the Affimetrix GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix Inc., Santa Clara, CA, USA).

Data processing and screening for differentially expressed genes (DEGs)

Further, microarray data were normalized using GeneSpring 14.5 software (Agilent Technologies, Foster City, CA, USA). MAS5 was used as a summarization algorithm. Baseline transformation was not performed. A lower cut-off value of 10 was used to filter entities in which at least 17 out of 34 samples had values within the range were retained. Only acceptable flags "Present" were used. I retained entities in which at least 17 out of 34 samples have acceptable values.

The threshold criterion for the selection of DEGs was fold change of >2.0. Statistical analysis was performed using a moderated t-test with $P<0.05$. Benjamini Hochberg False Discovery Rate procedure was used for controlling false-positives in multiple testing corrections. Selected DEGs were presented using volcano and scatter plots.

Functional enrichment analysis of DEGs

To examine functions and pathways that may be altered by the upregulated or downregulated DEGs, Gene Ontology (GO) analysis and WikiPathways analysis was performed using GeneSpring 14.5 software (Agilent Technologies). Pathway data of Homo sapiens were downloaded from WikiPathways. The cut-off value for the screening of significant functions and pathways was $P<0.05$.

Result and Discussion

Identification of DEGs between CRC and normal samples

Normalization of each 17 pair data of cancer (CRC) and non-cancerous tissues (normal) from CRC patients (Figure 1A) and Principal component analysis (Figure 1B) was performed using GeneSpring 14.5 software. Compared with normal samples, 3,856 DEGs were identified in CRC samples, including 2,015 up-regulated DEGs and 1,841 down-regulated DEGs (Figures 1C and 1D).

Analysis of biological functions and pathways altered by DEGs

Under p-value of 0.05, 448 GO terms were identified in the upregulated DEGs, and 606 GO terms were identified in the downregulated DEGs. Of these, the top 15 GO terms are presented in Table 1. Notably, cell-to-cell communication tool such as extracellular exosome ($P=1.11 \times 10^{-28}$) or extracellular vesicle ($P=2.67 \times 10^{-28}$) was significantly associated with the downregulated DEGs (Table 1). Exosomes are a subclass of extracellular vesicles that are released by all cell types, including cancer cells, and are involved in intercellular communication [10]. These exosomes have been detected in the cancer microenvironment, and emerging evidence suggests that they play a role in facilitating tumorigenesis by regulating angiogenesis, immunity, and metastasis [11]. This indicates that cell-to-cell communication is abnormal in the CRC microenvironment. Thus, analysis of exosomes is necessary for a better understanding of CRC carcinogenesis and progression in future studies.

Current CRC screening methods are divided into invasive and non-invasive tests. The non-invasive tests include stool and blood-based tests (fecal immunochemical test and fecal DNA test etc.) and radiologic tests (computed tomographic colonography etc.) [12]. Recently, exosomal components of blood samples have been studied for CRC biomarkers [13]. The ideal screening study should be efficient with high sensitivity and specificity, safe, available, convenient, and cheap.

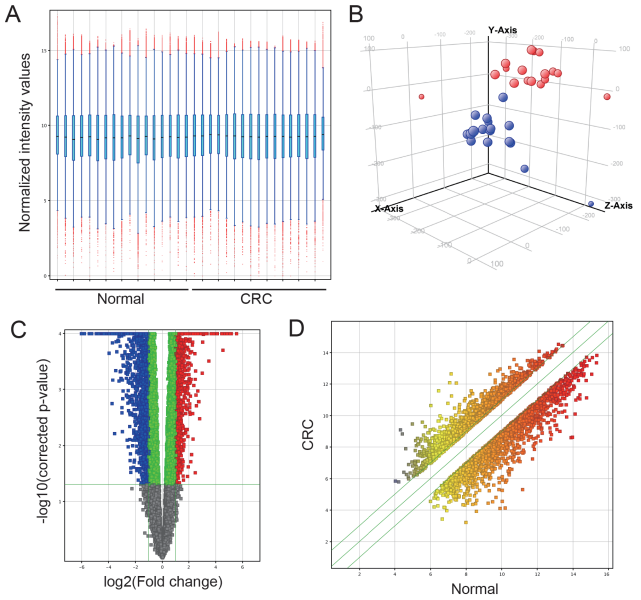

Figure 1. *Identification of DEGs between CRC and normal tissue samples. (A) Normalization of the 17 data pairs of cancerous (CRC) and non-cancerous tissues (normal) from patients with CRC. (B) Principal component analysis. Red dot represents normal samples. Blue dot represents CRC samples. (C) Volcano plot of DEGs. Red plot represents upregulated DEGs. Blue plot represents downregulated DEGs. (D) Scatter plot of DEGs.*

Using a cut-off p-value of 0.05, 240 pathways were detected that involved up-regulated and down-regulated DEGs. Of these, the top 30 pathways are presented in Table 2. Using one of these pathways, the relationship among TGF-β, PI3K-Akt, and vascular endothelial growth factor (VEGF)-A-VEGF receptor 2 (VEGFR2) signaling pathways was predicted. The TGF-β signaling pathway plays an important role in cancer cell proliferation, growth, metastasis, and apoptosis and elicits both pro- and anti-oncogenic effects [5]. This pathway is controlled by positive regulators such as Smad2, Smad3, and Smad4 and negative regulators such as Smad6 and Smad7. Mutations in the TGF-β signaling components, including TGF-β receptors and cytoplasmic signaling transducers, are frequently observed in CRC. The regulatory effects of the TGF-β/Smad signaling pathway on cell growth inhibition and apoptosis contribute to CRC progression. Further, Smad4 deletion is a poor response

marker in patients undergoing chemotherapy, whereas Smad7 deletion is a good prognostic marker in patients with CRC.

Table 1. *Gene Ontology (GO) analysis and significant top 15 GO terms of DEGs in human colorectal cancer.*

GO number	GO term	Count	P-value
Up-Regulation			
GO:0070013	Intracellular organelle lumen	652	0
GO:0034641	Cellular nitrogen compound metabolic process	672	0
GO:0006139|GO:0055134	Nucleobase-containing compound metabolic process	603	0
GO:0031974	Membrane-enclosed lumen	652	0
GO:0031981	Nuclear lumen	580	0
GO:0043233	Organelle lumen	652	0
GO:0044428	Nuclear part	620	0
GO:0005634	Nucleus	847	0
GO:0046483	Heterocycle metabolic process	618	1.40 x 10-45
GO:0006725	Cellular aromatic compound metabolic process	622	1.40 x 10-45
GO:0090304	Nucleic acid metabolic process	552	2.80 x 10-45
GO:0000278|GO:0007067	Mitotic cell cycle	196	4.20 x 10-45
GO:0005654	Nucleoplasm	464	4.20 x 10-45
GO:1901360	Oganic cyclic compound metabolic process	634	3.01 x 10-43
GO:0006807	Nitrogen compound metabolic process	915	9.17 x 10-38
Down-Regulation			
GO:0043230	Extracellular organelle	367	2.86 x 10-28
GO:0070062	Extracellular exosome	367	1.11 x 10-28
GO:1903561	Extracellular vesicle	367	2.67 x 10-28
GO:0071944	Cell periphery	530	1.14 x 10-26
GO:0044421	Extracellular region part	454	3.99 x 10-26
GO:0005615	Extracellular space	435	6.98 x 10-26
GO:0005886|GO:0005904	Plasma membrane	516	1.61 x 10-25
GO:0031982|GO:0031988	Vesicle	459	1.31 x 10-23
GO:0005576	Extracellular region	485	1.53 x 10-20
GO:0044425	Membrane part	641	4.48 x 10-19
GO:0070887	Cellular response to chemical stimulus	315	1.09 x 10-18
GO:0016020	Membrane	808	1.31 x 10-17
GO:0065008	Regulation of biological quality	406	1.52 x 10-17
GO:0006629	Lipid metabolic process	166	3.25 x 10-17
GO:0050896|GO:0051869	Response to stimulus	718	4.37 x 10-17

The PI3K–Akt signaling pathway leads to reduced apoptosis, stimulates cell growth and increases proliferation [8]. Genetic aberrations leading to PI3K-Akt hyper-activation are observed at considerable frequency in all major nodes in most tumors. In CRC the most commonly observed pathway changes are insulin-like growth factor 2 overexpression, phosphatidylinositol-4,5-bisphosphate-3-kinase catalytic subunit alpha mutations and phosphatase and

tensin homolog deleted from chromosome 10 mutations and deletions. VEGFR2 signal transduction and trafficking pathways are mediated by VEGF-A ligands [14]. VEGFA-VEGFR2 signaling pathway is involved in cell proliferation, chemotaxis and survival of endothelial cells. Angiogenesis is mainly regulated by this signaling pathway. In CRC, this pathway is associated with survival and distant metastasis of cancer cells.

Table 2. Top 30 of 240 pathways enriched with DEGs in CRC

Pathway name	P-value	Gene count
Hs_Glucuronidation_WP698_94183	6.32 x 10-11	26
Hs_miRNA_Regulation_of_DNA_Damage_Response_WP1530_98336	1.83 x 10-10	98
Hs_G1_to_S_cell_cycle_control_WP45_97000	1.94 x 10-10	65
Hs_DNA_Replication_WP466_96302	2.10 x 10-10	42
Hs_DNA_Damage_Response_WP707_94731	2.11 x 10-10	68
Hs_Hepatitis_C_and_Hepatocellular_Carcinoma_WP3646_102150	2.55 x 10-10	57
Hs_DNA_IR-damage_and_cellular_response_via_ATR_WP4016_101923	2.66 x 10-10	83
Hs_Retinoblastoma_Gene_in_Cancer_WP2446_98748	2.99 x 10-10	90
Hs_Cell_Cycle_WP179_97174	3.53 x 10-10	120
Hs_TGF-beta_Signaling_Pathway_WP366_90028	3.78 x 10-10	132
Hs_Focal_Adhesion-PI3K-Akt-mTOR-signaling_pathway_WP3932_102154	6.36 x 10-10	302
Hs_PI3K-Akt_Signaling_Pathway_WP4172_96453	6.54 x 10-10	340
Hs_Nuclear_Receptors_Meta-Pathway_WP2882_97489	6.57 x 10-10	318
Hs_VEGFA-VEGFR2_Signaling_Pathway_WP3888_102004	9.53 x 10-10	236
Hs_Myometrial_Relaxation_and_Contraction_Pathways_WP289_96894	1.07 x 10-9	156
Hs_Prostaglandin_Synthesis_and_Regulation_WP98_98180	1.24 x 10-9	46
Hs_Metapathway_biotransformation_Phase_I_and_II_WP702_102200	1.86 x 10-9	190
Hs_Gastric_Cancer_Network_1_WP2361_101906	2.17 x 10-9	29
Hs_Imatinib_and_Chronic_Myeloid_Leukemia_WP3640_89384	4.20 x 10-9	21
Hs_Adipogenesis_WP236_97606	5.13 x 10-9	131
Hs_Vitamin_D_Receptor_Pathway_WP2877_94793	5.38 x 10-9	186
Hs_Breast_cancer_pathway_WP4262_97132	6.51 x 10-9	156
Hs_NRF2_pathway_WP2884_94787	8.53 x 10-9	143
Hs_LncRNA_involvement_in_canonical_Wnt_signaling_and_colorectal_cancer_WP4258_97136	8.70 x 10-9	98
Hs_Integrated_Cancer_Pathway_WP1971_98351	8.87 x 10-9	49
Hs_Regulation_of_sister_chromatid_separation_at_the_metaphase-anaphase_transition_WP4240_96623	1.05 x 10-8	15
Hs_Focal_Adhesion_WP306_97459	1.84 x 10-8	198
Hs_Circadian_rythm_related_genes_WP3594_87161	2.43 x 10-8	210
Hs_Pyrimidine_metabolism_WP4022_95459	2.84 x 10-8	99
Hs_ESC_Pluripotency_Pathways_WP3931_102153	5.31 x 10-8	116

The above mentioned pathway analyses suggested that the microarray data obtained in this study was indicative for colon cancer. As a novel pathway, the involvement of lncRNA in canonical Wnt signaling in CRC was predicted (Table 2). Taken above, novel candidate genes and pathways in CRC were identified by integrated bioinformatical analysis. These candidate genes and pathways may act as potential therapeutic targets for CRC.

Conclusion

I have identified candidate DEGs in CRC tissues and pathways. These candidate genes and pathways could be therapeutic targets for CRC.

Acknowledgement

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*Correspondence to:

Mitsuru Chiba

Department of Bioscience and Laboratory Medicine,

Graduate School of Health Sciences, Hirosaki University,

66-1 Hon-cho, Hirosaki, Aomori 036-8564,

Japan