Revealing the molecular mechanism of colorectal cancer by establishing LGALS3-related protein-protein interaction network and identifying signaling pathways

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Received August 28, 2013; Accepted December 6, 2013

DOI: 10.3892/ijmm.2014.1620

Abstract. LGALS3 plays a role in colorectal cancer, however, the detailed molecular mechanism remains to be determined, while signaling pathways provide valuable information for understanding the underlying mechanism of the cancer. The purpose of this study was to explore the roles of LGALS3 and signaling pathways in the pathogenesis of colorectal cancer. In this study, microarray data GSE8671 were downloaded from the Gene Expression Omnibus database and differentially expressed genes (DEGs) in colorectal cancer were identified by Significant Analysis of Microarray. Gene ontology (GO) analysis was performed on the top 500 upregulated and 500 downregulated genes using DAVID. The signaling pathways were predicted by the signaling pathway impact analysis (SPIA) with pGFdr<0.05 and transcription factors were identified by TFats. The LGALS3-related protein-protein interaction network (PPI) was established by STRING and Cytoscape. In total, 6,593 upregulated and 5,897 downregulated DEGs were identified and 41 downregulated genes, including CLND8 and CLND23 were enriched in cell adhesion. In addition, 21 pathways, such as the cell cycle, p53 signaling pathway and NF-κB signaling pathway, were selected. MYC and TCF7L2 were found to be activated while FOXO3 was suppressed in colorectal cancer. Eight downregulated and 10 upregulated genes were identified in the LGALS3 PPI network. Results of the present study shed new light on the molecular mechanism of colorectal cancer and these findings have the potential to be used in colorectal cancer treatment.

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Key words: colorectal cancer, differentially expressed genes, gene ontology analysis, signaling pathway, protein-protein interaction network

Introduction

As the leading cause of death in economically developed countries and the second leading cause of death in developing countries, cancer is a major public health concern worldwide (1). Atkin *et al* have reported that colorectal cancer is the third most common cancer worldwide and has a high mortality rate (2). Grady and Carethers have confirmed that colorectal cancer developed as a consequence of the accumulation of genetic alterations, such as gene mutation and gene amplification, and epigenetic alterations, including aberrant DNA methylation and chromatin modification that is able to transform colonic epithelial cells into colonic adenocarcinoma cells (3). Due to the high mortality, there is a need to investigate the pathogenesis and molecular mechanism of colorectal cancer.

During the last 15 years, the focus has been on recognition of the 'serrated neoplastic pathway' and has led to a paradigm shift in our understanding of the molecular basis of colorectal cancer and significant changes in clinical practice (4). The changes that have occurred in the DNA sequence of the genomes of cancer cells result in the development of various types of cancer (5) and multiple gene expression patterns are altered during the evolution of normal cells to cancer cells. Furthermore, genome-wide analysis of the gene expression has been largely used to identify important genes of human cancers (6). The gene expression profile has been previously characterized in various types of human cancer, including prostate, colorectal and epithelial ovarian cancer (6-8).

In addition, it has been reported that genetically altered core pathways and regulatory processes become evident once the coding regions of the genome are analyzed in depth, while dysregulation of these core pathways and processes through mutation can explain the major features of tumorigenesis (7). The development of cancer depends on the abnormal activation of signal transduction pathways that control the growth and survival of cells (8). Therefore, various signaling pathways

are altered in the pathogenesis of cancer. Activation of the signaling pathway of hypoxia inducible factor (HIF) is crucial in the progression of physiological development and tumor growth (9). Activation of the Wnt signaling pathway promotes neoplastic transformation in humans (8). Other signaling pathways such as gefitinib-sensitizing EGFR, β-catenin-Tcf, and p53 have also been reported to be dysregulated in cancer (12-14).

By binding to specific DNA sequences within the promoter regions of target genes, transcription factors (TFs) are able to regulate DNA expression (10). Findings of previous studies identified several cancer-related TFs, such as *TMPRSS2* and *ETS* in prostate cancer (11). *KLF4* and *KLF5* affect proliferation, apoptosis and invasion in esophageal cancer cells by regulating a number of genes (12). NF-κB has an impact on the development and progression of cancer by affecting cell proliferation, migration, and apoptosis (13).

The transcriptome profile of human colorectal adenomas has been previously characterized (14), however, the molecular mechanism involved remains to be determined. Galectin-3 is a human galectin (galactose-binding lectin) family member and is expressed by many types of cells. The concentration of galectin-3 is increased to almost 31-fold in the blood circulation of colorectal cancer patients and the increased concentration of circulating galectin-3 correlates closely with cancer progression and metastasis (15). Recently, we revealed that galectin-3, at concentrations similar to those found in the circulation of cancer patients, interacts with mucin protein MUC1, promoting cancer metastasis (16,17). As the Galectin-3 protein is encoded by the *LGALS3* gene, the possibility that the LGALS3-related network likely represents a fundamental mechanism in promoting colon cancer metastasis was examined. In the present study, differentially expressed genes (DEGs) between colorectal cancer and normal cells were identified and functional analyses were subsequently performed. The TFs were then predicted and a LGALS3-related protein-protein interaction (PPI) network was constructed. Based on this bioinformatics information, the roles of LGALS3 and signaling pathways were analyzed in the pathogenesis of colorectal cancer.

Materials and methods

Affymetrix microarray data. The Affymetrix microarray data were accessible at the National Center for Biotechnology Information Gene Expression Omnibus data repository (http://www.ncbi. nlm.nih.gov/geo/) using the series accession number GSE8671 (14). In total, 32 adenomas and 32 normal colonic epitheliums were collected based on the GPL570 (HG-U133-Plus-2) Affymetrix Human Genome U133 plus 2.0 Array. The original data were converted into expression measures and normalized by the robust multiarray average (RMA) algorithm (18).

Identification and gene ontology analysis of DEGs. The DEGs were identified by using Significant Analysis of Microarray (SAM) with |logFC| >1.5 and a false discovery rate (FDR)<0.05 (δ=1) (19). GO analysis (20) was performed on the top 500 upregulated and 500 downregulated DEGs using DAVID (Database for Annotation, Visualization, and Integrated Discovery) (21). The biological process with P<0.05 considered statistically significant were screened in the present study.

Signaling pathway impact analysis. The signaling pathway impact analysis (SPIA) was performed to predict the signaling pathways that the DEGs would likely impact. SPIA combines the evidence obtained from the classical enrichment analysis with a novel type of evidence, which measures the actual perturbation on a given pathway under a given condition (22). In SPIA, pG combines enrichment pNDE and perturbation pPERT, and is then adjusted to pGFdr. In the present study, pGFdr<0.05 was set as a threshold.

Predication of transcription factors. TFatS (www.tfacts.org) was used as a bioinformatics tool to evaluate the transcription factor target genes among the list of regulated genes (23). The top 500 upregulated and 500 downregulated genes were mapped to TfactS to identify target genes with p<0.05, q<0.05, E<0.05 and FDR<0.05. In addition, the Fisher's exact test was used to examine whether the transcription factor was activated or suppressed.

Protein-protein interaction (PPI) network for LGALS3. LGALS3 was submitted to STRING database to predict the potential interacted proteins. STRING (www.//string.embl.de) is a database of predicted functional associations between proteins (24). STRING database produces a score to estimate the accuracy of each pairwise association from 0 to 1. In the present study, the PPIs were screened with score >0.7. The PPI network was subsequently visualized using Cyoscape software (25).

Results

Identification and GO analysis of DEGs. Based on SAM analysis, a total of 6,593 upregulated and 5,897 downregulated DEGs were identified. Subsequently, the GO analysis was performed to the top 500 upregulated and 500 downregulated genes, respectively (Table IA and B). The results showed that 41 downregulated DEGs, including CLDN8 and CLDN23, were enriched in cell adhesion (P=2.23E-06) (Table IA). The upregulated DEGs which included *KIF23*, *PRC1*, *TTK*, *AURKA*, *AURKB*, *PTTG1*, and *RUVBL1* were mainly enriched in the terms associated with cell cycle, such as the mitotic cell cycle (P=3.74E-34) and cell cycle process (P=3.49E-29) (Table IB).

KEGG pathways analysis. Based on SPIA analysis, a total of 21 KEGG signaling pathways were screened to determine whether they were dysregulated in colorectal cancer (Table II). Then the cell cycle (pGFdr=3.00E-04), p53 signaling pathway (pGFdr=8.82E-03), and NF-κB signaling pathway (pGFdr=3.77E-02), which significantly correlated with cancer were selected for subsequent investigation. In detail, cyclin-dependent kinase genes, such as *CDK1*, *CDK2*, *CDK4*, *CDK6* and *CDK7* were upregulated in the cell cycle pathway (Fig. 1). In the p53 signaling pathway, *ATR* and *p53* were upregulated (Fig. 2), while in the NF-κB pathway, TRAFs were significantly differentially expressed (Fig. 3).

Table I. The enriched GO terms.

A, The top 10 GO terms of the top 500 upregulated DEGs

Category	Term	Count	Genes	P-value
GO:0000278	Mitotic cell cycle	67	KIF23, PRC1, TTK	3.74E-34
GO:0022402	Cell cycle process	75	AURKA, AURKB, PTTG1	3.49E-29
GO:0000280	Nuclear division	49	KIF23, AURKA, PTTG1	4.99E-29
GO:0007067	Mitosis	49	KIF23, AURKA, PTTG1	4.99E-29
GO:0000087	M phase of mitotic cell cycle	49	KIF23, AURKA, PTTG1	1.19E-28
GO:0022403	Cell cycle phase	64	KIF23, PRC1, TTK	1.65E-28
GO:0048285	Organelle fission	49	KIF23, PTTG1, AURKA	3.43E-28
GO:0007049	Cell cycle	85	KIF23, PCR1, CDK2	3.01E-27
GO:0000279	M phase	65	PCR1, KIF23, AURKA	5.30E-27
GO:0051301	Cell division	46	PRC1, KIF23, CDK1	1.49E-20

B, The enriched terms of the top 500 downregulated DEGs

Category	Term	Count	Genes	P-value
GO:0007155	Cell adhesion	41	CLDN8, CLDN23	2.23E-06
GO:0022610	Biological adhesion	41	CLDN8, CLDN23	2.27E-06
GO:0007584	Response to nutrient	14	BMP2, A2M	7.14E-05

GO, gene ontology; DEGs, differentially expressed genes.

Table II. The 21 pathways identified based on signaling pathway impact analysis (pGFdr<0.05).

Pathway	Count	Genes	pGFdr
RNA transport	125	XPOT, NCBP1, DDX20	9.08E-09
HTLV-1 infection	195	NRP1, SLC2A1, TGFB3	3.51E-05
Natural killer cell-mediated cytotoxicity	87	NFNT5, PPP3CB, TNFSF10	3.00E-04
Cell cycle	97	CDK1, CDK2, MCM2	3.00E-04
Epstein-Barr virus infection	150	CR2, HLA-DRA, CD38	1.44E-03
Fanconi anemia pathway	43	FANCM, FANCI, FANCF	1.83E-03
Antigen processing and presentation	54	CD74, HLA-DMA, NFYA	2.35E-03
Chemokine signaling pathway	132	CXCR6, CCR1, CXCR3	8.80E-03
Staphylococcus aureus infection	37	CFD, FCGR2B, HLA-DMA	8.80E-03
P53 signaling pathway	56	P53, ATR, CDK2	8.82E-03
Fc γ R-mediated phagocytosis	66	FCGR2B, HCK, LYN	1.11E-02
Pathways in cancer	236	CASP3, CTNNB1, WNT2	2.29E-02
Protein processing in endoplasmic reticulum	124	MAPK9, SEC61B, VCP	2.29E-02
RNA degradation	57	EN01, TTC37, EXOSC9	2.29E-02
Oocyte meiosis	86	CDK1, MAD2L1, CCNB2	2.35E-02
Focal adhesion	139	ITGB3, ITGA8, FLNA	2.39E-02
Systemic lupus erythematosus	66	FCGR2B, C5, TNF	2.39E-02
Gap junction	64	CSNK1D, PRKCB, GNAI3	2.39E-02
NF-κB signaling pathway	70	TRAF5, BCL2L1, BCL2	3.77E-02
Lysosome	94	TCTRG1, ATP6VOA2, CTCS	3.85E-02
T-cell receptor signaling pathway	83	CDK4, TNF, CSF2	4.43E-02

Regulation of DEGs by transcription factors. TFactS analysis was performed to determine changes in transcription factor activity based on upregulated and downregulated genes in colorectal cancer (Table III). The results showed that *MYC* and *TCF7L2* were activated in colorectal cancer. A total of 26 target genes of MYC were identified, including 24 upregulated and

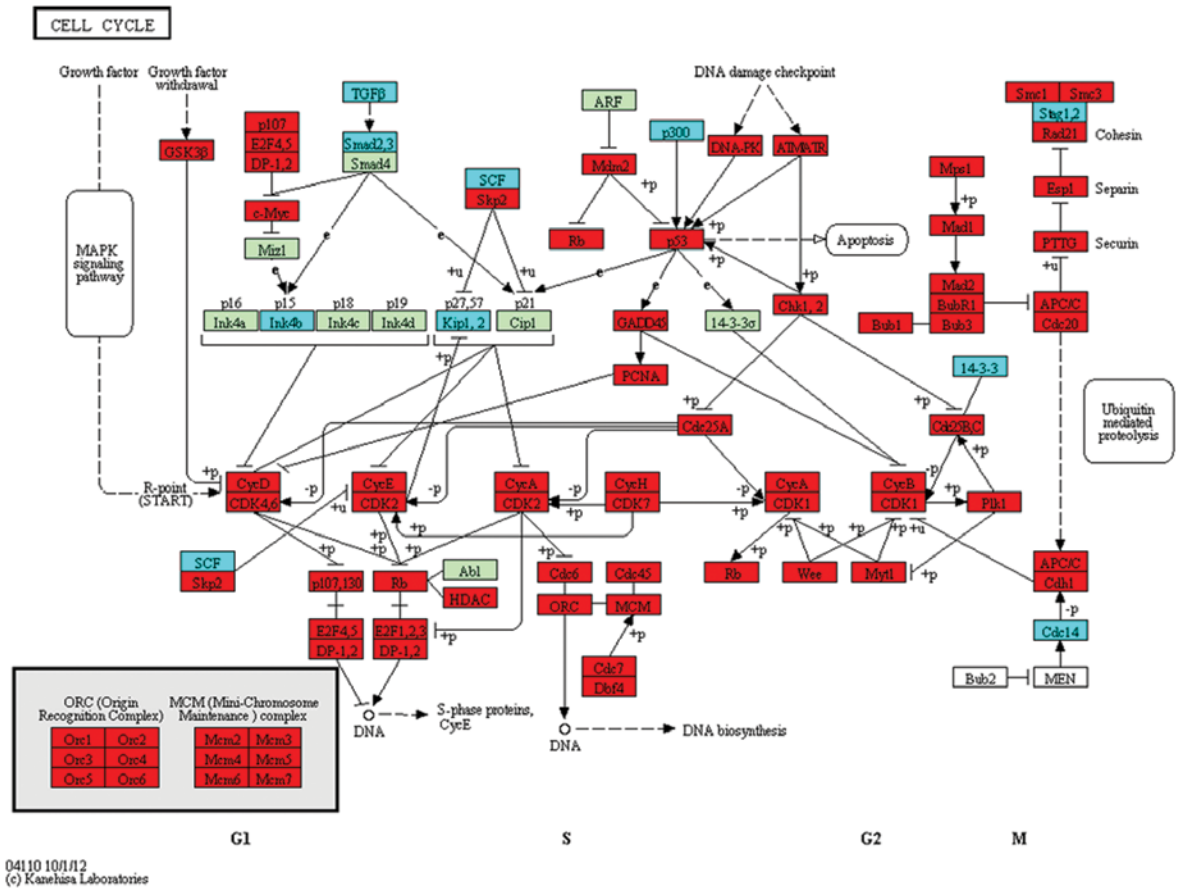

Figure 1. The cell cycle pathway which may be dysregulated in colorectal cancer. Red boxes, upregulated genes and blue boxes, downregulated genes.

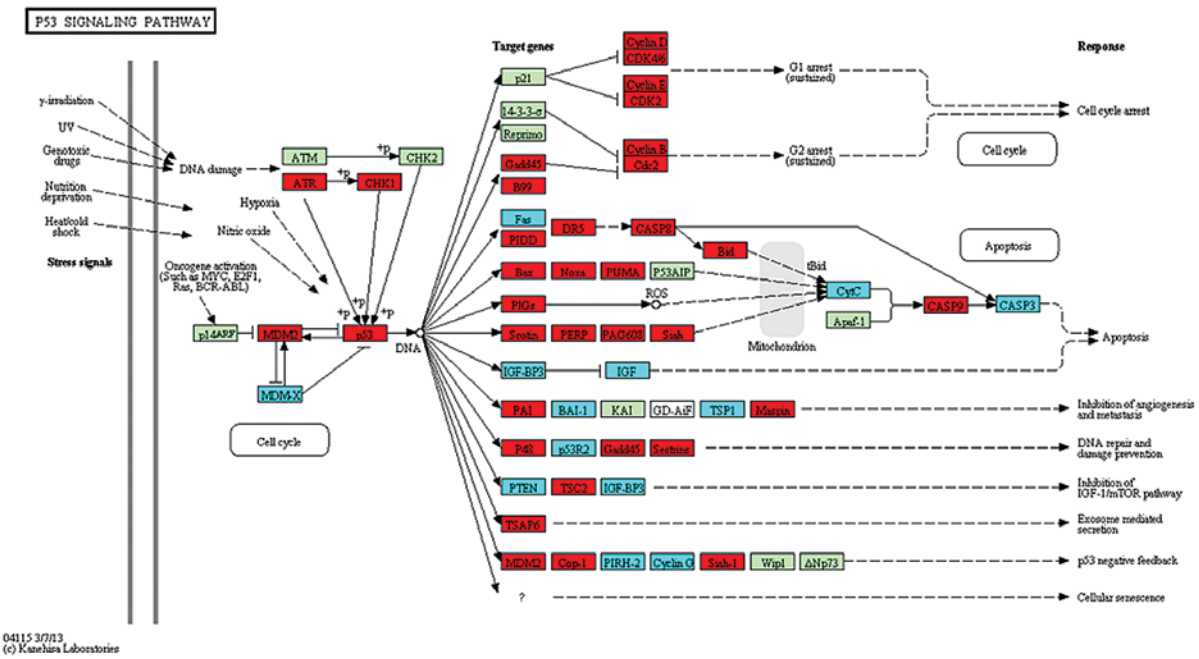

Figure 2. The p53 signaling pathway which may be dysregulated in colorectal cancer. Red boxes, upregulated genes and blue boxes, downregulated genes.

2 downregulated genes, while for TCF7L2, 8 target genes were upregulated and 2 genes were downregulated. Of note, *TCF7L2* was activated by *MYC*. Additionally, 9 target genes of *FOXO3* were downregulated and 1 gene was upregulated.

The LGALS3 PPI network. Tumor metastasis is the primary cause of mortality in patients with cancer (26). LGALS3, a member of a family of β-galactoside-binding lectins, has been found to promote tumor metastasis (22,23). To investigate the

Table III. Results of the TfactS analysis.

Gene name	TF	Regulation type	Differential expression type
ID1	FOXO3	Down	Up
TNFSF10	FOXO3	Up	Down
KLF4	FOXO3	Up	Down
BTG1	FOXO3	Up	Down
PINK1	FOXO3	Up	Down
SFRP1	FOXO3	Up	Down
BCL2L11	FOXO3	Up	Down
HPGD	FOXO3	Up	Down
CDKN2B	FOXO3	Up	Down
CITED2	FOXO3	Up	Down
MYC	MYC	Down	Up
DUSP1	MYC	Down	Down
CDKN2B	MYC	Down	Down
PCNA	MYC	Up	Up
RFC2	MYC	Up	Up
RCC1	MYC	Up	Up
NOP56	MYC	Up	Up
NME1	MYC	Up	Up
CCT6A	MYC	Up	Up
C1QBP	MYC	Up	Up
NPM1	MYC	Up	Up
CCNB1	MYC	Up	Up
CDK4	MYC	Up	Up
ODC1	MYC	Up	Up
CKS2	MYC	Up	Up
CCNA2	MYC	Up	Up
SNRPB	MYC	Up	Up
PPAT	MYC	Up	Up
APEX1	MYC	Up	Up
MIF	MYC	Up	Up
H2AFZ	MYC	Up	Up
TRAP1	MYC	Up	Up
MTHFD1	MYC	Up	Up
TP53	MYC	Up	Up
TYMS	MYC	Up	Up
UBE2C	MYC	Up	Up
CCT3	MYC	Up	Up
CASP7	TCF7L2	Down	Down
MXD1	TCF7L2	Down	Down
MYC	TCF7L2	Up	Up
ENC1	TCF7L2	Up	Up
MMP7	TCF7L2	Up	Up
MMP1	TCF7L2	Up	Up
AXIN2	TCF7L2	Up	Up
PTTG1	TCF7L2	Up	Up
CD44	TCF7L2	Up	Up
SP5	TCF7L2	Up	Up
SGK1	TCF7L2	Up	Down
CAPN2	TCF7L2	Up	Down
TAGLN	TCF7L2	Up	Down

Regulation means the regulation pattern of the transcription factor (TF) in TfactS to target gene. Differential expression type is the differential expression of DEGs in our study, 'Up' means upregulated and 'Down' is downregulated in colorectal cancer.

function of LGALS3 in colorectal cancer, the LGALS3-related PPI network was constructed (Fig. 4). The results predicated that 8 proteins (SUFU, RUNX2, ELN, MUC2, EGFR, TLR2, KRAS, and MMP2) which were encoded by downregulated genes interacted with LGALS3, while 10 proteins (HRAS, GEMIN4, GSK3B, CCND1, ANXA7, DDOST, LGALS3BP, DMBT1, IL1B, and AXIN1) encoded by upregulated genes interacted with LGALS3. In addition, no significant changes in the expression levels of *MMP9*, *KDR*, *DIF*, *PRKCSH*, *NRAS*, and *CDH5* were observed, however, the proteins encoded by these genes interacted with LGALS3.

Discussion

Colorectal cancer is the third most common type of cancer worldwide and has a high mortality rate (2). Although a number of studies have been conducted, the underlying mechanism of colorectal cancer remains to be clarified. In this study, the DEGs were identified between colorectal cancer and normal samples and their functions were predicted by GO analysis. The pathways which these DEGs dysregulated and the TFs were identified. A LGALS3-related PPI network was also established. Our findings provide a new angle for the prediction of the pathogenesis of colorectal cancer.

The GO enrichment analysis revealed that the upregulated genes were mainly enriched in cell proliferation processes, including mitotic cell cycle, cell cycle progression, nuclear division and cell division of tumor. The oncogene *AURKA*, enriched in the cell cycle, is an important protein that regulates G2 transit into M during mitosis (27). In addition, *AURKA* is associated with abnormal chromosome segregation, aneuploidy and predisposition (28). Previously, it was suggested that pituitary tumor transforming gene 1 (*PTTG1*) is an oncogene (29). The expression levels of *RUVBL1* and *RUVBL2* were increased in different types of cancer and interacted with oncogenic factors, including β-catenin and c-Myc to regulate their function (30). These upregulated genes led to abnormal cell accumulation in order to accelerate the process of colorectal cancer.

The downregulated genes, including CLDN8 and CLDN23, in colorectal cancer were significantly enriched in the cell adhesion biological process. Claudins, major components of the strands, promote cell-cell adhesion (31). CLDN8 codes for tight junction proteins expressed in distal nephron epithelium, and it is considered a candidate marker for distinguishing chromophobe renal cell carcinoma from other types of renal cancer (32). In addition, CLDN23 gene, frequently downregulated in intestinal-type gastric cancer, is a novel member of CLAUDIN gene family (33). Findings of the present study are consistent with those of previous studies.

The role of the signaling pathway in cancer pathogenesis has been previously investigated (34). Alterations in cyclin-dependent kinase (CDK) activity often leads to cell cycle defects in tumor growth (35). In the present study, CDK2, CDK4 and CDK6 were enriched in the cell cycle pathway. This result indicates that these DEGs are important in the development of colorectal cancer by dyregulating the cell cycle pathway. Previously, it has been shown that one of the most prominent regulators disrupted in cancer is the tumor suppressor, p53 (36). TRAF (TNF receptor-associated

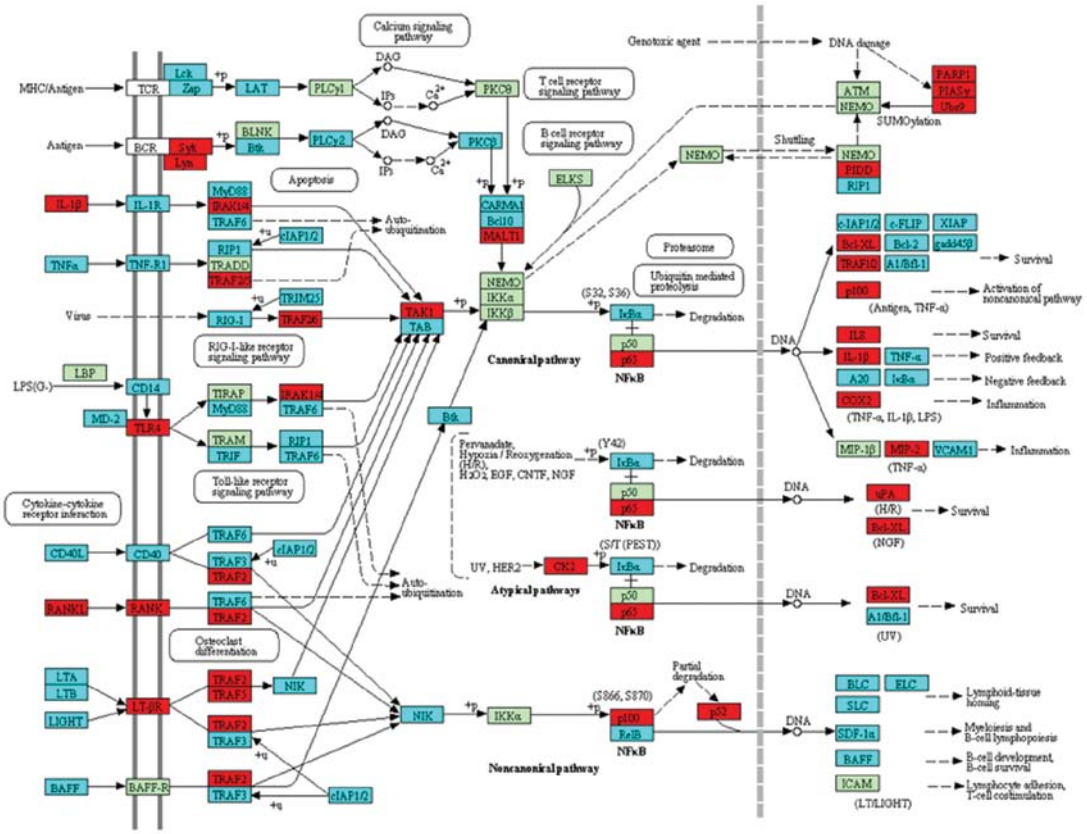

Figure 3. The NF-κB signaling pathway which may be dysregulated in colorectal cancer. Red boxes, upregulated genes and blue boxes, downregulated genes.

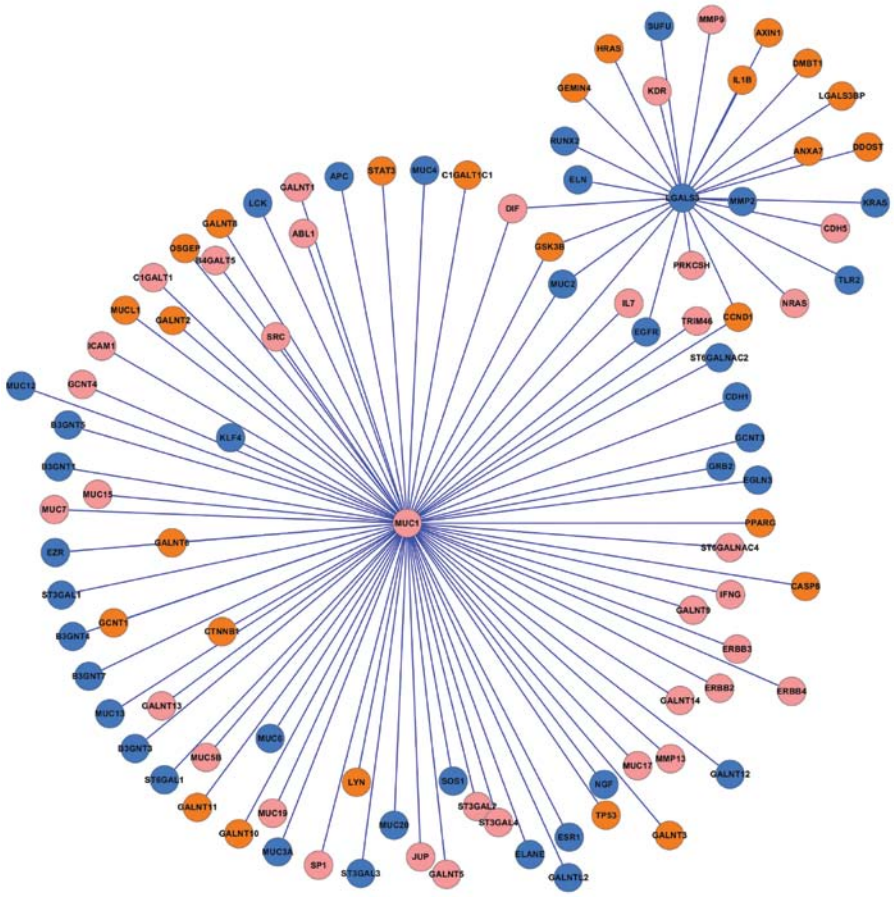

Figure 4. Protein-protein interaction (PPI) network of LGALS3. The interaction network was predicated by STRING and visualized by Cyoscape software. The protein in blue, orange, pink circles means encoded by the downregulated and upregulated genes, or no significant change, respectively.

factor) family member-associated NF-κB activator is a negative regulator of osteoclastogenesis and bone formation (37). NF-κB is one of the best-characterized transcription factors involved in the regulation of immune responses and inflammation (38,39). It has been previously suggested that inhibition of the NF-κB signaling pathway presents a notable therapeutic potential for the diagnosis of cancer (40). Results of this study have shown that genes enriched in the cell cycle, p53 signaling pathway and NF-κB signaling pathway were differentially expressed in colorectal cancer.

The list of transcription factors in most human cancer cells is limited and these factors usually serve as targets for anticancer drugs development (41). NF-κB has been used as a target for cancer drug development which induces drug resistance by changing *MDR1* expression in cancer cells (18,27). Transcription activation mediated by HIF-1α and STAT serve as targets for cancer drug development (29,30). In this study, we have shown that the transcription factors of *MYC*, *TCF7L2*, and *FOXO3* were regulators of some DEGs. *MYC* was activated in colorectal cancer and the overexpression pattern was identified as a downstream step at the end of the Wnt/APC/β-catenin signaling pathways is crucial in human cancer (42,43). The *TCF7L2* gene has been shown to be involved in renal cell carcinoma metastasis (44). Members of the FOXO transcription family were involved in several cell processes, including apoptosis, stress resistance, metabolism, cell cycle, and DNA repair (45,46). These findings are contributory to the development of cancer treatment.

Current investigations have focused on the molecular mechanism of tumor formation and metastasis (47). The expression of *LGALS3* is associated with neoplastic transformation and the differentiation of monocytes into macrophages. The present study result suggest that LGALS3 may be involved in colorectal cancer progression by interacting with upregulated and downregulated genes. Due to the LGALS3-related genes being mainly differentially expressed, LGALS3 is important in the development of colorectal cancer. The predicated network of the metastatic factor *LGALS3* may facilitate understanding of the mechanism of tumor cell metastasis to provide a therapeutic target in cancer treatment.

In conclusion, findings of the present study have demonstrated that, LGALS3, cell cycle, p53 signaling pathway and NF-κB signaling pathway are crucial in the development of colorectal cancer. Additionally, several genes that are potential candidate targets for colorectal cancer therapy have been identified. However, more studies with regard to other signaling pathway and key cancer-related proteins should be conducted in order to reveal the underlying molecular mechanism of colorectal cancer.

Acknowledgements

This study was funded by the Chongqing Natural Science Foundation (CSTC, 2011BB5120).

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