Dynamic activation of the key pathways: linking colitis to colorectal cancer in a mouse model

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An association between carcinogenesis and inflammation has long been appreciated. Chemically induced colitis-associated cancer (CAC) is a classical mouse model for investigating ‘inflammation–cancer link’ in the intestine. Diverse mechanisms behind this non-resolving inflammation model have been reported before, most of them were emphasized on key cancer genes, cytokines, and signal transduction abnormality based on prior knowledge. In this study, we dynamically and globally dissect the alteration of key pathways in the development from colitis to colorectal cancer. Striking evidence from gene expression profiling, serum cytokines detection, and immunohistochemistry analysis all reveals that different key pathways [NF-κB, STAT3, p38 mitogen-activated protein kinase (MAPK), and Wnt/β-catenin signaling] and their target genes are hyperactive in different phases of the inflammation–cancer link. Nuclear factor-kB (NF-κB) and STAT3 signaling are hyperactive in the whole process, while p38 MAPK and Wnt/β-catenin signaling are only hyperactive in the beginning and ending, respectively. Through this unbiased system biological approach, we provide strong evidence that different key pathways are specifically involved in different phases, which bridge the gap between inflammation and cancer.

Introduction

Inflammation plays important roles at different stages of carcinogenesis, from cancer initiation, to invasion and metastasis (1). Non-resolving inflammation makes individuals susceptible to many forms of cancer (2). ‘Inflammation–cancer link’ is a complex and multistep process. Global dissection of this process in different disease models could promote our understanding on inflammation and cancer, as well as improve medical professional’s awareness on inflammation prevention and treatment.

Colitis-associated cancer (CAC), arising as a result of the ‘inflammation–dysplasia–cancerina’ sequence (3), has been widely studied as a model for inflammation–cancer link. Key oncogenes and tumor suppressor genes mutations (p53, k-ras, APC etc.), hyperactivation of inflammation cytokines (IL-6, IL-1, and TNF-α), and pathways [nuclear transcription factor kappa B (NF-κB) and (signal transducers and activators of transcription) STAT3] (4–7) have been revealed and confirmed to be distinct important in CAC development. Abnormal signal transduction contributes substantially to the hallmarks of cancer (8) as well as inflammation. In this study, by using a chemically induced CAC mouse model, we dynamically and globally dissected the alteration of key pathways in the inflammation–low-grade dysplasia–high-grade dysplasia–cancerina’ sequence and found that different key pathways [NF-κB, STAT3, p38 mitogen-activated protein kinase (MAPK), and Wnt/β-catenin signaling] and their target genes were hyperactive in different phases of the inflammation–cancer link, both in messenger RNA (mRNA) levels and protein levels. Even the specific protein levels of signaling molecules that participate in these specific pathways also altered in different phases. NF-κB and STAT3 signaling are hyperactive in the whole process, while p38 MAPK and Wnt/β-catenin signaling are only hyperactive in the beginning and ending, respectively. Gene expression profiling revealed that the most dramatic changes happened in the beginning of the inflammation–cancer link, which may imply that on the resolution of inflammation, the beginning programs the end. This study provides new insights into the dynamic molecular and signaling changes in the development of CAC and suggests that alteration of key pathways links colitis to colorectal cancer.

Materials and methods

Animals and experimental procedure

The original group of Swiss mice that served as progenitors of this stock consisted of two male and seven female albino mice derived from a non-inbred stock in the laboratory of Dr. de Coulon, Centre Anticancereux Romand, Lausanne, Switzerland. These animals were imported into the United States by Dr. Clara Lynch of the Rockefeller Institute in 1926. The Haushka Ha/ICR stock was initiated in 1948 at the Institute for Cancer Research (ICR) in Philadelphia from “Swiss” mice of Rockefeller origin. To Dr. Edward Mirand of Roswell Park Memorial Institute where they were designated as HaM/ICR. To Charles River in 1959.

Five-week-old male ICR mice were intraperitoneal injected with a single dose of 10 mg/kg azoxymethane (AOM) (Sigma–Aldrich, St. Louis, MO) on day 1, followed by three cycles of Dextran sulfate sodium (DSS) (MP Biomedicals, Solon, OH, MW 36–50 kDa) administration (cycle 1: 2%, 7 days; cycle 2: 1.5%, 5 days; and cycle 3: 1.5%, 5 days), and then distilled water until the end of the experiment on day 140. Two groups of mice were sequentially killed randomly at day 14, 28, 42, 56, and 140 (at the end of the 2nd, 4th, 6th, 8th, and 20th week), and at each time point, at least five mice were killed for each group. Experimental procedure was shown in Figure 1A. All mice procedures were performed in accordance with institutional guidelines. Sample preparation

Serum from each mouse was collected for inflammatory cytokines detection. The large bowels were removed and cut open longitudinally along the main axis and flushed with saline. The masses, inflamed and normal colonic mucosa were cut and immediately placed in five volumes of RNA later (Ambion, Austin, TX), then stored in liquid nitrogen for RNA extraction. The other part of the large bowel was fixed as Swiss-roll in 4% paraformaldehyde overnight. Paraffin-embedded sections (4 μm) of the large bowel were then made by routine procedures for histopathological analysis and immunohistochemistry. RNA extraction and purification

Total RNA was isolated using Trizol or PureLink RNA Mini Kit (Invitrogen, Carlsbad, CA) following manufacturer recommendations. Quality of RNA was...
examined by gel electrophoresis and the Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA).

**Quantitative real-time reverse transcription PCR**

Quantitative real-time reverse transcription PCR (qPCR) was performed using SYBR Green Master Mix. The primers for Il6, Il10, csf3, Il1b, Cxcl1, Cxcl2, Mmp3, Mmp9, Spp1, S100a8, S100a9, Saa3, Cxcl5, Mmp7, and Postn were synthesized as shown in Supplementary Table S1, available at *Carcinogenesis* Online. qPCR was performed on the IQ5 real-time PCR detection system (Bio-Rad, Hercules, CA). The data were analyzed using IQ5 software. The relative gene expression was quantified based on the threshold cycle value and normalized to the housekeep gene *Gapdh* (n ≥ 5).

**Complementary DNA microarray analysis**

For each group, at each time point, three mice’s RNAs were detected for gene expression profiling separately. Biotinylated complementary RNA (cRNA) was prepared according to the standard Affymetrix protocol from 1 µg total RNA. Following fragmentation, 15 µg of cRNA were hybridized for 16 h at 45°C on GeneChip mouse 430 2.0 Genome Array (with 45 101 probes). GeneChips were scanned using the GeneChip Scanner 3000. The data were analyzed with Microarray Suite version 5.0 using Affymetrix default analysis settings and global scaling as normalization method. The trimmed mean target intensity of each array was arbitrarily set to 500. These data along with design parameters have been deposited in NCBI’s Gene Expression Omnibus (GEO) and are accessible through GEO Series accession number GSE30016 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE31106).

**Identification and bioinformatic analysis of differentially expressed transcripts**

Group differences in the tested samples were analyzed with the significance analysis of microarrays (SAM) 3.11 algorithm (http://www-stat.stanford.edu/~tibs/SAM/). Unsupervised hierarchical clustering of filtered, normalized, and mean-centered log2-transformed data was performed by using GeneCluster 3.0 with an average linkage clustering method and viewed by using TreeView version 1.60. We used Gene Set Enrichment Analysis (GSEA) (9) (http://www.broadinstitute.org/gsea/index.jsp) to compute gene set enrichment for gene ontology (GO) annotations of our data and visualized the enrichment results as an Enrichment Map (10) (http://baderlab.org/Software/EnrichmentMap/), a Cytoscape (11) plugin for functional enrichment visualization. Moreover, to compare our data to the data that based on the human inflammatory bowel disease (IBD) and colorectal cancer, or search on the similarities of the gene expression of DSS only

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**Fig. 1.** Experimental procedure, pathological observation, and disease activity index (DAI) curves of the CAC mouse model. (A) Experimental procedure of the AOM/DSS group and the control group. (B) General observation of the colorectums of the AOM/DSS group at the end of the 2nd, 4th, 6th, 8th, and 20th week. (C) Histopathological examination (H and E staining, top panel: ×40 original magnification, bar = 500 µm; bottom panel: ×200 original magnification, bar = 100 µm). (D) DAI of the AOM/DSS-treated mice and littermate controls [DAI = (Weight loss score + Stool characters score + Hematochezia score)/3]. *P*-value < 0.05 compared with control group.
treated mice, we downloaded two data sets from the NCBIs GEO with the GEO Series accession number GSE4183 and GSE 22307 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4183, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE22307). Finally, Kyoto Encyclopedia of Genes and Genomes (KEGG) database were used for signaling pathway analysis on differentially expressed genes by CapitalBio Molecule Annotation System (MAS) V3.0. P-value heatmap was generated for visualizing the pathway enrichment analysis.

**Histopathological analysis and immunohistochemistry**

Paraffin-embedded sections were stained with hematoxylin and eosin (H and E). Colonic mucosa dysplasia (low and high grade) and adenocarcinomas were diagnosed according to the criteria described by Boivin et al. (12). Immunohistochemistry was carried out on 4 μm-thick paraffin-embedded sections from the colorectums in mice. The deparaffinized sections were incubated with primary antibody at 4°C overnight followed by incubation with biotinylated goat antirabbit or mouse IgG antibody (Vector Laboratories, Burlingame, CA) for 30 min. Primary antibodies were purchased from the listed vendors: p65 (Santa Cruz Biotechnology, Santa Cruz, CA); p-STAT3, β-catenin, proliferating cell nuclear antigen (PCNA), B-cell lymphoma-extra large (Bcl-xL), and cyclooxygenase-2 (COX-2) (Cell Signaling Technology, Danvers, MA); and p52 and p-p38 (Boster, Wuhan, China). Omission of the primary antibody was used as negative control. The immunostained slides were observed under a microscope. Images were taken by a digital camera and semi-quantity of the signal was analyzed by counting mean density of the immunoreactivities against all primary antibodies. Brown or yellow was regarded as positive signal. Image data were analyzed with NIS-Elements AR 3.0 software (Nikon, Japan).

**Inflammatory protein array**

Comprehensive analysis of inflammatory protein levels were performed by using commercially available RayBio Mouse Inflammation Antibody Array Kit (13) (RayBiotech, Norcross, GA) as described previously (14). Briefly, for each sample, one nitrocellulose membrane, each containing 40 different antibodies in duplicate spots, were blocked, incubated with appropriately diluted sera (pooled of five mice blood per group, the initiate amount of pooled blood was 250 μl), washed, and then incubated with a cocktail of biotin-conjugated antibodies specific for the different proteins. The chemiluminescent signal was detected using a charge-coupled device imaging system (Alpha Innotech Fluochem, San Leandro, CA). The resulting images were analyzed using Quantity One software (Bio-Rad) to measure expression of various cytokines. Positive control spots and negative control spots within the membranes were used to normalize the results from different membranes being compared.

**Results**

GSEA depicted a dynamic molecular and functional landscape of the link between inflammation and cancer

Many previous studies on AOM/DSS-induced CAC mouse model focused on some oncogenes and tumor suppressor genes, cytokines and chemokines, as well as key signaling. Indeed, this model presented inflammation-low-grade dysplasia-high-grade dysplasia-carcinoma sequential pathological process (Figure 1C), and with a relatively high level of disease activity index (15) compared with their normal littermates (Figure 1D). These pathway processes closely mimicked human ulcerative colitis and CAC. So, we used it to dynamically dissect the molecular events that link the colitis and CAC through a global high level of disease activity index (15) compared with their normal controls at each time point have been shown in Supplementary Figure S3, available at Carcinogenesis Online.

**Sequential activated key pathways drove the initiation and progression of CAC**

Signaling pathways are the main ways for genes to perform a variety of biological functions. As we found that the functional gene sets changed between inflammation and cancer, we presume that chronic inflammation and cancer arise from dysfunction of multiple key pathways.

SAM algorithm was used to analyze gene expression profiles changes in the different time points. Probe sets with q-value (%) ≤ 5 and fold change ≥ 2 or ≤ 0.5 were chosen for further analysis. Compared with normal colon mucosa, a storm of gene expression changed, with a burst at the end of the second week of AOM/DSS group (i.e. acute inflammation period), as 2245 probe sets showed significantly elevated expression and 2294 probe sets were down-regulated in the inflamed mucosa. This changing was extremely greater than other time points. The number of probe sets up/down regulation between each group had been shown in Supplementary Figure S2A, available at Carcinogenesis Online. Some of the significantly expressed genes (il6, csf3, Cxcl1, Mmp3, Sl100a8, Pstn, etc) had been validated by qPCR (Supplementary Figure S2B is available at Carcinogenesis Online).

Because little changes occurred among the last three time points, with related pathological diagnosis with high-grade dysplasia and adenocarcinoma, the CAC carcinogenesis process was divided into three distinguished phases in this study, named inflammatory phase (the 2nd week), low-grade dysplastic phase (the 4th week), and high-grade dysplastic/cancerous phase (the 6th-20th week). The latter two collectively named as neoplastic phase.

To define an integrity view of the gene ontology (biological processes, molecules function, and cellular component) changes among the inflammation–cancer link, we used GSEA to compare gene set enrichment for inflammatory phase (the second week) and neoplastic phase (the sixth week), then visualized the gene set enrichment results as a single integrated enrichment map (10) (Figure 2), as to simplify identification both similarities and differences between inflammation and tumor. The node center and node border color represents the enrichment obtained for the inflammatory phase and the neoplastic phase, respectively. Uniform up-regulation of biological processes, such as apoptosis, neural system development, actin cytoskeleton organization, and down-regulation of transmembrane transporter activity, generation of precursor metabolites and energy, oxidoreductase activity were observed in both inflammatory and neoplastic phase. The differentially expressed genes in apoptosis annotated to the GO terms positive regulation of apoptosis process and negative regulation of apoptosis process compared with the normal controls at each time point were shown in Supplementary Figure S3, available at Carcinogenesis Online.

Other gene sets enriched in this map mainly activated in the inflammatory phase, such as protein kinase cascade, in which components of MAPK and NF-kB signaling were significantly elevated in this phase, suggesting these pathways were highly activated from the very start. Gene sets included response to external stimulus, inflammatory response, and response to wounding were persistent till the neoplastic phase, while regulation of response to stimulus and regulation of immune response seems failed after tumors formed. Homeostatic process was an erratic one, as the homeostasis, cellular cation homeostasis, homeostasis of number of cells, and regulation of body fluid level/blood pressure activated in the inflammatory phase, they became non-responsive in the neoplastic phase. Further, the signaling pathways that regulate these biological effects brought to our attention.

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Differentially altering pathways in each time point were analyzed by MAS 3.0 (http://bioinfo.capitalbio.com/mas3/) based on KEGG database. Pathway enrichment analysis was shown as a P-value heat-map, which implied changed activity of the specific interested pathways that play a distinct role at different stages of CAC (Figure 3). The representative gene lists of the activated pathways in each time point were shown in Supplementary Table S2, available at Carcinogenesis Online.
Pathways such as cytokine–cytokine receptor interaction, cell communication, complement and coagulation cascades, hematopoietic cell lineage, and leukocyte transendothelial migration were activated from inflammation to cancer, highlighted the key roles of inflammation and immune system in tumorigenesis. Toll-like receptor signaling pathway and vascular endothelial growth factor signaling pathway were activated mainly in the inflammatory phase. Although Jak-STAT signaling pathway only significantly activated
Fig. 3. P-value heatmap of signaling pathways during CAC progression. (A) Hierarchical clustering was performed to make a P-value heatmap of 31 significantly changed signaling pathways. The P-value score was obtained by MAS 3.0 based on KEGG database. P-value is indicated as yellow to red boxes, according to the color bar shown on the top right. Gray boxes signify P-value > 0.05. (B) Fold change of expression levels of differentially expressed genes relative to the important pathways ‘cytokine–cytokine receptor interaction’ (a), and ‘Wnt signaling pathway’ (b). Green boxes signify low expression and red boxes signify high expression, according to the color bar shown on the bottom right. (C) Gene expression levels of Cxcl1, Cxcl5, and Mmp7 at serial time points. The left panel shows the gene array data, and the right panel shows the gene expression levels detected by qPCR. *Statistical significant compared with normal.

**Abbreviations**
- Norm. = Normal
- Infla. = Inflammation
- LD. = Low grade dysplasia
- HD. = High grade dysplasia
- Ca. = Adenocarcinoma
at very early time point in mRNA level, we detected persistent activation of STAT3 by immunohistochemistry (see below, as well as Supplementary Figure S4 available at Carcinogenesis Online). Most noteworthy was Wnt signaling pathway, though appeared activated in early stage, the inhibitors of this pathway, Wnt inhibitory factor (WIF) and secreted frizzled-related proteins (SFRPs), were also activated significantly before high-grade dysplastic phase, which implies Wnt signaling is hyperactive only when high-grade dysplasia presented. Some signaling pathways related to carcinoma, such as basal cell carcinoma, melanoma, and colorectal cancer, etc were dramatically changed in tumors as well (data not shown). NF-κB signaling pathway is not an independent pathway in the KEGG database; so, it has not been shown in the heatmap, however, the downstream target genes of this pathway were strikingly differentially expressed in the whole process of CAC carcinogenesis, such as Cxcl1 (16), Il1b (17), Il6 (18), Csf3 (19), Vcam1, Icam1 (20), Egfr (21), S100a8, S100a9 (22), and Cd40 (23). p38 MAPK signaling was upregulated in the inflammatory and low-grade dysplastic phases, but backed to normal level in the high-grade dysplastic and cancerous phase.

Furthermore, expression levels at each time points of the genes involved in the cytokine–cytokine receptor interaction and Wnt signaling pathway were shown in Figure 3B. Some of the important genes were confirmed by qPCR (Figure 3C and Supplementary Figure S2, available at Carcinogenesis Online). The target genes of NF-κB pathway, STAT3 pathway, p38 MAPK pathway, and Wnt signaling pathway were listed in the Supplementary Tables S3~S6, available at Carcinogenesis Online, respectively, and discussed in Figure 5.

As shown in the Supplementary Table S2, multiple and different pathways were hyperactive in different disease phases, while in different phases, the genes (i.e. differentially expressed genes compared with control group) involved in the same pathways are varied, which implicated the regulatory outcomes of the same signaling pathway may varied in different phases and may have different functions among the link between inflammation and cancer.

Key molecules of specific signaling pathways in protein level in the colorectum with different pathological stages

Proteins are the ultimate enforcer of signaling pathways. To confirm the signaling abnormality observed in mRNA levels, we tested the related protein levels in different time points along the inflammation–cancer progression. We assayed five signaling pathways enriched in the mRNA expression profiles, the canonical NF-κB pathway, alternative NF-κB pathway, STAT3 pathway, p38 MAPK pathway, and Wnt/β-catenin signaling pathway through immunohistochemistry detection of p65, p52, p-STAT3, p-p38, and β-catenin, respectively (Supplementary Figure S4, available at Carcinogenesis Online).

Expression of p65 and p-STAT3 were increased in both epithelial cells and stromal cells. The expression level and localization of p65 and p-STAT3 changed along with the course of colitis-associated carcinoma development, implied persistent activation of canonical NF-κB and STAT3 signaling in both epithelial cells and stromal
cells. Alternative NF-κB signaling pathway were mainly activated in the inflammatory cells as the immunopositive staining of p52 located in nuclears of stromal cells infiltrated in the mucosa and submucosa. No p52 staining was observed in normal/transformed epithelial cells, which suggested alternative NF-κB signaling is only hyperactive in the inflammatory cells. Phosphorylated p38 (p-p38) was highly expressed both in the nuclears of epithelial cells and stromal cells in the inflammatory phase. Although p-p38 was moderately expressed by dysplastic colonic epithelium, adenocarcinoma showed dramatically reduced expression by the malignant epithelial cells, implying that p38 MAPK signaling is back to normal levels when cancer had been established in this model.

β-catenin was restricted to cell membrane in non-cancerous epithelial cells, whereas carcinoma cells and part of early phase inflammatory cells showed cytoplasmic redistribution and exclusive nuclear staining, suggesting that Wnt signaling was mainly upregulated in the late phase of the inflammation–cancer link.

The gradually strengthened immunopositivity in the nucleus of PCNA, reflected both DNA synthesis and DNA repair, were increased during the process of inflammation to cancer. In addition, the crucial downstream targets of both p65 and STAT3 signaling, COX-2 and Bcl-xL, also elevated significantly during this process same to the above observation on p65 and p-STAT3.

Serum signaling protein expression profiles validated the major pathways activated during colitis-associated carcinogenesis

Striking changes of the key signaling happened in the intestine revealed their importance in colitis-associated carcinogenesis. We hypothesized that the characteristic changes of inflammation cytokines and chemokines in blood could reflect the disease status, generating a detectable stage-specific molecular phenotype. For this reason, we measured 40 cytokines and chemokines levels in the blood samples of AOM/DSS-treated mice and controls at serial time points with the RayBio Mouse Inflammation Antibody Array.

Surprisingly, NF-κB, STAT3, and/or p38 MAPK signaling pathway-dependent cytokines, such as IL-1β, IL-6, KC/CXCL1 (24–26), eotaxin (27), and eotaxin-2, were especially upregulated in the inflammatory phase; the cytokines that activated or regulated by both Wnt/β-catenin signaling and NF-κB, such as granulocyte-macrophage colony stimulating factor (28), granulocyte colony stimulating factor (29), SDF-1/CXCL12 (30), RANTES/CCR5 (31), were upregulated in different phases of CAC, but especially in the high-grade dysplastic/cancerous phase (Figure 5). These results well matched the pathway changes detected in the intestine (Figure 3) (Supplementary Figure S4, available at Carcinogenesis Online) and also confirmed dynamic activation of the key pathways link inflammation and cancer in the CAC model.

Fig. 5. Schema of the key pathways alteration during the colitis-associated carcinogenesis processes of the AOM/DSS mouse model. The molecules listed in the figure are significantly upregulated in this study by means of mRNA expression profiling, protein array, and immunohistochemistry analysis. For example, in the left panel (inflammatory phase), chemokines (CCL2, 4, 6, CXCL1, 2, 3, 5, etc) and chemokine receptors (CCR1, 2, 5, 6, etc) were upregulated in the inflammatory phase in AOM/DSS mice, which can induce STAT3 signaling activation, and, then increase the transcription of STAT3 downstream target genes (Il10, Il12, CD86, Tgf, Bcl2 etc). All of the target genes at the bottom of the left panel can continue to affect the inflammation environment and contribute to linking inflammation and cancer.
Cross-species expression profiles comparison of mouse and human intestine diseases

As a mutagen, the role of single, low dose of AOM treatment in inflammation promoted carcinogenesis has not been shown. So, we compared the inflammation phase (DSS treated at day 7) of our data with another research data (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE22307), which was derived from the C57BL/6J mice's colon tissue treated with 3% DSS on day 6. The enriched gene sets were surprisingly similar (Supplementary Figure S5, available at Carcinogenesis Online), which indicated this model has good stability and reproducibility. What is more, the carcinogen AOM may minimally affect the molecular function in the inflammatory phase.

We next used GSEA to compare the expression profiles of mouse model with human beings, by using expression profile data set GSE4183 of human IBD and sporadic colorectal carcinoma (SCC) (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4183). Due to more serious inflammation, the current CAC mouse model presented more obvious gene sets variation, though most gene sets were, on the whole, the same as human IBD, e.g. response to external stimulus, cytokine activity, homeostasis, apoptosis, angiogenesis, and protein kinase cascade (Supplementary Figure S6A available at Carcinogenesis Online). On the other hand, only part of gene sets such as apoptosis, angiogenesis, and protein dimerization activity were upregulated in both mouse CAC model and human SCC. The gap between these two process was widening, as response to stimulus, cell–cell signaling, homeostasis, kinase regulator activity, etc. (Supplementary Figure S6B available at Carcinogenesis Online) were highly enrichment in the CAC mouse model but unremarkable in human SCC.

This expression profiles comparison revealed that the CAC mouse model can well mimic human IBD. Otherwise, the molecular mechanisms underlying IBD-associated cancer is different from the SCC. We presumed the obvious differences between CAC and SCC mostly due to the inflammatory background existed in the CAC mouse model.

Discussion

Non-resolving inflammation like IBD is associated with an increased incidence of colorectal cancer (32). It has been widely accepted that inflammation plays a critical role in colorectal cancer initiation, progression, and as a multistage disease that has been defined as an inflammation–low-grade dysplasia–high-grade dysplasia–carcinoma sequence, mechanisms underlying CAC development seems complicated for there is no clear genetic basis has been identified to explain colorectal cancer predisposition in IBD.

AOM/DSS-induced CAC mouse model has been used to elucidate the mechanisms and test the therapeutic effects of CAC. By taking advantage of this chemically induced CAC mouse model, we employed a global view and a time-course manner to dissect the molecular events bridging the gap between inflammation and cancer.

Gene expression profiling and GSEA of different time points found that different key pathways were dynamically altered during the colitis–low-grade dysplasia–high-grade dysplasia–carcinoma process, mechanisms underlying CAC development seems complicated for there is no clear genetic basis has been identified to explain colorectal cancer predisposition in IBD.

Conclusion

In conclusion, we presented a global view of molecular events happened in the process of colitis-associated carcinogenesis in a mouse model. NF-κB and STAT3 signaling are hyperactive in the whole inflammation–cancer link, while p38 MAPK and Wnt/β-catenin signaling are only hyperactive in the beginning and ending, respectively.
Striking changes of the key pathways in the different time points confirm a solid link between inflammation and cancer and suggest the possibility that the epithelial cells undergo malignant transformation by taking advantage of the non-resolving inflammation environment or direct stimulation from the cancer-favorable inflammation environment. The massive information obtained from high-throughput technology provided new insights into the understanding the actual space and temporal sequence of the key signaling pathways in the colitis–dysplasia–carcinoma process (Figure 5), creating new insights into the therapeutic and preventive methods for CAC.

**Supplementary material**

Supplementary Tables S1–S6 and Figures S2–S6 can be found at http://carcin.oxfordjournals.org/.

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**Conflict of Interest Statement**: None declared.

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