Transcriptomic Analysis Provides Insights into the Molecular Mechanisms of Epigallocatechin-3-gallate to Attenuate Schistosomiasis Hepatic Fibrosis in Mice

Jinmei Feng¹, Fahu Yuan¹, Stephen Hsu² and Wenjian Song*¹
¹Department of Pathogenic Biology, School of Medicine, Jianghan University, Wuhan 430056, China  
²Dental College of Georgia, Augusta University, Augusta, GA 30912, USA  
*For correspondence: songwenjian@outlook.com

Abstract

Epigallocatechin-3-gallate (EGCG) was shown to possess anti-inflammatory, anti-infection, and anti-fibrotic effects. Our previous study showed EGCG could attenuate Schistosoma japonicum hepatic fibrosis, however, its underlying molecular mechanisms are still elusive. In this work, RNA-sequencing (RNA-seq) and bioinformatics analyses were used to detect the gene expression alterations in S. japonicum hepatic fibrosis mouse model following EGCG treatment. Our results showed 106 common differentially expressed genes (DEGs) were upregulated in EGCG_LF_vs_Mod_LF (EGCG treatment group relative to S. japonicum infected model group) and downregulated in Mod_LF_vs_Nor_LF (model group relative to normal group), and they are enriched in cytochrome and metabolic genes involving in the metabolism pathways, implying EGCG could improve the S. japonicum egg induced hepatic injury and recover the metabolic functions of the liver. The 410 common DEGs, downregulated in EGCG_LF_vs_Mod_LF and upregulated in Mod_LF_vs_Nor_LF, are associated primarily with inflammation and immunology. Among them, proinflammatory cytokines, chemokines to activate the hepatic stellate cells (HSCs), profibrotic genes, and extracellular matrix (ECM) production and accumulation genes, together with part components of TLR2 and NF-κB signaling pathways, were upregulated in Mod_LF_vs_Nor_LF and downregulated in EGCG_LF_vs_Mod_LF. These results indicate that EGCG treatment could ameliorate S. japonicum egg induced hepatic fibrosis progression via inhibiting the HSCs activation and reducing ECM production and accumulation, which may in part result from suppressing the TLR2 and NF-κB signaling pathways. Our results reveal the molecular mechanisms of EGCG to be used as a potential drug to regress S. japonicum egg induced hepatic fibrosis. © 2018 Friends Science Publishers

Keywords: EGCG; Hepatic fibrosis; Mechanism; RNA-Seq; Schistosoma japonicum

Introduction

Human schistosomiasis is still considered to be one of the most prevalent and important parasitic diseases causing severe morbidity and mortality in infected individuals in the worldwide. The World Health Organization (WHO) reported that about 800 million people are at risk of schistosomiasis, and approximately 260 million individuals afflicted in over 70 countries and territories in the worldwide (Engels et al., 2002; WHO, 2016). In China, Schistosoma japonicum is mainly prevalent and endemic in the south of China, which gives rise to a serious public health problem with the intestinal and hepatosplenic schistosomiasis (Engels et al., 2002). By the end of 2015, 77,194 cases of schistosomiasis were calculated, and 30,843 cases of advanced schistosomiasis were reported in China (Zhang et al., 2016).

In human schistosomiasis, the pathology is mostly caused by the eggs trapped in the hepatic and intestinal tissues of host. The toxic egg material damages cells in host tissues, and the host tissues lodged with eggs generate persistent antigenic stimulation. Subsequently, the inflammatory and immune cells were recruited to the infected locates, which induce the production of egg granulomas and progressively replaced by chronic and advanced fibrosis in some infected cases (Chuah et al., 2014). The most severe consequence of S. japonicum infection is given rise to hepatic fibrosis, which primarily induced by hepatic stellate cells (HSCs) to remodel the extracellular matrix (ECM) and deposit the collagens (Carson et al., 2018). The persistent hepatic fibrosis of schistosomiasis usually links with liver-related mortality (Takeamura et al., 1998). Therefore, effective drug treatments to control and regress hepatic fibrosis of schistosomiasis is urgently needed.

Currently, praziquantel (PZQ) is still chosen as the first-line anti-schistosome drug for chemotherapy of schistosomiasis (Gryseels, 2012). However, the effect of
PZQ on *Schistosoma* egg induced hepatic fibrosis is still a controversial issue. Previous studies reported that the PZQ had anti-fibrotic effects on mice in infected with *S. japonicum* (Liang et al., 2011) and *S. mansoni* (El-Lakkany et al., 2012). However, other study revealed that the hepatic fibrosis persists to develop even after the PZQ treatment (Abdel-Hafeez et al., 2012). Thus, no clear consensus has been reached on anti-fibrotic effects of PZQ on mice which were infected with *Schistosoma*. Furthermore, PZQ induces considerable side effects in clinical treatments (Pinlaor et al., 2008). Hence, developing high efficiency and low side-effect anti-fibrosis drugs to control and regress hepatic fibrosis is imperious demands for the schistosomiasis.

Green tea polyphenols have antioxidant activity, which results in the potential promise of using them as dietary agents to prevent and treat several human chronic diseases (Khan and Mukhtar, 2007). Among the mixture of biologically-active green tea polyphenols, one of the ampltest constituent is the Epigallocatechin-3-gallate (EGCG) (Steinmann et al., 2013). EGCG has been shown to possess antioxidant, anti-viral, anti-bacterial, anti-fungal, anti-inflammatory and anti-fibrotic functions, and neuroprotective properties in various diseased individuals (Dona et al., 2003; Chakrawarti et al., 2016; Ying et al., 2017; Muhammed et al., 2018). Numerous previous reports showed that EGCG is vital in anti-fibrotic effect by suppressing the production of collagen and regulating the collagenase activity in HSCs (Yasuda et al., 2009; Tipoe et al., 2010; Sriram et al., 2015; Hsieh et al., 2017). More importantly, we previously revealed that EGCG intervention on mice infected with *S. japonicum* can decrease the egg granulomas and collagen deposition significantly, and furtheral alleviated the *S. japonicum* egg induced hepatic fibrosis (Yuan et al., 2016). However, the underlying molecular mechanisms of EGCG to ameliorate the *S. japonicum* egg induced hepatic fibrosis are still elusive.

To elucidate the underlying molecular mechanisms of EGCG to attenuate the *S. japonicum* egg induced hepatic fibrosis, we focused on bioinformatics analyses of RNA-Seq sequence data by using differentially expressed gene (DEG) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and gene ontology (GO) enrichment analysis to identify the genes and pathway altered, which provide insight into gene expression alternations in EGCG administrated *Schistosoma*-infected mouse livers after the formation of hepatic fibrosis. Our results revealed that EGCG significantly ameliorates *S. japonicum* egg induced hepatic fibrosis by suppressing the toll-like-receptors 2 (TLR2) and NF-κB signaling pathway to repress the activation of HSCs and decrease the accumulation of ECM. Our results reveal the molecular mechanisms of EGCG to be used as a potential drug to treat and regress *S. japonicum* egg induced hepatic fibrosis.

**Materials and Methods**

**Ethics Statement**

Experimental mice were kept and cared under the guidelines of the Jianghan University Ethics Committee for using experimental animals. Mice experiments were carried out under the international accepted regulations and with the approval of Jianghan University Ethics Committee. To minimize the suffering, sodium pentobarbital anesthesia were carried out for all surgery.

**Mice and Parasites**

The male BALB/c mice weighing 20-25 g and aged 7–8 weeks were provided by the Hubei Provincial Center for Disease Control and Prevention (Wuhan, China). The animals were fed with the standard pellet food and water *ad libitum* in an automatically controlled animal house with at 23-25°C room temperature.

The *S. japonicum* infected Oncomelania hupensis snails was supplied by the Jiangsu Institute of Schistosomiasis Control (Jiangsu, China). The cercariae used for modeling mice were shed from the infected *O. hupensis* snails in dechlorinated water with artificial light at 28°C.

**Experimental Design and Sample Preparation**

Mice were randomly divided into normal control (Nor_LF), *Schistosoma* model (Mod_LF) and *Schistosoma*+EGCG (400 mg/kg) (EGCG_LF) groups with ten mice in each group. Following the methods used in previous studies (Liu et al., 2011), 25 ± 2 cercariae of *S. japonicum* were put on the shaved abdominal skin of mice to infect them percutaneously in both the Mod_LF group and EGCG_LF group. At the same time, treatments were also performed on mice in the Nor_LF group but with no cercariae infection. The infected mice were orally administered PZQ (500 mg/kg) on week 7 for 2 days to eliminate the adult worms. Then, mice in the EGCG_LF group were treated intragastrically with EGCG (400 mg/kg) daily from week 7 to week 11 after infection. During the same time, mice in both the Nor_LF group and Mod_LF group were treated intragastrically with sterile water daily. All animals were sacrificed after intraperitoneal injection with 0.75% sodium pentobarbital at 24 h after the last oral gavage. Then they were immediately dissected, and the right lobe of the livers of the animals were removed, frozen and stored at -80°C until further processing.

**RNA Sequencing and Analysis**

Trizol reagent (Invitrogen, USA) was used to extract the total RNA individually from the cryopreserved mice liver samples, and the DNase I was used to treat the extracted total RNA. Subsequently, poly-T oligo-attached magnetic
beads were used to purify mRNA from total RNA. The mRNA was fragmented into short fragments, which were used as templates to synthesize the cDNA. After purifying and resolving the short fragments and connecting with adapters, the 150–200 bp fragments were used as templates to perform PCR amplification. The quantification and qualification of the library was assessed by using the Agilent Bioanalyzer 2100 system. The library was paired-end sequenced on an Illumina HiSeq2000 platform to produce 150 bp read.

Raw reads were subjected to removing low quality reads, reads with unknown nucleotides larger than 5% and reads containing adapters to obtain clean reads. The low quality sequencing reads were filtered with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) . Then the high quality clean reads were mapped to the reference genome. Both the reference mouse genome and gene model annotation files were obtained from genome website (ftp://ftp.ensembl.org/pub/release-87/fasta/musculus/dna/). Bowtie v2.2.3 was used to build index of the reference genome and TopHat v2.0.12 was used to align the paired-end clean reads to the reference genome.

Screening DEGs and Functional Analysis

The read numbers mapped to each gene were counted by HTSeq v0.6.1. The FPKM (fragments per kb per million reads) method were used to measure the gene expression level of each gene (Trapnell et al., 2010). DESeq R package (1.18.0) was used to conduct the differential expression analysis. Two-fold change (log2 fold-change ≥1 or ≤-1) was used to screen the DEGs of each biological replicate, with referring to the significance of digital gene expression profiles for statistical test (Audic and Claverie, 1997) and a 0.001 Benjamini and Hochberg False Discovery Rate (FDR) (Benjamini and Hochberg, 1995) corrected P-value cut-off. The DEGs were identified with a corrected P-value less than 0.05.

GO functional enrichment and KEGG pathway analyses were implemented with a hypergeometric distribution model to reveal the functions of the DEGs. The GOseq R package was used to perform GO enrichment analysis of differentially expressed genes. The corrected P-value 0.05 was used as cut-off to assign the significantly enriched GO terms. The KEGG pathway analysis was performed by using the KEGG database (http://www.genome.jp/kegg/) (Kanehisa and Goto, 2000), and the pathways enrichment analysis was conducted by the KOBAS software (Xie et al., 2011).

Results

Gene Expression Analysis of the RNA-seq

We assessed the correlations of three biological replicates of liver samples from each group. We found the correlation among the nine samples was from 0.85 to 0.99, and among the three biological replicates of each group was more than 0.96 (Fig. 1). In the Nor_LF group, Mod_LF group, and EGCG_LF group, hierarchical clustering heat-map of the significant DEGs were drawn by Genespring software (Fig. 2). Two major expression patterns (upregulation and downregulation in infected mice compared to control uninfected mice) are shown by the hierarchical clustering. These expression data showed that the greatest number of DEGs were present in mice between the Nor_LF group and the Mod_LF group, and significant differences of DEGs were also identified in mice between the Nor_LF group and EGCG_LF group or between the Mod_LF group and EGCG_LF group, illustrating that the intragastric administration of EGCG affects the liver gene expression in Schistosoma-infected mice (Fig. 2).

Comparisons of DEGs Among different Groups

Between any two groups of mouse livers, genes which were upregulated or downregulated with at least two-fold changes in expression were assigned as DEGs. The venn diagrams of upregulated and downregulated DEGs among different groups are shown in Fig. S1a and b, respectively. The number of shared DEGs between groups is shown in the overlapping part, and the number of unique DEGs between groups is presented in the non-overlapping part. We found 2066 and 1322 genes were specifically upregulated and downregulated in livers of mice in the Mod_LF group relative to those in the Nor_LF group, respectively. Furthermore, in mouse livers of the EGCG_LF group relative to those of the Nor_LF group, the specifically upregulated and downregulated gene numbers were ten and two, respectively. Compared the Mod_LF group with the EGCG_LF group, numbers of the specifically upregulated and downregulated genes of the mouse livers were 119 and 431, respectively. As compared with those of the Nor_LF group, 22 common upregulated and 76 common downregulated genes were found in mouse livers of both infected groups.

Functional Enrichment Analysis of DEGs

DEGs between the Nor_LF group and the Mod_LF group: There were 3,486 DEGs between the Nor_LF group and the Mod_LF group. Compared with the samples from the Nor_LF group, 2,088 upregulated and 1,398 downregulated DEGs in the Mod_LF group, respectively (Fig. S1). GO terms of the upregulated DEGs in the Mod_LF group were focused on immune system process, regulation of response to stimulus, positive regulation of biological process, immune response and inflammatory response, which are mainly related to inflammatory and immune response (Fig. S2a), while GO terms of the downregulated DEGs were focused on metabolism, such as oxidation-reduction process, small molecule metabolic process, carboxylic acid metabolic process, fatty acid metabolic process, and lipid metabolic process (Fig. S2b).
Results of the KEGG pathway enrichment analysis show that the upregulated DEGs in the Mod_LF group were predominantly related to infection and immune pathways, including chemokine signaling pathway, cytokine-cytokine receptor interaction, focal adhesion, NF-kB signaling pathway, ECM-receptor interaction, platelet activation, and so on (Fig. S3a), while the downregulated DEGs were mainly associated with drug metabolism, metabolic pathways, retinol metabolism, and oxidative phosphorylation (Fig. S3b). To sum up, compared with the Nor_LF group, the upregulated genes in the Mod_LF group are predominantly related to the inflammatory and immune processes, while the downregulated genes are primarily associated with the metabolism.

DEGs between the EGCG_LF group and the Mod_LF group: There were 553 DEGs between the EGCG_LF group and the Mod_LF group. As compared the EGCG_LF group with the Mod_LF group, 122 upregulated DEGs and 431 downregulated DEGs were found (Fig. S4). The GO function analysis of the upregulated DEGs in the EGCG_LF group
group showed that the primarily enriched GO terms are associated with metabolism, such as small molecule metabolic process, carboxylic acid metabolic process, lipid metabolic process, and fatty acid metabolic process (Fig. 4a). While the downregulated DEGs in the EGCG_LF group were mainly correlated with inflammatory and immune response, such as immune system process, cytokine production, cell adhesion, biological adhesion, regulation of cell adhesion and cytokine production (Fig. 4b). Further pathway analysis revealed that the upregulated DEGs are focused on the metabolism pathways, metabolism of xenobiotics by cytochrome P450, steroid hormone biosynthesis, and retinol metabolism (Fig. 5a), while the downregulated DEGs are focused on the cytokine-cytokine receptor interaction, ECM-receptor interaction, rheumatoid arthritis, malaria, and NF-kappa B signaling pathway (Fig. 5b). Altogether, relative to the Mod_LF group, the downregulated genes in the EGCG_LF group are predominantly related to the inflammatory and immune processes, while the upregulated genes are primarily associated with the metabolism. Compared these results of DEGs between the EGCG_LF group and Mod_LF group (EGCG_LF_vs_Mod_LF) with those of DEGs between the Mod_LF group and Nor_LF group (Mod_LF_vs_Nor_LF), we found that the GO functional and KEGG pathway enrichment results between them are just the reverse.

Comparisons of the DEGs between the EGCG_LF_vs_Mod_LF and Mod_LF_vs_Nor_LF: The above results revealed that the GO functional enrichment and KEGG pathways of the up-down-regulated DEGs in the EGCG_LF_vs_Mod_LF are almost contrary to those in the Mod_LF_vs_Nor_LF. Therefore, we further compared the DEGs between them in detail. We found 106 common genes are shared by the 122 upregulated DEGs in the EGCG_LF_vs_Mod_LF and 1398 downregulated DEGs in the Mod_LF_vs_Nor_LF (Table S1). The 106 common genes are involved predominantly in metabolic processes (Fig. S5a) and metabolism related pathways (Fig. S5b), consistent with the results of the upregulated DEGs in EGCG_LF group compared with the Mod_LF group. Notably, among them, expression levels of five cytochrome genes were shown different significantly between the 122 upregulated DEGs in EGCG_LF_vs_Mod_LF and 1398 downregulated DEGs in Mod_LF_vs_Nor_LF. Cytochromes 2c29 (Cyp2c29), Cyp4f14, Cyp2j5, Cyp2j2, and Cyp2c67 were significantly upregulated in the EGCG_LF group relative to the Mod_LF group, while downregulated in the Mod_LF group compared with the Nor_LF group (Table 1.).

There are 410 common genes shared by the 431 downregulated DEGs in the EGCG_LF_vs_Mod_LF and 2088 upregulated DEGs in the Mod_LF_vs_Nor_LF (Table S2). The significant difference between the expression patterns of DEGs in EGCG_LF_vs_Mod_LF and Mod_LF_vs_Nor_LF was the opposite expression profiles. The opposite expressed genes were predominantly focused on the biological process including immune system process, cytokine production, cell adhesion, biological adhesion, cell surface receptor signaling pathway, regulation of cytokine production, and collagen metabolic process (Fig. S6a). Prominent genes of interest were related to neutrophils, HSC activation and hepatic fibrosis (Table 1).

Three genes related to neutrophils, the neutrophilic granule protein (NGP), myeloperoxidase (MPO), and cathepsin G (CTSG), were upregulated significantly in Mod_vs_Nor_LF, while they were downregulated in EGCG_vs_Mod_LF (Table 1). Two chemotactic molecules for neutrophils, S100A8 and S100A9, were also significantly upregulated in Mod_vs_Nor_LF and downregulated in EGCG_vs_Mod_LF (Table 1). Activation of toll-like-receptors (TLRs) facilitates neutrophil recruitment and it was reported that TLR2 and TLR4 have increased expressions and important functions in liver disease (Seki et al., 2011). Here, we found TLR2 and TLR4 are significantly upregulated in Mod_vs_Nor_LF (Table 1), and TLR2 is significantly downregulated in EGCG_vs_Mod_LF.

Activated HSCs produce ECM to progress the hepatic fibrosis. In this study, we revealed that many genes implicated in the ECM production and accumulation were upregulated significantly in Mod_vs_Nor_LF, such as the laminin (Lamb1 and Lam2), integrin (Itgα4 and Itgβ7), thrombospondine (Thbs1, Thbs2 and Thbs3), and profibrotic cytokines (Il17ra, Ccl12, Ccl21 and Ccl6) (Table 1). However, they were significantly downregulated in EGCG_vs_Mod_LF. Furthermore, we detected that NF-κB2 and RelB were significantly upregulated in Mod_vs_Nor_LF and downregulated in EGCG_vs_Mod_LF (Table 1), which is in accordance with the expression patterns of genes implicated in the ECM production and accumulation. Previous study reported that NF-κB plays a vital role in fibrosis and regulates multiple essential functions of HSCs (Luedde and Schwabe, 2011). Therefore, our results imply that NF-κB may be involved in regulating the activation of HSCs with EGCG treatment.

Our previous study detected more severe hepatic fibrosis in livers of schistosoma-infected model group mice than those of EGCG treatment group mice (Yuan et al., 2016). Thus, hepatic fibrosis associated genes were inspected in the two comparisons. Tissue inhibitor of metalloproteinase 1 and 2 (TIMP1, 2) and matrix metalloproteinases 2, 3, 8, 9, 11 and 23 (MMP2, MMP3, MMP8, MMP9, MMP11, MMP23) were significantly upregulated in Mod_vs_Nor_LF, while they are downregulated in EGCG_vs_Mod_LF (Table 1). Moreover, the profibrogenic genes, platelet-derived growth factor receptor-β (PDGFR-β), transforming growth factor-β (TGF-β), TNFα, Col11a1 and Col6a5, showed significantly upregulated in Mod_vs_Nor_LF, while they are significantly downregulated in EGCG_vs_Mod_LF (Table 1).
KEGG pathway enrichment analysis showed that the 410 common genes shared by the 431 downregulated DEGs in the EGCG_LF_vs_Mod_LF and 2088 upregulated DEGs in the Mod_LF_vs_Nor_LF are predominantly involving in four pathways (Fig S6b), including cytokine-cytokine receptor interaction and ECM-receptor interaction, indicating that EGCG intervention altered the ECM deposition.

Discussion

EGCG, the amplest and most active polyphenolic catechin found in green tea, has attracted considerable attentions (Steinmann et al., 2013). Numerous beneficial biological effects of EGCG consumption have been revealed as regards physiological and pharmacological health benefits. The anti-inflammatory mechanisms of EGCG has been partly attributed to decrease the pro-inflammatory response (Tipoe et al., 2010) or suppress the activation of TLR-4/NFκB p65 signal pathway (Marinovic et al., 2015). Moreover, EGCG has been shown anti-fibrotic effect via different mechanisms in various diseases. Sriram had demonstrated that EGCG can suppress TGF-β1 signaling in pulmonary fibrosis to suppress the activation of fibroblast and reduce the collagen accumulation (Sriram et al., 2015). Lin found that EGCG attenuated the myocardial fibrosis via inhibiting the inflammatory effect through JNK/AP-1 pathway (Lin et al., 2016). Wang revealed that EGCG attenuating renal interstitial fibrosis in mice might be associated with its inhibition effects on inflammatory responses and TGF-β/Smad signaling pathway (Wang et al., 2015). Previous studies have demonstrated that EGCG attenuates hepatic fibrosis in rat by regulating the TGF/SMAD, PI3 K/Akt/FoxO1 and NF-κB pathways (Xiao et al., 2014) and suppressing the expression of the PDGFR and insulin-like growth factor receptor (IGF-1R) (Yasuda et al., 2009).
More importantly, we have found that EGCG could alleviate the hepatic fibrosis induced by *S. japonicum* egg in mice (Yuan et al., 2016). However, the mechanistic basis for EGCG to improve the *S. japonicum* egg induced hepatic fibrosis is still elusive. In order to explore the vital differential genes and pathways in *S. japonicum* egg induced hepatic fibrosis following EGCG intervention, in this work, based on the RNA-Seq sequence data, the differentially expressed genes and pathways were identified by using bioinformatics analyses such as the DEG analysis, KEGG pathway analysis and GO enrichment analysis. Our aim is to reveal the gene expression alternations in the *S. japonicum* egg induced hepatic fibrosis mouse model following the EGCG intervention.

In previous study, we observed moderate granulofibrotic pathology in EGCG treatment group mice and severe one in the *S. japonicum* infected model group mice (Yuan et al., 2016). Here, the 106 common DEGs which are upregulated in EGCG_LF_vs_Mod_LF and downregulated in Mod_LF_vs_Nor_LF are primarily involved in the metabolic pathways, metabolism of xenobiotics by cytochrome P450 and drug metabolism - cytochrome P450 (Fig. S5).
The present study, the expression is presented by value of log2FoldChange. Negative values represent down-regulation, and positive values represent up-regulation.

More importantly, the 410 common DEGs downregulated in EGCG_LF_vs_Mod_LF and upregulated in Mod_LF_vs_Nor_LF play a vital role in attenuating the S. japonicum egg induced hepatic fibrosis following EGCG treatment. The pathology of schistosomiasis is the T-helper (Th) cell induced fibro-granulomatous inflammatory response to eggs deposited in host tissues and the subsequent fibrogenesis (Lewis and Tucker, 2014). Thus, the inflammation response plays a vital role in exacerbating liver injury and promoting the subsequent fibrogenesis, and reducing the levels of proinflammatory cytokines may be favourable for hepatic fibrosis. In the present study, the expression levels of proinflammatory cytokines, such as...
Ccl4, Cxcl1, and Cxcl2, were significantly upregulated in Mod_LF_vs_Nor_LF, while they were downregulated in EGCG_LF_vs_Mod_LF, suggesting that EGCG treatment ameliorates liver injury, possibly due to reduced expression levels of inflammatory cytokines. Moreover, the neutrophil markers NGP, MPO and CTSG were significantly upregulated in Mod_LF_vs_Nor_LF and downregulated in EGCG_LF_vs_Mod_LF, indicating EGCG treatment ameliorates S. japonicum egg induced hepatic fibrosis might be associated with neutrophils.

In Schistosome infected livers, the activated HSCs are the major cells to produce the profibrogenic molecules and collagen secreted by the fibroblasts (Bartley et al., 2006). The profibrotic cytokines CCL21 and CCL12 have been found to be chemoattractive for HSCs or myofibroblasts and have been involved in the pathogenesis of various fibrotic diseases (Bonacchi et al., 2003; Moore et al., 2006). Here, the chemokines CCL12 and CCL21 were significantly upregulated in Mod_LF_vs_Nor_LF and significantly downregulated in EGCG_LF_vs_Mod_LF. Coincidentally, the expression patterns of profibrotic genes TGF-β, TNFα, and PDGFR presented a striking relevance to the expression patterns of the chemokines CCL21 and CCL12. TGF-β is considered as a key profibrogenic gene in the progression of hepatic fibrosis (Seki et al., 2007). TNFα signaling elicits necroptosis in activated HSCs to ameliorate hepatic fibrosis (Chang et al., 2015). PDGF is a potent mitogen to mediate the HSC proliferation during liver injury (Kinnman et al., 2001). Moreover, studies revealed that EGCG might inhibit HSC proliferation and activation by interfering with PDGF/PDGFRβ signaling (Chen and Zhang, 2003; Yasuda et al., 2009). Thus, our results indicate that EGCG treatment may be contributed to improve the HSC-associated fibrosis by regulating the expression of the chemokines of fibrogenic effector cells and the profibrotic genes.

Hepatic fibrosis results from the excessive accumulation of collagens and other ECM constituents. In liver pathologies, activated HSCs are the primary source of MMPs, TIMPs, and various ECM constituents, such as collagen, laminin, fibronectin, proteoglycan and adhesive glycoproteins (Hemmann et al., 2007; Seki and Brenner, 2015). We revealed that genes involved in ECM production and accumulation (Lamb1, Lama2, Itga4, Itgb7, Tbs1, Tbs2 and Tbs3), collagen deposition (Col11a1 and Col6a5), and profibrotic cytokines (Il17ra, Ccl12, Ccl21 and Ccl6) were significantly upregulated in Mod_LF_vs_Nor_LF, but they were all significantly downregulated in EGCG_LF_vs_Mod_LF (Table 1). Furthermore, our result showed that the expression levels of multiple MMPs (MMP-2, -3, -9, -11, -23) and TIMPs (TIMP-1, -2) were upregulated significantly in the Mod_LF_vs_Nor_LF (Table 1), which is in accordance with the studies that multiple MMPs and TIMPs are upregulated in S. japonicum egg induced hepatic fibrosis (Burke et al., 2010). However, they were downregulated in the EGCG_LF_vs_Mod_LF (Table 1). The altered expressions of MMPs and TIMPs in EGCG_LF_vs_Mod_LF and Mod_LF_vs_Nor_LF indicated that the wound healing responses are different between livers of the S. japonicum infected mice and EGCG intervention ones, which may partly account for the less severe of hepatic fibrosis observed in the EGCG treated mice. Collectively, all these results imply that EGCG might exhibit the anti-fibrosis property to S. japonicum egg induced hepatic fibrosis via inhibiting the HSCs activation and subsequent ECM deposition. To confirm the conclusion, we will isolate the primary HSCs of EGCG-treatment mice for checking the mRNA expressions in the future.

To further investigate the underlying mechanism of EGCG attenuating the hepatic fibrosis, we examined the activation of the NF-κB signaling pathway, which had been reported to regulate the hepatic fibrogenesis mainly via modulating the activation of HSCs and hepatocyte injury (Luedde and Schwabe, 2011). Herein, NF-κB2 and Relb were significantly upregulated in Mod_LF_vs_Nor_LF, implying S. japonicum egg induced hepatic fibrosis significantly increased NF-κB signaling activation. Interestingly, EGCG treatment significantly decreased S. japonicum egg induced NF-κB activation as evidenced by the downregulation of NF-κB2 and Relb in EGCG_LF_vs_Mod_LF. Taken together, these results suggest that EGCG treatment alleviates the S. japonicum egg induced hepatic fibrosis by reducing HSC activation, possible through inhibiting the NF-κB signaling pathway.

TLRs are implicated in the modulation of inflammation and fibrosis in liver (Mencin et al., 2009). TLR2 has been reported to promote hepatic inflammation and fibrogenesis (Miura et al., 2013), and TLR2 deficiency alleviates hepatic fibrosis via inhibiting NF-κB signaling pathway (Ji et al., 2014). Here, the transcriptional levels of TLR2 and 4 were significantly upregulated in Mod_LF_vs_Nor_LF, consistent with the previous report that TLR2 and 4 were important and expressed increasingly in acute and chronic liver disease (Seki et al., 2011). However, we revealed that TLR2 was significantly downregulated in EGCG_LF_vs_Mod_LF. Interestingly, the downregulation of TLR2 in EGCG_LF_vs_Mod_LF was accompanied with the downregulation of the proinflammatory and profibrotic genes including Ccl4, Cxcl1, Cxcl2, PDGFR, TNFα, TGFβ, MMPS and TIMPS in EGCG_LF_vs_Mod_LF (Table 1). TLR2 is highly expressed in the Kupffer cells and HSCs (Seki and Brenner, 2008), which are known as the primary cells to produce the profibrotic molecules and ECM components (Coenen et al., 2011). Thus, the downregulated TLR2 in EGCG_LF_vs_Mod_LF might be contributed to alleviate S. japonicum egg induced hepatic fibrosis with EGCG treatment, indicating EGCG alleviates S. japonicum egg induced hepatic fibrosis may be associated with the downregulation of TLR2.

To check the reliability of the RNA-Seq data analysis, qRT-PCR analysis is needed to check expression profiles of
genes produced by Illumina. The main limitation of the study is lacking of experimental confirmation in mouse. However, our previous studies revealed that EGCG intervention on mice infected with *S. japonicum* can decrease the expression level of TIMP1 (Yuan et al., 2016), which is consistent with the result from the RNA-Seq in this work, suggesting the RNA-Seq data were reliable.

**Conclusion**

Our studies indicate that EGCG intervention could ameliorate *S. japonicum* egg induced hepatic fibrosis progression, which might be result from the inhibition of HSC activation and subsequently decreased expression of procollagen and profibrotic genes to reduce ECM production and accumulation, at least partly by suppressing the TLR2 and NF-κB signaling pathways. Our results reveal the molecular mechanisms of EGCG to be used as a potential drug to reverse *S. japonicum* egg induced hepatic fibrosis. However, at the present time, EGCG has only been used to treat *S. japonicum* egg induced hepatic fibrosis in mice. Therefore, in order to use EGCG as clinical agent to treat hepatic fibrosis of human schistosomiasis, the clinical trial and data to favor the present results of EGCG are needed in the future.

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**Reference**


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